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ABSTRACT BOOK

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ABSTRACT BOOK

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BEST ABSTRACTS

B001

A PHASE 3 STUDY OF ENASIDENIB (ENA) VERSUS CONVENTIONAL CARE REGIMENS (CCR) IN OLDER PATIENTS WITH LATE-STAGE MUTANT-IDH2 (MIDH2) RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (R/R AML)

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Prognosis is poor for older patients (pts) with R/R AML, especially if multiple treatments (Tx) have failed. This open-label phase 3 trial enrolled pts age ≥60 yrs who received 2-3 prior AML Tx. Pts were first preselected to a CCR–azacitidine (AZA; 75 mg/m^2 ×7d), intermediate-dose Ara-C (IDAC; 0.5–1.5 g/m^2 ×3–6d), low-dose Ara-C (LDAC; 20 mg BID ×10d), or best supportive care (BSC) only—and then randomized 1:1 to ENA (100 mg QD) or preselected CCR (28d cycles). Endpoints included overall survival (OS), event-free survival (EFS), time to Tx failure (TTF), overall response rate (ORR), hematologic improvement (HI), and transfusion independence (TI). Endpoints were assessed in the ITT population, and OS was also estimated in efficacy-evaluable (E-E) pts (≥1 dose study drug and ≥1 response assessment on-Tx). 158 pts were randomized to ENA and 161 to CCR (AZA 69, IDAC 33, LDAC 37, BSC-only 22). Median age was 71 yrs, 21% of pts received ≥3 prior AML Tx, 40% had primary refractory AML, and 63% were adverse-risk. Baseline (BL) characteristics were similar between Tx arms. In the ENA and CCR arms, median Tx durations were 142d (3–1270) and 36d (1–1166). 20 CCR pts (12%) and 1 ENA pt did not receive study Tx. 47 ENA pts (30%) and 69 CCR pts (43%) received subsequent AML Tx, including 19 CCR pts who received subsequent ENA. Median OS (ITT) was similar with ENA and CCR: 6.5 and 6.2 mo (HR 0.86; *p*=0.23); 1-yr survival rates were 37.5% *vs* 26.1%. Compared with CCR, ENA significantly improved EFS, TTF, ORR, and CR and HI rates; RBC and platelet TI favored ENA (Table 1). For pts preselected to lower-intensity Tx (AZA, LDAC, or BSC; ENA 139, CCR 128), median OS was 6.8 *vs* 6.2 mo with ENA *vs* CCR (HR 0.74; p=0.029). Median OS in pts with *IDH2*-R172 mutations was ~2-fold longer with ENA (n=43) *vs* CCR (n=45): 14.6 *vs* 7.8 mo (HR 0.59; p=0.039). In E-E pts (ENA 147; CCR 129), median OS was 6.8 *vs* 5.7 mo (HR 0.77; p=0.047). ENA safety was consistent with prior studies.

OS (ITT) results may be confounded by pts randomized but not treated, early discontinuation, and subsequent Tx (including ENA)—all higher in the CCR arm. When the effect of no Tx or early discontinuation was reduced (E-E population), OS was improved with ENA. ENA also prolonged OS for pts preselected to lower-intensity Tx and pts with m*IDH2*-R172 AML, and meaningfully improved other efficacy endpoints. HI and TI benefits also support ENA outpatient Tx for pts with m*IDH2* R/R AML.

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Table 1.

Efficacy	ENA N=158	CCR N=161
Overall survival (ITT), months, median [95%CI]	**6.5 [5.5, 9.5]**	**6.2 [4.6, 7.7]**
HR [95%CI]; log-rank *P*	0.86 [0.67, 1.10]; *P* = 0.23	
Overall survival (Efficacy Evaluable)	n=147	n=129
months, median [95%CI]	6.8 [5.7, 9.8]	5.7 [4.6, 7.6]
HR [95%CI]; log-rank *P*	0.77 [0.59, 1.00]; *P* = 0.047	
Event-free survival,* months, median [95%CI]	4.9 [3.7, 5.9]	2.6 [1.9, 4.4]
HR [95%CI]; log-rank *P*	0.68 [0.52, 0.91]; *P*=0.008	
Time to treatment failure,† months, median [95%CI]	4.9 [4.0, 6.0]	1.9 [1.4, 2.5]
HR [95%CI]; log-rank *P*	0.53 [0.41, 0.67]; *P*<0.0001	
Overall response rate (ORR),‡ n (%)	64 (40.5)	16 (9.9)
Odds ratio [95%CI]; Fisher's exact *P*	6.1 [3.3, 11.1]; *P*<0.0001	
CR rate, n (%)	37 (23.4)	6 (3.7)
Fisher's exact *P*	*P*<0.0001	
Stable disease, n (%)	64 (40.5)	54 (33.5)
Disease progression, n (%)	13 (8.2)	29 (18.0)
Not evaluable,§ n (%)	17 (10.8)	62 (38.5)
Time to first response (ORR), days, median (range)	92 (24–337)	59 (29–177)
Duration of response (ORR),¶ months, median [95%CI]	7.3 [5.6, 11.1]	NE [2.5, NE]
RBC-Transfusion Independence (TI),‖ n/N (%)		
RBC-TD at BL, achieved TI on-study	33/104 (31.7)	9/97 (9.3)
RBC-TI at BL, retained TI on-study	32/53 (60.4)	7/44 (15.9)
Platelet-TI,‖ n/N (%)		
Platelet-TD at BL, achieved TI on-study	26/88 (29.5)	8/74 (10.8)
Platelet-TI at BL, retained TI on-study	48/69 (69.6)	22/67 (32.8)
Any Hematologic Improvement (HI),‖ n (%)	67 (42.4)	18 (11.2)
Fisher's exact *P*	*P*<0.0001	
HI-Erythroid	21 (13.3)	9 (5.6)
HI-Platelet	31 (19.6)	7 (4.3)
HI-Neutrophil	57 (36.1)	13 (8.1)

*Time from randomization to relapse, PD, or death. †Treatment discontinuation for any reason. ‡ORR includes CR, CRi/CRp, PR, and MLFS, per International Working Group (IWG) 2003 response criteria for AML. §No postbaseline marrow collected (considered nonresponders; included in denominator for response assessments) ¶Date of first morphologic response to relapse, PD, or death. ‖Per IWG 2006 response criteria for MDS.

BL, baseline; CCR, conventional care regimens; CR, complete remission; CRi/CRp, CR with incomplete blood count/platelet recovery; ENA, enasidenib; HR, hazard ratio; MLFS, morphologic leukemia-free state; PR, partial remission; PD, progressive disease; RBC, red blood cell; TD, transfusion-dependent.

B002

EFFICACY AND SAFETY OF AVAPRITINIB IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS: INTERIM RESULTS FROM THE OPEN-LABEL, SINGLE-ARM, PHASE 2 PATHFINDER STUDY

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Background: Treatment outcomes remain poor for patients (pts) with advanced systemic mastocytosis (AdvSM). PATHFINDER (NCT03580655) is a pivotal open-label, single-arm, phase 2 study evaluating avapritinib in pts with AdvSM.

Methods: Pts aged ≥18 years with centrally confirmed diagnosis of an AdvSM subtype were enrolled. Primary endpoint was overall response rate (ORR) by modified IWG-MRT-ECNM criteria. Secondary endpoints included overall survival (OS), mean baseline change in AdvSM-Symptom Assessment Form Total Symptom Score (TSS), and safety.

Results: As of June 23, 2020, 62 pts with AdvSM received avapritinib primarily at 200 mg orally once daily (QD); 84% pts remained on treatment. Median age was 69 years (range 31–88) and 68% had prior systemic therapy (55% with midostaurin). The primary endpoint was met with an ORR of 75% (95% confidence interval [CI] 57–89) in 32 ORR-evaluable pts at the pre-specified interim analysis (median follow-up 10.4 months; Table 1). Median time to response was 2 months (range 0.3–12); responses deepened over time. Median OS in the safety population (N=62) was not reached; estimated 12-month OS was 86%. In the safety population, there were ≥50% reductions from baseline for serum tryptase (54/58; 93%), marrow mast cells (44/50; 88%), and *KIT* D816V variant allele fraction (33/55; 60%). Mean TSS at baseline (n=56) was 18.3/80 (0 represents no symptoms and 80 the most severe symptoms). Most severe symptoms were fatigue, spots, itching, flushing, and abdominal pain. TSS improved rapidly from baseline at Cycle 3 which was sustained through Cycle 11 (mean 36% improvement [P<0.001]). Common (≥25%) adverse events (AEs; any grade, Grade ≥3) were peripheral (50%, 3%) and periorbital (48%, 3%), edema, thrombocytopenia (45%, 16%), and anemia (32%, 16%). Overall, 3 (5%) pts discontinued due to a treatment-related AE and 3 (5%) pts discontinued due to disease progression, including 1 pt with transformation to secondary acute myeloid leukemia. There were 3 (5%) deaths, all considered unrelated to treatment. Seven (11%) pts had cognitive AEs (all Grade 1–2). One pt with pre-treatment severe thrombocytopenia (platelets <50×10^9/L) had Grade 4 subdural hematoma.

Summary: Avapritinib at a 200 mg QD starting dose induced a high rate of rapid responses (2 months) that deepened over time regardless prior therapy. Avapritinib was generally well tolerated with few pts discontinuing treatment due to AEs.

Table 1. Confirmed ORR and best response by mIWG-MRT-ECNM criteria in pts with AdvSMa .

Outcome, n (%)	All (n=32)	ASM (n=2)	SM-AHN (n=26)	MCL (n=4)	All (n=32)			
					Prior therapy (n=23)	No prior therapy (n=9)	Prior midostaurin (n=17)	No prior midostaurin[b] (n=15)
ORR[c]	24 (75)	2 (100)	21 (81)	1 (25)	17 (74)	7 (78)	14 (82)	10 (67)
95%CI	57–89	16–100	61–93	1–81	52–90	40–97	57–96	38–88
CR	0	0	0	0	0	0	0	0
CRh	6 (19)[d]	1 (50)	5 (19)	0	3 (13)	3 (33)	3 (18)	3 (20)
PR	10 (31)	1 (50)	8 (31)	1 (25)	7 (30)	3 (33)	5 (29)	5 (33)
CI	8 (25)	0	8 (31)	0	7 (30)	1 (11)	6 (35)	2 (13)
SD	4 (13)	0	2 (8)	2 (50)	2 (9)	2 (22)	0	4 (27)
PD	1 (3)	0	0	1 (25)	1 (4)	0	0	1 (7)
NE	3 (9)	0	3 (12)	0	3 (13)	0	3 (18)	0

[a]The PATHFINDER pre-specified interim analysis occurred in 32 ORR-evaluable pts with a diagnosis of AdvSM, who received at least 1 dose of avapritinib and were evaluable per mIWG-MRT-ECNM criteria at baseline and had ≥2 complete post-baseline response assessments and ≥6 cycles of therapy (or came off study).

[b]Includes pts previously treated with other anti-neoplastic therapies

[c]CR + CRh + PI + CI, all lasting ≥12 weeks.

[d]Median time to CRh: 5.6 months (range 1.8–6.1).

AdvSM, advanced systemic mastocytosis; ASM, aggressive systemic mastocytosis; 95%CI, 95% confidence interval; CI, clinical improvement; CR, complete remission; CRh, complete remission with partial hematologic recovery; MCL, mast cell leukemia; mIWG-MRT-ECNM, modified International Working Group-Myeloproliferative Neoplasms Research and Treatment and European Competence Network on Mastocytosis; NE, not evaluable for response; ORR, overall response rate; PD, progressive disease; PR, partial remission; pts, patients; SD, stable disease; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm.

B003

PEVONEDISTAT (P) PLUS AZACITIDINE (A) VS A ALONE IN HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS): EFFICACY AND SAFETY RESULTS FROM STUDY P-2001 (NCT02610777)

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P, an investigational first-in-class inhibitor of NEDD8-activating enzyme, disrupts degradation of select proteins leading to cancer cell death. Patients (pts) with HR-MDS/chronic myelomonocytic leukemia (Revised International Prognostic Scoring System risk >3, including intermediate [≥5% blasts], high or very high risk) or low-blast acute myeloid leukemia (AML) naive to hypomethylating agents were randomized 1:1 to receive P (20 mg/m^2 intravenously [IV] on days 1, 3, 5) + A (75 mg/m^2

IV/subcutaneously on days 1–5, 8, 9) (n=58) or A alone (n=62) in 28-day cycles until unacceptable toxicity, relapse from complete remission (CR) or partial remission (PR), transformation to AML or progression. The study was powered for event-free survival (EFS: time from randomization to death/transformation to AML, whichever occurred first). These analyses focus on clinical, cytogenetic and genetic factors affecting efficacy and safety in pts with HR-MDS. In the intent-to-treat population (n=120), EFS trended longer (median 21.0 *vs* 16.6 months [mos]; hazard ratio [HR] 0.67; 95% confidence interval [CI] 0.42–1.05; p=.076) with P+A *vs* A. In pts with HR-MDS (n=67), baseline characteristics were balanced between arms. EFS was longer with P+A *vs* A (median 20.2 *vs* 14.8 mos; HR 0.54; 95% CI 0.29–1.00; p=.045). For pts with high-risk MDS, as assessed by the Cleveland Clinic model formula (n=16 per arm), median EFS was 20.2 *vs* 11.7 mos (HR 0.39; 95% CI 0.17–0.90; p=.023) and median overall survival (OS) was 24.2 *vs* 14.2 mos (HR 0.45; 95% CI 0.19–1.05; p=.056) with P+A *vs* A. Overall response rate in response-evaluable pts (n=59, CR+PR+hematologic improvement) was 79% with P+A *vs* 57% with A, with a CR rate of 52% *vs* 27% (p=.050); median duration of response was 34.6 *vs* 13.1 mos (p=.106). Median (range) time to AML transformation in pts who transformed (P+A [n=5] *vs* A [n=9]) was 12.2 (4.6–12.6) *vs* 5.9 (1.7–14.8) mos. Pts received 13.5 *vs* 10 cycles (median) of P+A *vs* A; A dose intensity was 98% (median) in both arms. Exposure-adjusted adverse event (AE) rates were lower with P+A *vs* A (Table 1). Clinical activity of P+A was noted in pts with adverse-risk mutations.

In pts with HR-MDS, P+A *vs* A led to longer EFS, delayed transformation to AML, nearly double the CR rate and triple the duration of response. EFS and OS favored P+A *vs* A in pts with MDS assessed as high-risk by the Cleveland Clinic model formula. Exposure-adjusted AE rates were lower with P+A *vs* A, without added myelosuppression.

Table 1. Rates of AEs, SAEs and grade ≥3 AEs normalized by mean number of A cycles dosed.

	P+A (n=32)	A (n=35)
Any AE, normalized n* (n)	1.96 (32)	3.27 (35)
Treatment-related AE, normalized n* (n)	1.35 (22)	2.52 (27)
SAE, normalized n* (n)	1.47 (24)	1.87 (20)
Treatment-related SAE, normalized n* (n)	0.25 (4)	0.28 (3)
Grade ≥3 AE, normalized n* (n)	1.84 (30)	2.71 (29)

*Normalized n=AE (n)/A cycles dosed (mean). A, azacitidine; AE, adverse event; P, pevonedistat; SAE, serious adverse event.

**Normalized n=AE (n)/A cycles dosed (mean). A, azacitidine; AE, adverse event; P, pevonedistat; SAE, serious adverse event.*

B004

ECHELON-2 (NCT01777152), A RANDOMIZED, DOUBLE-BLIND, PHASE 3 STUDY OF BRENTUXIMAB VEDOTIN + CHP VS CHOP IN PREVIOUSLY UNTREATED PATIENTS WITH CD30-POSITIVE PERIPHERAL T-CELL LYMPHOMA: 5-YEAR RESULTS

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The ECHELON-2 study established the superiority of frontline brentuximab vedotin + cyclophosphamide, doxorubicin and prednisone (A+CHP) *vs* cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) in patients (pts) with untreated systemic anaplastic large cell lymphoma (sALCL) or other CD30-expressing peripheral T-cell lymphomas (PTCL). At the primary analysis, A+CHP significantly improved progression-free survival (PFS), the primary endpoint, and overall survival (OS) *vs* CHOP; A+CHP was the first treatment regimen to increase OS *vs* CHOP in this population. We report 5-year data from ECHELON-2. Adults with untreated CD30-positive PTCL were randomised 1:1 to receive 6–8 cycles of A+CHP or CHOP. We report PFS per investigator (INV) and the following key secondary endpoints: OS, PFS in sALCL, complete remission (CR) and objective response rates (ORR) in re-treated pts. Of 452 pts enrolled, most had sALCL (n=316 [70%]) and most had advanced disease (27% Stage III, 53% Stage IV; 78% international prognostic index ≥2). At data cutoff (2 Oct 2021), median follow-up was 47.6 months for PFS and 66.8 months for OS. Hazard ratios (HRs) for PFS per INV (0.70 [95% confidence interval [CI]: 0.53–0.91], p=0.0077) and OS (0.72 [95% CI: 0.53–0.99], p=0.0424) favoured A+CHP *vs* CHOP. Median PFS was 62.3 months (95% CI: 42.0–not evaluable) for A+CHP *vs* 23.8 months (95% CI: 13.6–60.8) for CHOP. Estimated 5-year PFS was 51.4% (95% CI: 42.8–59.4) for A+CHP *vs* 43.0% (95% CI: 35.8–50.0) for CHOP. Median OS was not reached in either arm. Estimated 5-year OS was 70.1% (95% CI: 63.3–75.9) for A+CHP *vs* 61.0% (95% CI: 54.0–67.3) for CHOP. In pts with sALCL, the HR for PFS (0.55 [95% CI: 0.39–0.79]) also favoured A+CHP *vs* CHOP, estimated 5-year PFS was 60.6% (95% CI: 49.5–69.9) for A+CHP *vs* 48.4% (95% CI: 39.6–56.7) for CHOP. In the A+CHP arm overall, median time to retreatment was 15.0 months (range, 3–64); 17 pts (ORR: 59%) had CR (n=11) or partial remission (n=6) after retreatment with brentuximab vedotin monotherapy (n=25) or brentuximab vedotin-containing regimen (n=4). Treatment-emergent peripheral neuropathy (PN) occurred in the A+CHP (n=117) and CHOP arms (n=124), of which, 72% and 78% had resolved or improved, respectively.

At 5 years, frontline A+CHP continued to provide clinically meaningful improvement in PFS and OS *vs* CHOP, including ongoing remission in ~60% of pts with sALCL, and a manageable safety profile, including continued resolution or improvement of PN.

B005

PRELIMINARY RESULTS OF THE SEQUENTIAL CHEMOTHERAPY-BLINATUMOMAB FRONT-LINE TRIAL FOR NEWLY DIAGNOSED ADULT PH-NEGATIVE B-LINEAGE ALL PATIENTS

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Outcome of adults with Ph-negative acute lymphoblastic leukemia (ALL) has improved, with survival rates nowadays being more than 50%, particularly for minimal residual disease (MRD) negative subjects. To improve the rate of MRD response and of survival, in the GIMEMA LAL2317 trial 2 doses of blinatumomab were added to chemotherapy. The study was designed for adults with B-lineage CD19+ Ph- ALL aged between 18 and 65 years, with the final goal of evaluating the efficacy of blinatumomab in increasing early MRD negativity, measured by RQ-PCR (cut-off <10-4). The trial was based on the same backbone of GIMEMA LAL1913 with two additional blinatumomab cycles given after early consolidation cycle 3 and late consolidation cycle 6. While conversion to MRD negativity following blinatumomab 1 was the primary objective, the drug was given to all study patients regardless of MRD being assessable or not. Patients were stratified for risk-oriented therapy: very high risk (VHR, for early switch to allo-SCT) with a WBC count >100x109/l and/or highly adverse cytogenetics/genetics; high risk (HR) with >30x109/l WBC, a pro-B phenotype, or a late complete remission (CR); and standard risk (SR) with no risk factors. HR and SR patients were allocated to allo-SCT only if MRD-positive at weeks 10-22. Recruitment closed in June 2020: 149 cases were enrolled (146 evaluable). 78 were male (54%), median age was 41 years (18% >55 years). 39 patients (48%) were VHR/HR, with 8.5% KMT2A+ and 28% Ph-like. A hematological CR was achieved in 131 patients (90.4%), 7 were resistant, 7 died early and 1 was not evaluable. 85 patients were evaluable for the primary endpoint, *i.e.* MRD clearance after blinatumomab 1. After early consolidation, 73% of patients were MRD-negative (<10-4). This increased to 96% after blinatumomab 1 (P=0.018), with a conversion rate of 87% among MRD+ patients (n=20/23), including 10/10 MRD+ Ph-like ALL cases. With a short median follow-up of 10 months (range 0.5-27.4), 12-month overall and disease-free survival (OS, DFS) are 83.8% and 71.6%, respectively. Favorable prognostic factors are age <56 years (OS 65.4% in >55 years, 88% 41-55 years and 91% in 18-40 years, P<0.001 *vs* age >55) and week 10 MRD negativity (OS 94%, P=0.0073; DFS 88%, P=0.0036). 15 relapses occurred, with a 12-month relapse incidence of 11%. This preliminary analysis highlights the efficacy of blinatumomab added to chemotherapy in increasing MRD negativity, translating into a low early relapse rate.

B006

MINIMAL RESIDUAL DISEASE (MRD)-DRIVEN TREATMENT PERSONALIZATION WITH SEQUENTIAL ADDITION OF IBRUTINIB (IBR) TO VENETOCLAX (VEN) IN RELAPSED/REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): THE IMPROVE STUDY

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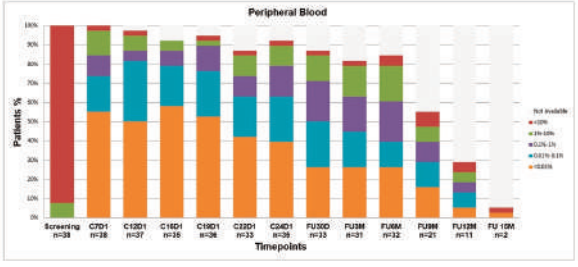
Undetectable MRD (uMRD) has become an achievable endpoint for patients (pts) with CLL, in particular using the BCL2 inhibitor VEN. uMRD can be reached in a proportion of pts with VEN mono, and in a larger fraction in combination with the BTK inhibitor IBR. This phase 2 multicenter MRD-driven Italian study aims at discontinuing treatment upon reaching uMRD in pts with R/R CLL treated with VEN mono or through the addition of IBR in pts who did not achieve uMRD with VEN alone. VEN 400 mg/day was administered for 12 months. MRD in peripheral blood (PB) and bone marrow (BM) was evaluated using the ERIC 6-color flow cytometry panel. Pts with uMRD in both PB and BM at C12D1 discontinued VEN. Pts with detectable MRD added IBR 420 mg/day and continued both drugs up to C24D28, uMRD, progression or toxicity. After C24D28, pts with detectable MRD continued IBR. 38 pts started VEN, 61% were previously treated with FC+/-R; 24% carried del(17p); 33% TP53 mutations, and 80% unmutated IGHV. Overall response rate with VEN was 36/38 (94.7%), 19 CR and 17 PR. 17 pts (45%) with uMRD4 in PB and BM at C12D1 discontinued VEN at C12D28. 19 (55%) cases with detectable MRD at C12D1 added IBR to VEN from C13D1. By combining IBR and VEN for a median of 7 months (range 3-10), 5/10 pts in PR improved to CR, 16/19 (84%) achieved uMRD in both PB and BM (Figure 1), thus stopping both therapies. The remaining 3 (16%) continued IBR. After a median follow up of 30 months, median PFS was not reached; 3 pts progressed without treatment need, 1 pt restarted VEN mono, 2 pts developed Richter transformation. 11/33 pts (33.3%) who discontinued treatment in uMRD, after a median observation of 30 months remain uMRD (6 treated with VEN only). No cases of tumor lysis syndrome were reported. With prolonged follow-up no new relevant toxicities occurred. Our updated results demonstrated that a sequential MRD-guided approach leads to an overall uMRD in 33/38 pts (87%) with either VEN mono or in combination with IBR. Interestingly, 84% of pts who did not achieve uMRD after VEN alone obtained uMRD after the addition of IBR and the remaining 3 pts who did not obtain uMRD even after the combination, could be selected for continuous IBR. This MRD-driven strategy allows to reach identical depth of response in each patient with an individualized time-limited approach, avoiding treatment intensification in those who achieve uMRD, and ultimately identifying the few pts that may benefit from continuous treatment.

Figure 1. MRD evaluation (CLL cells%) in PB in the all evaluable patients (n = 38).



C = Cycle; D = Day; FU = follow-up

Figure 1.

B007

IDECABTAGENE VICLEUCEL (IDE-CEL, BB2121), A BCMA-DIRECTED CAR T CELL THERAPY, IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED KARMMA RESULTS

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RRMM pts previously exposed to immunomodulatory agents (IMiD agents), proteasome inhibitors (PIs) and anti-CD38 antibodies (mAbs) have poor outcomes with subsequent treatments. In the phase 2 KarMMa trial (NCT03361748), ide-cel, a BCMA-directed CAR T cell therapy, showed frequent, deep, and durable responses in heavily pretreated pts with RRMM (Munshi NC, et al. *N Engl J Med* 2021). Here we report updated results for pts who had received ≥3 prior regimens (including an IMiD agent, a PI, and an anti- CD38 mAb) and were refractory to their last regimen per IMWG criteria. After 3 days of lymphodepletion (cyclophosphamide 300mg/m^2+fludarabine 30mg/m^2) pts received 150-450×10^6 CAR+ T cells (target dose levels). Endpoints included overall response rate (ORR; primary) and complete response (CR) rate (key secondary).

Dose, × 10^6 CAR+ T cells	150 (n = 4)	300 (n = 70)	450 (n = 54)	300-450 (n = 124)	Total (N = 128)
ORR, n (%)	2 (50)	48 (69)	44 (81)	92 (74)	94 (73)
CR/sCR, n (%)	1 (25)	20 (29)	21 (39)	41 (33)	42 (33)
Median DOR, mo*	†	9.9	11.3	10.7	10.7
Median PFS, mo*	†	5.8	12.2	8.8	8.8

*Kaplan-Meier estimate. †Not reported due to small n.

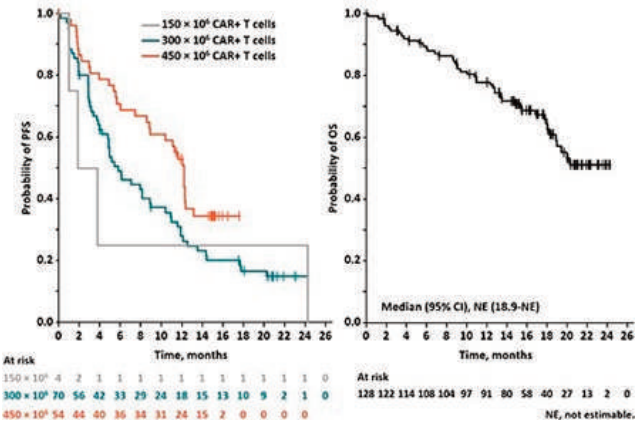

Figure 1 and Table 1.

Other secondary endpoints included duration of response (DOR), progression-free survival (PFS), overall survival (OS) and safety. Of 140 pts enrolled, 128 received ide-cel (median age of 61 y; median of 6 [range, 3-16] prior regimens); 84% were triple-class refractory, 26% were penta-refractory; 88% had received bridging therapy. Median follow-up was 15.4 mos (data cutoff, 7 Apr 2020). ORR was 73% and the median PFS was 8.8 mos; both increased with higher dose (Table 1). At the highest target dose (450×10^6 CAR+ T cells), ORR was 81%, CR rate was 39%, and median PFS was 12.2 mos. Responses were observed in all subgroups, including pts with high tumor burden (71%), extramedullary disease (70%) and R-ISS stage III disease (48%). OS continues to mature and the median has not been reached (Figure 1); the estimated 15-mo OS rate was 71%. The most common any-grade (gr) toxicities were cytopenia (97%) and cytokine release syndrome (CRS; 84%). CRS was mostly gr 1/2; 5 pts (4%) had gr 3, 1 had gr 4 (at 300×10^6) and 1 had gr 5 (at 300×10^6) events. Investigator-identified neurotoxicity was reported in 23 pts (18%; 4 pts (3%) had gr 3 and 0 had gr ≥ 4 events. Tocilizumab was used in 67 and 3 pts with CRS and neurotoxicity, respectively. Similarly, steroids were used in 19 and 10 pts with CRS and neurotoxicity, respectively.

Summary/Conclusion: Updated results from the KarMMa trial continue to demonstrate deep, durable responses with ide-cel in heavily pretreated pts with RRMM. Efficacy and safety results reflect prior reports and support a favorable clinical benefit-risk profile for ide-cel across the target dose levels.

Accepted for presentation at EHA 2021.

B008

CPI-0610, A BROMODOMAIN AND EXTRATERMINAL DOMAIN (BET) PROTEIN INHIBITOR, IN COMBINATION WITH RUXOLITINIB, IN JAK INHIBITOR-NAÏVE MYELOFIBROSIS PATIENTS: UPDATE OF MANIFEST PHASE 2 STUDY

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CPI-0610 is first-in-class, oral, small-molecule inhibitor of BET proteins with potential for disease-modifying activity through altered gene regulation of key oncogenic, fibrotic, and inflammatory factors and may transform the standard of care in myelofibrosis (MF). CPI-0610 in combination with ruxolitinib (CPI-0610 +rux) is currently being studied in JAK-inhibitor (JAKi) treatment-naïve MF patients (pts) in Arm 3 of MANIFEST, a global, open-label, phase 2 study. Here we report the safety and efficacy data from Arm 3 of the ongoing MANIFEST study.

Eligibility: JAKi-treatment-naïve MF pts with DIPSS score ≥Int-2; platelet ≥100 x 10^9/L; spleen volume ≥ 450 cc by CT/MRI; ≥2 symptoms measurable (score ≥3) or a total symptom score (TSS) of ≥10 using the MFSAF v4.0. Primary endpoint: SVR35 response (≥35% reduction in spleen volume) at wk 24; key secondary endpoint: TSS50 response (≥50% reduction in TSS) at wk 24. As of 29 September 2020, 78 pts treated, 66 pts ongoing. Baseline characteristics: mean age: 67 years old; 72% male; primary MF: 54% pts; DIPSS ≥Int-2: 76% pts; IPSS ≥Int-2: 83%; 65% pts anemic (Hgb <10g/dL); median platelet: 294 x 10^9/L (range: 100, 1849); median spleen volume: 1719 cc (range: 451, 4782); median TSS: 16 (range: 0, 38); high-molecular-risk mutations: 55% pts,

JAK2 mutation: 72%; *ASXL1* mutation: 45%. At week 24, 67% (42/63) pts achieved SVR35 (median % change from baseline: -50%; range: -84.4%, 23.7%) and 57% (34/60) pts achieved TSS50 (median % change from baseline: -59%; range: -100%, 225%). Additionally, 33% (16/48) of pts had at least one grade improvement in bone marrow fibrosis. 78 pts were evaluable for safety. The most common hematological TEAEs of any grade were anemia (33%, ≥Gr3: 30%) and thrombocytopenia (32%, ≥Gr3: 8%). These cytopenias were generally manageable with dose modifications. CPI-0610 + rux combination is generally well-tolerated in JAKi-treatment-naïve MF pts. The encouraging clinical data demonstrate the potential for the combination treatment to provide enhanced efficacy as evidenced by higher SVR35 and TSS50 rates at wk 24 compared with historical data from pivotal phase 3 studies. Overall, the data suggest that the addition of CPI-0610 to rux is potentially synergistic in JAKi-naïve MF pts. A phase 3, randomized, double blind, active-control study to further evaluate this combination is initiated.

B009

REAL-LIFE PROSPECTIVE OBSERVATIONAL STUDY "CAR-T CELL IN DIFFUSE LARGE B-CELL (DLBCL) AND PRIMARY MEDIASTINAL LYMPHOMAS (PMBCL)" OF THE ITALIAN SOCIETY OF HEMATOLOGY (SIE)

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Introduction: Axicabtagene ciloleucel (axi-cel) and tisagenlecleucel (tisa-cel) are anti-CD19 chimeric antigen receptor T cells (CAR-T) registered for the treatment of relapsed/refractory (R/R) DLBCL and PMBCL patients (pts). Methods. SIE is conducting an observational trial aimed to: 1. register all DLBCL and PMBCL candidate to CAR-T in the Italian authorized centers; 2. evaluate the intention to treat overall response rate (ORR, complete [CR] and partial response [PR]), duration of response (DOR), progression free survival (PFS) and overall survival (OS); 3. evaluate safety in terms of cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and long-term cytopenia; 5. evaluate different CAR-T products.

Results: Since March 2019 to January 2021, 126 pts were enrolled and leukapheresed; 113 were infused. Clinical characteristics were: median age 53 years (19-70), stage III/IV 77 (68%); median number of prior lines was 3 (2-7), including 33 (29%) prior autologous stem cell transplantation. For histologies, 59 (52%) were DLBCL, 18 (16%) high-grade B-cell (HGBCL), 23 (20%) PMBCL, 13 (12%) transformed Follicular (tFL). Bridging therapy was delivered to 97 pts (86%); all pts received lymphodepletion. Fifty-nine (52%) infused axi-cel and 54 (48%) tisa-cel. Median follow-up time for infused pts was 6.9 months (IQR: 3.13-11.78). At 30-days after the infusion, the response was: 45 (40%) CR, 35 (31%) PR, with ORR of 71%. Median DOR was not reached for CR pts, 6.6 months in PR pts. In the whole series, 6 and 12-months PFS and OS were 54% (95% CI:45-65); 46% (95%CI:35-59) and 80% (95%CI:71-88); 75% (95%CI:65-86), respectively. Six-months PFS and OS by histotype were: 48% and 78% for DLBCL, 62% and 80% for HGBCL, 66% and 80% for PMBCL, 59% and 92% for tFL. No differences between axi-cel and tisa-cel were reported. Severe (grade 3-4) CRS was observed in only 6 (5%) pts, and severe ICANS in 11 (10%). Sixty-one (54%) pts received tocilizumab and 38 (34%) steroids. Cytopenia beyond 30 days was reported in 30 (27%) pts; 27 of them (24%) experienced viral or bacterial infections. No toxic deaths were recorded so far.

Conclusions: In the real-life, axi-cel and tisa-cel showed an ORR similar to those of the registrative trial, with no differences across histotypes and CAR-T products. Toxicities are manageable, relapse beyond 6 months is a rare event. Cytopenias are an emerging problem in real-life setting.

B010

STANDARDIZATION OF NEXT GENERATION SEQUENCING (NGS) FOR ADVANCED MOLECULAR DIAGNOSIS OF MYELOID NEOPLASMS, A GIMEMA LABNET (GRUPPO ITALIANO MALATTIE EMATOLOGICHE DELL'ADULTO) PROJECT

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NGS is widely used in the molecular diagnostic but there are no clear and unique indications in the context of somatic variants. Main issues are identifying low variant allele fraction (VAF) and the interpretation of clinically relevant variants. Standardizing NGS methodology is crucial to increase the reliability and reproducibility of diagnostic results in myeloid neoplasms (MN). From 2019 GIMEMA group started the NGS standardization activity across the board of LabNet (CML, AML, MDS) and JakNet projects, aiming to develop shared standard procedures for advanced molecular analysis and interpretation of variants in MN in order to establish the analytical and clinical sensitivity and limit of detection/quantification of methods. To develop such an approach, a NGS committee including 4 referral centers (Firenze, Roma, Palermo and Bologna) was established. Proficiency Test (PT) was developed by inter-laboratory comparisons to determine the performance of each center. In a 1st round of validation, the reference Seraseq Myeloid Mutation DNA was serially diluted, by Coordinating Laboratory (CL; Firenze), with the wild-type reference DNA (NA24385) obtaining a spectrum of 11 single nucleotide variants (SNV) and 10 indels with VAF from 1% to 15%. After confirming by NGS the presence of all variants, aliquots were delivered to each Test Laboratory (TL) to perform the in-house NGS myeloid panel in triplicate inter/intra runs, following routine diagnostic procedures. A 2nd round of PT was performed with 3 custom reference DNA samples with Sanger validated critical variants. Results from 1st PT showed that a total of 90.4% (N=684/756) variants were correctly detected by TLs: 99.6% in the whole sample; 96.4% and 75.3% in the 1:2-1:5 diluted samples respectively. The global performance analysis showed a positive percent agreement (PPA) value ranging from 89.4-100% for variants with VAF from 1% to 10%. Analyzing variants according to the type and VAF, best results of PPA was obtained for variants with VAF>5% while the percentage decreased in indels compared to SNVs for VAF 1-2.5% (89.4 *vs* 95% and 98.5 *vs* 99% respectively). The

positive predicted value (PPV) was calculated only for the CL and it was >95%. The analysis of the 2nd PT round of validation is ongoing, and results will be showed during the meeting. Next efforts will address development of standardized interpretation criteria for variant reporting.

B011

MUTATIONS OF THE EXPORTIN 1 (XPO1) GENE PREDICT SHORTER TIME TO FIRST TREATMENT IN 1092 EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS. TRAINING/VALIDATION STUDY

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Introduction and aim: Approximately 70% of newly diagnosed chronic lymphocytic leukemia (CLL) patients present in early Binet or Rai stage and are managed with the watch and wait strategy. Two studies have identified clinical and immunogenetic variables associated with shorter time to first treatment (TTFT). We aimed at identifying novel molecular biomarkers that may predict early treatment requirement.

Methods: In the training cohort, tumor genomic DNA, isolated at the time of diagnosis, was analyzed in the most frequently mutated genes in CLL with a next-generation sequencing (NGS) approach. In the validation series, the *XPO1* gene was analyzed by NGS or by Sanger sequencing.

Results: In the training cohort (N=295), NGS mutational analysis showed that *XPO1* was mutated in 7 (2.4%) patients. By multivariate analysis, *XPO1* mutations (HR 4.24; 95% CI 1.72-10.44; p=0.002) and unmutated IGHV genes (HR 3.43; 95% CI 2.08-5.67; p<0.0001) maintained an independent association with a shorter TTFT. In the Binet A validation cohort (N=402), *XPO1* was mutated in 15 (3.7%) patients and was associated with a shorter TTFT (HR 2.59; 95% CI 1.36-4.96; p=0.004) (Figure 1B). Similarly, in the Rai 0 validation cohort (N=395), *XPO1* was mutated in 8 (2.0%) patients and was associated with a shorter TTFT (HR 6.02; 95% CI 15.03-4.96; p<0.001) (Figure 1C). In the Rai 0 validation cohort, *XPO1* mutations maintained an independent association with a shorter TTFT when corrected in multivariate analysis by the IGHV mutational status (HR 3.31; 95% CI 1.30-8.44; p=0.012). By combining the training and the validation cohorts (N=1092), a total of 30 somatically acquired *XPO1* mutations, affected codon E571 or D624, were identified (2.7%). Patients carrying either *XPO1* E571 or D624 mutations showed superimposable outcome in terms of TTFT (p=0.345) (Figure 1D). The integration of *XPO1* mutations into the prognostic models for Binet A (Condoluci *et al*., 2020) and for Rai 0 (Cohen *et al*., 2020) CLL improved the C-statistics of both models (0.775 *vs* 0.751 and 0.755 *vs* 0.748, respectively).

Conclusions: Mutations of the *XPO1* gene are an independent predictor of shorter TTFT in early stage treatment naïve CLL patients. *XPO1* mutations are conceivably gain-of-function and may enhance cell proliferation. *XPO1* mutational analysis might be incorporated in other prognostic scores and help clinicians to refine the management of the watch and wait strategy for early stage CLL.

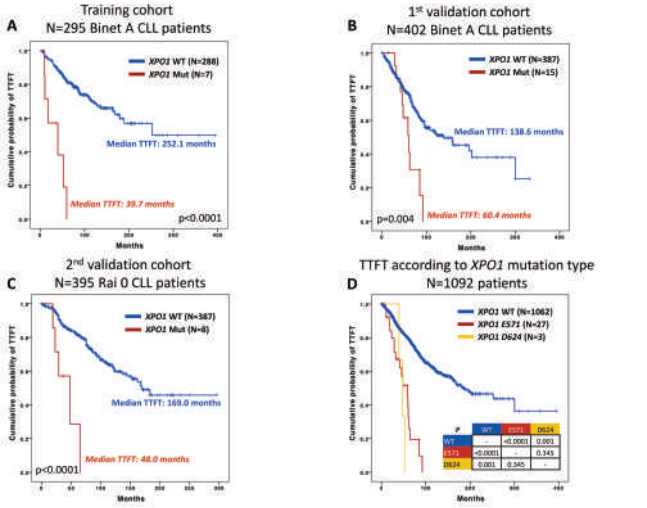

Figure 1.

B012

BETIBEGLOGENE AUTOTEMCEL (BETI-CEL) GENE THERAPY FOR THE TREATMENT OF TRANSFUSION-DEPENDENT β-THALASSEMIA (TDT): UPDATED LONG-TERM EFFICACY AND SAFETY RESULTS

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Background: Beti-cel *ex vivo* gene therapy adds copies of a modified HBB gene (HbA^{T87Q}) into hematopoietic stem cells of patients with TDT, aiming to correct the underlying cause of the disease to enable lifelong, stable production of functional adult hemoglobin (Hb). A total of 63 patients received beti-cel in 2 completed phase 1/2 (Ph1/2) and 2 ongoing Ph3 studies; subsequently, patients were enrolled in a long-term safety and efficacy follow-up study (LTF-303; NCT02633943). Here, we report LTF-303 interim results of 44 patients with up to 6.4 years [y] follow-up (planned total: 15 y).

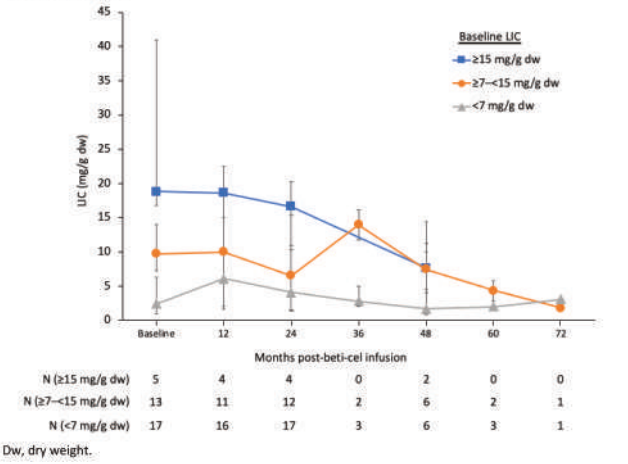
Methods: Endpoints include Hb levels, erythropoiesis, iron concentration, transfusion independence (TI; weighted average Hb ≥9 g/dL without packed red blood cell transfusions for ≥12 months [mo]) and safety. Data shown as median (min–max).

Results: By 30/11/2020, 44 patients (22 each from Ph1/2 and Ph3) had enrolled in LTF-303, with 45.6 (22.9–76.4) mo follow-up. Age at enrollment in Ph3/Ph1/2 was 19.5 (7–35) years. TI was achieved and maintained in 15/22 (68.2%; Ph1/2) and 20/22 (90.9%; Ph3) patients. Weighted average Hb during TI was 10.3 (Ph1/2) and 11.9 g/dL (Ph3). In patients who achieved TI, unsupported total Hb and HbA^{T87Q} were stable over time in Ph1/2 (Hb, HbA^{T87Q} [g/dL] at 24 mo: 10.3 [8.6–13.7;

n=14], 5.8 [3.4–9.6; n=15]; 60 mo: 10.6 [8.5–12.8; n=12], 7.6 [3.7–11.2; n=10]) and Ph3 (Hb, HbA^{T87Q} [g/dL] at 24 mo: 12.5 [9.7–14.0; n=19], 9.4 [5.0–12.4; n=19]; 36 mo: 12.3 [11.7–13.5; n=4], 10.6 [8.6–13.0; n=7]), with higher Hb levels in Ph3 due to refinement of beti-cel manufacturing process. In Ph3 in patients who achieved TI, markers of dyserythropoiesis trended towards normal levels; soluble transferrin receptor decreased from baseline to 24 mo (129.4 [65.9–235.3] nmol/L [n=20] to 60.0 [17.7–121.2] nmol/L [n=19]). Liver iron concentration decreased over time in patients who achieved TI (Figure 1). No drug product-related AEs were reported >2y post-beti-cel infusion. Serious AEs after 2 y of follow-up included gonadotropic insufficiency, ectopic pregnancy, fetal death, gallbladder wall thickening/polyp, bacteremia with neutropenia, and major depression (all n=1). No deaths, replication competent lentivirus, or insertional oncogenesis were reported.

Conclusions: After beti-cel treatment, patients with TDT maintained TI over time with normal or near-normal Hb levels, suggesting that beti-cel is a potentially curative treatment option for patients with TDT.

Figure. Liver iron concentration (LIC) over time by baseline value in patients who achieved transfusion independence and enrolled in LTF 303



N (≥15 mg/g dw)	5	4	4	0	2	0	0
N (≥7–<15 mg/g dw)	13	11	12	2	6	2	1
N (<7 mg/g dw)	17	16	17	3	6	3	1

Dw, dry weight.

Figure 1.

ORAL COMMUNICATIONS

Non Hodgkin Lymphoma 1

C001

GLOFITAMAB STEP-UP DOSING: UPDATED EFFICACY DATA SHOW HIGH COMPLETE RESPONSE RATES IN HEAVILY PRE-TREATED RELAPSED/REFRACTORY (R/R) NON-HODGKIN LYMPHOMA (NHL) PATIENTS (PTS)

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Introduction: Glofitamab, a T-cell-engaging, bispecific, full-length antibody, allows bivalent binding to CD20 (B-cells), and monovalent binding to CD3 (T-cells). In an ongoing multicenter, Phase I dose-escalation / expansion study (NCT03075696), 0.6–25mg glofitamab fixed-dosing with obinutuzumab pretreatment (Gpt), showed high, durable complete responses and manageable safety in heavily pretreated R/R NHL. Glofitamab step-up dosing (SUD), in addition to Gpt, allowed dose escalation up to 30mg to maximize efficacy, while mitigating cytokine release syndrome (CRS). We present updated efficacy data from glofitamab monotherapy (mono-tx) SUD cohorts.

Methods: Gpt (1000mg) was given 7 days pre-glofitamab, i.v. SUD of glofitamab on Day (D) 1 and 8 of Cycle (C) 1 and then at the target dose from C2D1 (2.5/10/16mg or 2.5/10/30mg); tx continued for up to 12 C, every 21 days.

Results: 52 pts received glofitamab SUD; 17 / 35 pts received 2.5/10/16mg and 2.5/10/30mg, respectively. 28 (54%) had aggressive (aNHL) and 24 had indolent NHL (iNHL). Median age was 68 (44–85) years with a median of 3 (1–12) prior tx-lines. 40 (77%) / 38 (73%) pts were refractory to their most recent / any prior CD20 tx. An updated efficacy analysis was done after a median follow-up of 6.3 months. In aNHL, best overall response (OR) / complete metabolic response (CMR) rates were 64% / 57%; a trend of improved response was observed with increased target dose, with a CMR rate of 71% at 2.5/10/30mg (N=14). Notably, 4/5 pts with mantle cell lymphoma (2.5/10/16mg, n=2; 2.5/10/30mg, n=2) had CMR. For aNHL, 13/16 CMRs are ongoing, with 8 CMRs >3 months. For pts with iNHL (N=24), OR and CMR rates were 79% and 71%, respectively; 14/17 CMRs are ongoing, with 10 CMRs >3 months. Common AEs (Aug 2020) were CRS (64%), neutropenia (39%), and pyrexia (33%). CRS was mostly confined to C1: 24/50 pts had CRS after 2.5mg; 20/49 pts after 10mg; 2/16 and 8/32 pts had CRS after 16 and 30mg (C2D1). Grade [Gr] 1/2 CRS was reported in 35%/23%. 3 pts had Gr 3 CRS; none had Gr 4/5 events. Updated data, including biomarker data on baseline CD20 expression and CD8 levels in the tumor, will be presented.

Conclusions: Updated data for glofitamab mono-tx SUD show higher preliminary response rates than previously reported in pts with R/R NHL who have failed multiple lines of tx. CRS was mostly manageable, of low grade, and confined to the first cycle of treatment.

C002

ASPEN: RESULTS OF A PHASE 3 RANDOMIZED TRIAL OF ZANUBRUTINIB VERSUS IBRUTINIB FOR PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA (WM)

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Background: Bruton tyrosine kinase (BTK) inhibition is an emerging standard of care for WM.

Aim/Objective: ASPEN (NCT03053440) is a randomized phase 3 study comparing zanubrutinib (ZANU), a potent and selective BTK inhibitor, versus ibrutinib (IBR), a first generation BTK inhibitor, in patients with WM.

Methods: Patients with *MYD88* mutation–positive (*MYD88*$^{mut+}$) WM were randomly assigned 1:1 to receive ZANU (160 mg twice daily) or IBR (420 mg once daily). Patients without *MYD88* mutations were assigned to a separate cohort to receive ZANU; these results are reported separately. Randomization was stratified by *CXCR4* mutational status and lines of prior therapy (0 *vs* 1-3 *vs* >3). The primary end point was the proportion of patients achieving a complete response or very good partial response (CR+VGPR). Sample size was calculated to provide 81% power to detect a difference in CR+VGPR rate of 35% *vs* 15% in the subset of patients with relapsed or refractory WM. Primary analysis was planned to occur at ~12 months after the last patient enrolled.

Table 1.

	Zanubrutinib (N=102)	Ibrutinib (N=99)
Efficacy (overall population)		
CR+VGPR rate	28.4	19.2
12-mo PFS	89.7	87.2
12-mo OS	97.0	93.9
Efficacy (R/R population)[a]		
12-mo PFS, n (95% CI)	92.4 (83.8-96.5)	85.9 (75.9-91.9)
12-mo OS, n (95% CI)	98.8 (91.6-99.8)	92.5 (84.1-96.6)
Safety/tolerability profile[b]		
AEs leading to discontinuation	4.0	9.2
≥Grade 3 AEs	58.4	63.3
Grade 5 AEs	1.0	4.1
Neutropenia	29.7	13.3
Hypertension	10.9	17.3
Major bleeding[c]	5.9	9.2
Atrial fibrillation/flutter	2.0	15.3

Data presented as %, unless otherwise designated.
[a]R/R population (n=83, zanubrutinib; n=81, ibrutinib).
[b]Safety population included 101 patients treated with zanubrutinib and 98 treated with ibrutinib.
[c]Includes grade ≥3 hemorrhage and central nervous system bleeding of any grade.
AE, adverse event; CR+VGPR, complete response or very good partial response; OS, overall survival; PFS, progression-free survival; R/R, relapsed or refractory.

Results: In total, 201 patients were randomized to receive ZANU (n=102) or IBR (n=99) between Jan 2017 and Jul 2018. While the treatment groups were well balanced for most of the important baseline factors, more elderly patients (aged >75 years, 33.3% *vs* 22.2%) and more patients with anemia (hemoglobin ≤110 g/L, 65.7% *vs* 53.5%) were randomized to receive ZANU. At a median follow-up of 19.4 months, the rate of VGPR was 28.4% with ZANU and 19.2% with IBR (2-sided *P*=.09; Table). No CRs were observed. Rates of atrial fibrillation, contusion, diarrhea, edema peripheral, hemorrhage, muscle spasms, pneumonia, and adverse events leading to discontinuation or death were lower with ZANU compared with IBR. Although the rate of neutropenia was higher with ZANU (Table 1), grade ≥3 infection rates were similar between treatment arms (17.8% *vs* 19.4%).

Conclusions: ASPEN is the largest phase 3 trial of BTK inhibitors in WM and the first head-to-head comparison of BTK inhibitors in any disease. Although not statistically significant, compared with IBR, ZANU was associated with a higher VGPR response rate and demonstrated clinically meaningful advantages in safety and tolerability.

C003

ABSTRACT WITHDRAWN

C004

OUTCOME OF PRIMARY MEDIASTINAL B CELL LYMPHOMA (PMBCL) AFTER DIFFERENT INDUCTION REGIMENS IN THE PRELIMINARY ANALYSIS OF THE IELSG37 TRIAL

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Introduction: Primary mediastinal B-cell lymphoma (PMBCL) is characterized by poor prognosis if an inadequate response is achieved or if the disease relapses after induction therapy. Therefore, dose□intensive chemoimmunotherapy regimens are widely used but their superiority over the standard RCHOP21 regimen has not been proven by randomized trials. Radiotherapy (RT) can consolidate responses after induction, but it may increase the risk of second malignancies and heart diseases. IELSG37 trial was planned according to a non-inferiority design to demonstrate that RT may be unnecessary in patients achieving a metabolic complete remission. The primary-endpoint analysis per arm will be reported when >80% will have a minimum post-treatment follow-up of 30 months. Herein, we present a preliminary analysis of the outcomes after different induction regimens in the overall population.

Methods: Patients with untreated PMBCL were enrolled in the study. Induction therapy was chosen according to local practice among different regimens containing a combination of rituximab and anthracyclines. Upon central review of post-induction PET scans, responding patients were randomized to observation versus consolidative RT. Responses were defined according to the Lugano classification using the Deauville 5-point scale (DS). Patients progressing during induction did not have central PET review and were assigned as DS5.

Results: 545 patients (209 men, 336 women) were enrolled and treated with R-V/MACOP-B (n=168,31%), RCHOP14 (n=146,27%), RCHOP21 (n=98,18%), DAEPOCH-R (n=88,16%), RmegaCHOP (n=19,3%), other dose-intensive regimen (n=26,5%). Induction treatment was completed in 511 patients, while 34 had an early failure. At a median follow-up of 3 years, almost 95% of patients are alive. The rate of complete metabolic responses (CR, defined by DS1-3) did not differ significantly across regimens. However, the rate of patients with a probable induction failure (DS5) was more than 2 times higher for RCHOP21 compared to the other regimens. At univariate analysis, there was no significant association of older age, poor PS, advanced stage, extranodal disease, bulky, higher international prognostic index (IPI) and larger metabolic tumor volume (MTV) with the use of RCHOP21 (Table 1).Survival analysis and Cox regression will be presented at the meeting.

Conclusions: Induction therapy has a critical role on PMBCL outcome. According to our preliminary results RCHOP21 appeared inferior to other dose-dense/dose-intensive regimens.

Table 1.

IELSG37 preliminary analysis: Complete metabolic response and risk factor rates by induction regimens in PMBCL

Regimen	Median age years (IQR)	Age >40 years	CR rate (DS1-3)	DS5	ECOG PS>1	Bulk >10 cm	High LDH	Extranodal infiltration	R-IPI very good risk	Median MTV ml (IQR)
N analyzed	*545*	*545*	*526*	*526*	*533*	*536*	*499*	*534*	*495*	*486*
R-CHOP21	32 (27-45)	34%	53%	25%	7%	65%	75%	24%	21%	316 (186-482)
R-CHOP14	37 (30-47)	45%	56%	7%	8%	78%	67%	36%	30%	360 (224-593)
R-V/MACOP-B	34 (28-45)	38%	54%	10%	12%	70%	69%	36%	24%	320 (202-498)
DA-EPOCH-R	33.5 (26-39)	25%	65%	6%	10%	68%	70%	28%	22%	333 (204-521)
Other, intensive	33 (29-38)	22%	60%	7%	19%	64%	78%	33%	22%	280 (172-443)
P-value (Fisher exact)	*0.220*	***0.006***	*0.546*	***0.001***	*0.231*	*0.150*	*0.568*	*0.262*	*0.488*	*0.521*

C005

THE ADDITION OF ROMIDEPSIN TO CHOEP PLUS UP-FRONT STEM-CELL TRANSPLANTATION IS NOT EFFECTIVE IN PERIPHERAL T-CELL LYMPHOMA (PTCL): FIRST ANALYSIS OF THE PHASE II FIL-PTCL13 STUDY

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Introduction: Peripheral T-cell lymphomas (PTCL) are a rare disease with a poor prognosis, even when treated with high dose chemotherapy and stem cell transplantation (HDC + SCT). Romidepsin (Ro), a histone deacetylase inhibitor, showed activity in relapsed or refractory PTCLs.

Methods: In the phase Ib FIL-PTCL13 (NCT02223208), we defined 14 mg/ms the maximum tolerated dose of Ro with cyclophosphamide, doxorubicin, etoposide, vincristine, dexamethasone (CHOEP) plus HDC + SCT in young PTCLs patients eligible to transplant. Aim of the phase II part of the study was to evaluate the efficacy (response rate, progression free survival, PFS, at 18-months and overall survival, OS) of the experimental combination. The primary objective was to demonstrate a 15% increase in 18-months PFS for the combination Ro-CHOEP plus HDC+SCT, compared to the literature data (from 55% to 70%, planned sample size=110). Patients aged 18-65 eligible to SCT, with advanced PTCL-NOS, angioimmunoblastic/T-helper follicular and ALK negative anaplastic large cell lymphoma were eligible. Treatment plan consisted of 6 courses of Ro-CHOEP every 21 days (14 mg/ms Ro day 1 and 8), followed by cisplatin-cytarabine-dexamethasone (DHAP) with stem cell harvest and SCT. Patients in complete response (CR) after induction proceeded to autoSCT, while those in partial response (PR), with an available HLA-matched donor, proceeded to alloSCT upfront.

Results: From September 2017 to October 2020, 83 patients were enrolled into the phase II part of the study; median age was 55 years (IQR 49;59); 74 (89%) had stage III-IV and 29 (35%) IPI risk >2. An interim analysis was performed, according to the statistical plan, when the first 75 patients were enrolled. At a median follow-up of 12 months, the estimated 18-months PFS was 53% (95% CI: 0.39-0.64) and the OS was 79% (95% CI: 0.66-0.87). On 74 patients evaluable for response after induction, the overall response rate (ORR) after 6 Ro-CHOEP was 72% (53 patients), with 59% (44 patients) complete response (CR). On 65 patients evaluable for response at the end of treatment, the ORR after SCT was 42/65 (65%), with 36 (55%) CR. No unexpected toxicities were recorded.

Conclusions: The interim analysis demonstrated an 18-months PFS of 53%, that is similar to those obtained with conventional HDC+SCT, the enrollment was stopped. In conclusion, the addition of Romidepsin to CHOEP did not improve PFS in PTCLs eligible to SCT.

Myeloma and Monoclonal Gammopathies 1

C006

RISK OF EARLY SEVERE INFECTIONS IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS TREATED WITH NOVEL AGENTS: A POOLED ANALYSIS

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Background: Infections are a major cause of toxicity in myeloma patients. We investigated the incidence of severe infections and associated risk-factors in NDMM patients receiving novel agents.

Methods: We pooled together data from Italian patients enrolled in clinical trials and receiving carfilzomib-based (IST-CAR-506, IST-CAR-561), bortezomib-based (EMN02) and lenalidomide-based (EMN01, RV-MM-PI-0752, RV-MM-EMN-441) induction treatment. We assessed the incidence of severe infections, defined as any grade (G)3-5 event or G2 if involving lung/lower respiratory tract (CTCAE version 4.0). Early Severe Infections (ESI, occurring during the first 4 months of therapy) were analyzed. Secondary aims were to identify risk factors for ESI and to evaluate the impact of ESI on outcome.

Results: 1892 patients were included in the analysis. Median age was 65 years, 970 (51%) patients were transplant eligible and 922 (49%) transplant ineligible. Overall, 1059 (56%) patients received IMiD-based and 833 (44%) PI-based induction therapy. Median follow-up was 68 months. We recorded 436 severe infections, mainly represented by lung/lower respiratory tract infections (50%), febrile neutropenia (23%) and sepsis/septic shock (10%). 377 (20%) patients reported at least one severe infection, and 129 patients (6.8%) one ESI. In a multivariate analysis (Table 1), factors associated with increased risk of ESI were ISS stage 3 (OR 2.14, 95% CI 1.32-3.48), presence of del17p by FISH (OR 1.80, 95% CI 1.1-2.96), intermediate fit status (OR 1.88 95% CI 1.1-3.21) and frail status (OR 2.12, 95% CI 1.08-4.18) according to IMWG frailty score. No difference in risk of ESI was observed according to induction therapy with PI *vs* IMiD (OR 1.10, 95%CI 0.68-1.78). In a time-dependent Cox regression analysis adjusted for potential confounders (age, RISS stage and performance status), the risk of disease progression/death was higher in patients with *vs* without ESI (median PFS 21.3 *vs* 31.3 months, HR 1.32, 95% CI 1.07-1.63, p<0.01). A significant impact was observed also on survival (median OS 45.8 *vs* 95.8 months, HR 1.72, 95% CI 1.34-2.21, p<0.01).

Conclusions: 34% of patients experiencing severe infections had the event within the first 4 months of therapy. Aggressive disease and frailty confer a higher risk of ESI, which hamper treatment adherence and affect PFS and OS. Risk-adapted antimicrobial prophylaxis for patients at higher risk of infections should be evaluated in clinical trials.

Table 1.

Table. Incidence of severe infections over time and multivariate analysis of baseline predictors of early severe infections (ESI) in the study population.

Incidence of severe infections n (%)		
Overall	377 (20%)	
At 12 months	280 (15%)	
At 4 months (early infection)	129 (6.8%)	
	Multivariate analysis	
Variable	**Odds ratio (95% CI)**	**p value**
ISS stage 2 vs 1	1.48 (0.94 - 2.33)	0.09
ISS stage 3 vs 1	2.14 (1.32 - 3.48)	< 0.01
Deletion 17q13 yes vs no	1.80 (1.1 - 2.96)	0.02
NTE fit* vs TE	1.13 (0.62 - 2.04)	0.69
NTE intermediate fitness* vs TE	1.88 (1.1 - 3.21)	0.02
NTE frail* vs TE	2.12 (1.08 - 4.18)	0.03
Induction PI vs IMiD	1.10 (0.68 - 1.78)	0.68

Abbreviations: ISS International Staging System. NTE transplant ineligible. TE transplant eligible. PI proteasome inhibitor. IMiD immunomodulatory drug

* Assessed through International Myeloma Working Group Frailty Score

C007

R2-ISS, A NEW RISK STRATIFICATION MODEL IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): ANALYSIS OF 7077 PATIENT DATA BY THE EUROPEAN MYELOMA NETWORK (EMN) WITHIN HARMONY BIG DATA PLATFORM PROJECT

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HARMONY Alliance

Background: In the Revised International Staging System (R-ISS) (Palumbo, JCO 2015) 60% of pts are R-ISS2, possibly including pts with different risk of progression/death. The EMN, within the HARMONY project, revised the R-ISS, including also 1q copy number alterations (CNAs).

Methods: The EMN collected NDMM data from 15 European clinical trials in HARMONY platform. All pts received IMiD and/or PI upfront. We evaluated the impact of each single risk feature on OS and PFS and used the hazard of death conferred by the most significant variables to create an additive risk score.

Table 1. Multivariate analysis on OS and PFS of the most impacting prognostic variables in the overall population (n=7077). Score calculation and stratification into 4 risk groups according to the total additive score in pts with complete data (n= 2227) is shown as well.

Risk feature	OS Hazard ratio*	PFS Hazard ratio*	Score value**
ISS2	1.55 (1.42-1.69)	1.35 (1.26-1.44)	1
ISS3	2.02 (1.83-2.24)	1.53 (1.42-1.66)	1.5
Del(17p)	1.74 (1.56-1.94)	1.41 (1.29-1.55)	1
High LDH	1.65 (1.50-1.83)	1.33 (1.23-1.45)	1
t(4;14)	1.56 (1.40-1.74)	1.49 (1.36-1.63)	1
1q CNAs	1.45 (1.29-1.63)	1.37 (1.25-1.50)	0.5
Group	**Number of patients (%)**		**Total additive score**
Low	429 (19.3%)		0
Low-Intermediate	686 (30.8%)		0.5-1
Intermediate-High	917 (41.2%)		1.5-2.5
High	195 (8.8%)		3-5

*Cox Model adjusted for Age, Sex, therapy, performance status, isotype, t(14:16) and renal function.
**calculated on the risk of death in patients with complete data only (n=2227), value rounded at the nearest 0.5 with ISS 2 vs 1 comparison as reference (score = 1).

Results: 7077 NDMM pts were registered in HARMONY platform and analyzed. Median follow-up was 75 months, median age 62 years.

In a multivariate Cox model, ISS (2 *vs* 1 HR 1.55 p<0.001, 3 *vs* 1 HR 2.02 p<0.001), del(17p) (HR 1.74, p<0.001), LDH (HR 1.65, p<0.001), t(4;14) (HR 1.56, p<0.001) and 1q CNAs (HR 1.45, p<0.001) had the highest impact on OS. ISS (2 *vs* 1 HR 1.35 p<0.001, 3 *vs* 1 HR 1.53 p<0.001), t(4;14) (HR 1.49, p<0.001), del(17p) (HR 1.41, p<0.001), 1q CNAs (HR 1.37, p<0.001) and LDH (HR 1.33, p<0.001) had the highest impact on PFS. These prognostic variables were simultaneously present in 2227 pts and most of the remaining pts were excluded because 1q CNAs were missing. Based on the OS impact of these risk features in pts with complete data, we built an additive scoring system (Table 1). Pts were stratified into 4 groups: Low [n=429 (19.3%), score 0], Low-Intermediate [n=686 (30.8%), score 0.5-1], Intermediate-High [n=917 (41.2%), score 1.5-2.5] and High [n=195 (8.8%), score 3-5]. Each group had significantly different OS and PFS (Figure 1A-B). Median OS was not reached *vs* 109.2 *vs* 68.5 *vs* 37.9 months and median PFS was 68 *vs* 45.5 *vs* 30.2 *vs* 19.9 months in the above 4 risk groups, respectively. Using this new model, R-ISS2 pts (n=1372) were better stratified into Low-Intermediate (n=517), Intermediate-High (n=811) and High risk (n=44) groups, confirming their highly different prognosis. Its prognostic value was maintained also in transplant-eligible and ineligible pts, and in pts receiving IMiDs, PIs or both.

Conclusion: This new model called "R2-ISS" may improve the current R-ISS. About 50% of the pts are Low or Low-Intermediate risk and 50% Intermediate-High or High risk, paving the way to risk-adapted approaches in a high number of pts. New prognostic variables can easily be included in the future and validation in an independent cohort is planned.

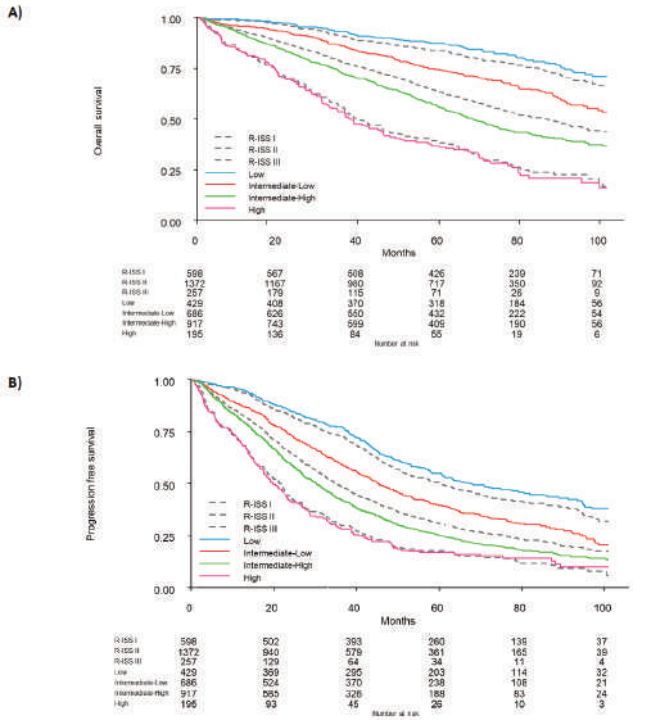

Figure 1. OS (A) and PFS (B) according to the newly defined risk groups. The dotted grey lines show the outcome of the same cohort of pts stratified by R-ISS.

C008

HIGH LEVELS OF CIRCULATING PLASMA CELLS ARE AN INDEPENDENT HIGH-RISK FEATURE IN MULTIPLE MYELOMA PATIENTS AND THEIR PROGNOSTIC IMPACT IS MODULATED BY THE ACHIEVEMENT OF MINIMAL RESIDUAL DISEASE NEGATIVITY

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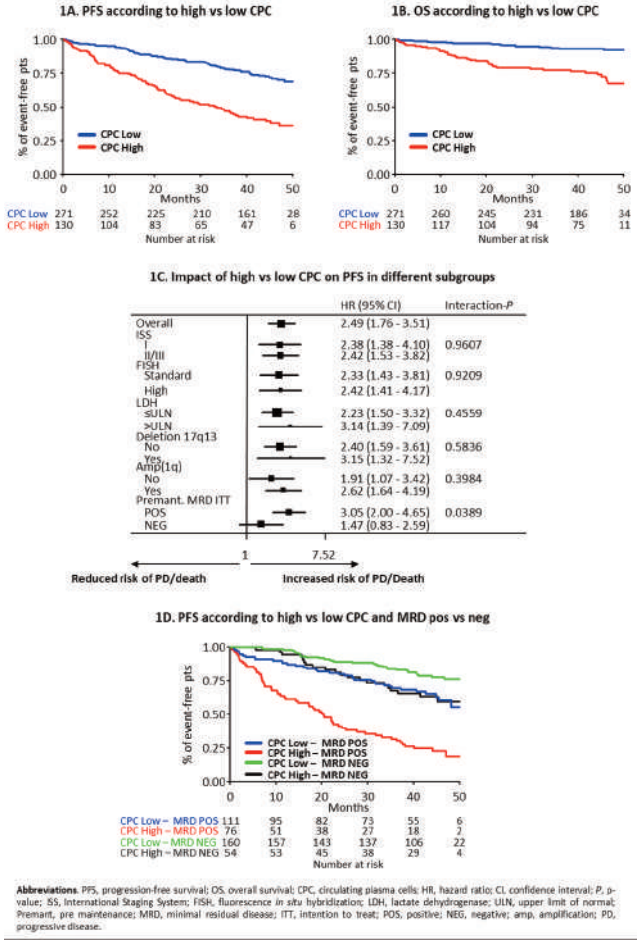
Introduction and aims: High levels of circulating plasma cells (CPC) have been long known to be a marker of aggressive disease associated with poor outcome in multiple myeloma (MM). We aimed to identify the best prognostic cut-off value for CPC levels and to assess the impact of high CPC levels (CPC-High) on the clinical outcome of newly diagnosed (ND)MM patients (pts) in the context of concomitant risk factors and minimal residual disease (MRD) evaluation.

Methods: At diagnosis, single-platform flow cytometry was used to sort and count CPC in patients enrolled in the multicenter randomized FORTE clinical trial (474 NDMM pts ≤65 years). MRD was assessed by 2nd-generation multiparameter flow cytometry (MFC, sensitivity 10^{-5}). Receiver Operating Characteristic (ROC) analysis was used to define a cut-off based on PFS as outcome. Correlations between CPC-High and

the most important baseline prognostic features were explored, and a multivariate (MV) analysis assessed the impact of CPC-High on PFS and OS. Finally, we evaluated the impact of baseline CPC and MRD achievement.

Results and conclusions: At diagnosis, CPC were analyzed in 401/474 pts; the median follow-up was 44.2 months (39.6-47.9). The optimal CPC cut-off to predict PFS was 0.07% (5 cells/ul, 0.005 x10^9/l). CPC-High pts (>0.07%) were 130/401 (32%). Baseline features significantly associated with CPC-High in a MV analysis were: high lactate dehydrogenase, International Staging System stage II/III, amp1q, t(4;14), t(14;16), and bone marrow PC (>60%). CPC-High, as compared with CPC-Low, were associated with lower PFS (3-year PFS 47% *vs* 78%, HR 2.49, 95% CI 1.76-3.51, *P*<0.0001; Figure 1A) and OS (3-year OS 78% *vs* 93%, HR 2.85, 95% CI 1.56-5.19, *P*<0.001; Figure 1B) in a MV analysis including all the baseline features and treatment arm. The prognostic impact of CPC levels on PFS was consistent in all high-risk subgroups (Figure 1C), except in patients who achieved pre-maintenance MRD negativity [(neg); interaction *P*=0.03]. CPC-Low_MRD-neg pts showed the best outcome (3-year PFS 84%), while CPC-Low_MRD-positive (pos) and CPC-High_MRD-neg pts had similar 3-year PFS (70% *vs* 68%). CPC-High_MRD-pos pts had a dismal outcome (3-year PFS 32%; Figure 1D). Elevated CPC levels with a cut-off of 0.07% (5 cells/ul, 0.005 x10^9/l) are a strong and independent high-risk factor predicting shorter PFS and OS even in the context of other high-risk features. MRD negativity improved the poor prognosis of CPC-High patients.



Abbreviations. PFS, progression-free survival; OS, overall survival; CPC, circulating plasma cells; HR, hazard ratio; CI, confidence interval; *P*, p-value; SS, International Staging System; FISH, fluorescence *in situ* hybridization; LDH, lactate dehydrogenase; ULN, upper limit of normal; Premant, pre maintenance; MRD, minimal residual disease; ITT, intention to treat; POS, positive; NEG, negative; amp, amplification; PD, progressive disease.

Figure 1.

C009

SURVIVAL ANALYSIS OF NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA PATIENTS ENROLLED IN THE RANDOMIZED FORTE TRIAL

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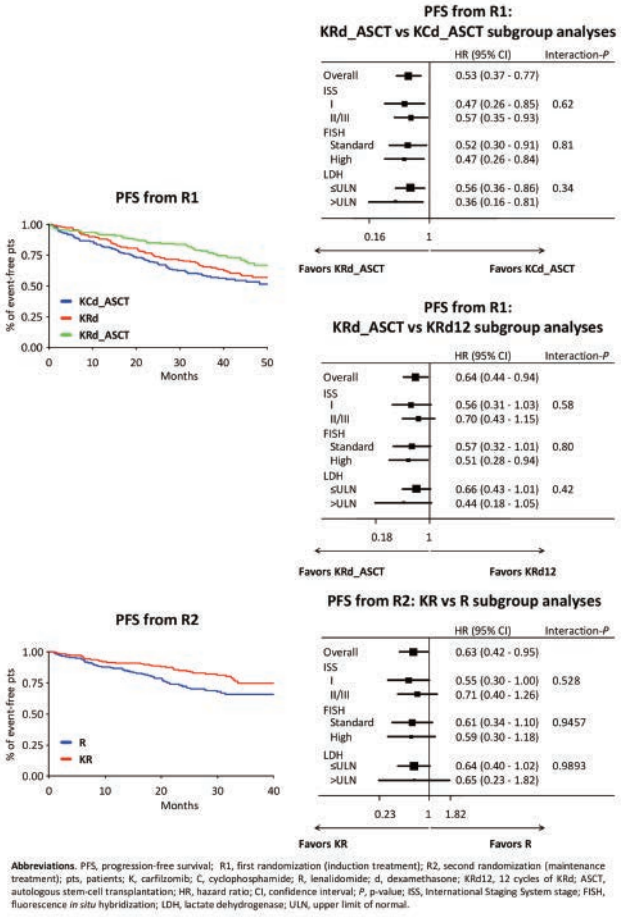
Background: The phase II FORTE study investigated the role of KRd induction-ASCT-KRd consolidation (KRd_ASCT) *vs* 12 KRd cycles (KRd12) *vs* KCd induction-ASCT-KCd consolidation (KCd_ASCT) in newly diagnosed multiple myeloma (NDMM) patients (pts) eligible for ASCT and that of KR *vs* R maintenance.

Methods: Pts ≤65 years were first randomized (R1) to 4 KRd induction cycles, MEL200-ASCT and 4 KRd consolidation cycles (KRd_ASCT) or 12 KRd cycles without ASCT (KRd12) or 4 KCd induction cycles, MEL200-ASCT and 4 KCd consolidation cycles. Thereafter, pts were randomized (R2) to maintenance with KR or R alone.



Abbreviations. PFS, progression-free survival; R1, first randomization (induction treatment); R2, second randomization (maintenance treatment); pts, patients; K, carfilzomib; C, cyclophosphamide; R, lenalidomide; d, dexamethasone; KRd12, 12 cycles of KRd; ASCT, autologous stem-cell transplantation; HR, hazard ratio; CI, confidence interval; *P*, p-value; ISS, International Staging System stage; FISH, fluorescence *in situ* hybridization; LDH, lactate dehydrogenase; ULN, upper limit of normal.

Figure 1.

Results: A total of 474 NDMM pts were randomized to KRd_ASCT (n=158), KRd12 (n=157) or KCd_ASCT (n=159). After a median follow-up from R1 of 45 months (m), median progression-free survival (PFS) was not reached with KRd_ASCT, 57 m with KRd12 and 53 m with KCd_ASCT (KRd_ASCT *vs* KCd_ASCT: HR 0.53, *P*<0.001; KRd_ASCT *vs* KRd12: HR 0.64, *P*=0.023; KRd12 *vs* KCd_ASCT: HR 0.82, *P*=0.262). The PFS benefit of KRd_ASCT *vs* both KCd_ASCT and KRd12 was consistent in most subgroups (Figure 1). The 3-year overall

survival (OS) was 90% with KRd_ASCT and KRd12 *vs* 83% with KCd. 356 pts (KR, n=178; R, n=178) were randomized to maintenance. After a median follow-up from R2 of 31 m, 46% of MRD-positive pts at randomization in the KR arm turned negative, as compared to 32% in the R arm (P=0.04). By ITT analysis, the 3-year PFS from R2 was longer in KR *vs* R (75% *vs* 66%; HR 0.63; P=0.026). The PFS benefit of KR *vs* R was observed in most subgroups (Figure 1). 3-year OS was 90% in both arms. During maintenance, the rate of ≥1 grade (G)3-4 hematologic adverse events (AEs)/serious (S)AEs was similar in the 2 arms (KR 22% *vs* R 23%); the most frequent were neutropenia (KR 18% *vs* R 21%) and thrombocytopenia (KR 3% *vs* R 3%). The rate of ≥1 G3-4 non-hematologic AEs/SAEs was higher with KR (27%) than with R (15%, P=0.012); the most frequent were infections (KR 4% *vs* R 7%); all other events were reported in ≤5% of pts and included: gastrointestinal (KR 5% *vs* R 2%), cardiac (KR 4% *vs* R 1%), hypertension (KR 3% *vs* R 0%) and thrombotic microangiopathy (3% *vs* 0%). Dose reductions of R were reported in 23% of KR and 29% of R pts; dose reductions of K were reported in 20% of pts. The rate of discontinuation due to AEs was similar in the 2 arms (KR 10% *vs* R 9%).

Conclusions: Treatment with KRd_ASCT significantly improved PFS, as compared with both KRd12 and KCd_ASCT. Maintenance with KR also improved PFS *vs* R.

C010

HORIZON (OP-106): MELFLUFEN PLUS DEXAMETHASONE (DEX) IN 55 PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) WITH EXTRAMEDULLARY DISEASE (EMD)—SUBGROUP ANALYSIS

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Background: Prognosis for pts with EMD is poor, particularly in the RRMM setting and no standard therapy has been established for this high-unmet need population. Melphalan flufenamide (melflufen) is a first-in-class peptide-drug conjugate that targets aminopeptidases and rapidly releases alkylating agents into tumor cells. In the phase 2 HORIZON study (NCT02963493), melflufen + dex showed clinically meaningful efficacy and a manageable safety profile in pts with heavily pretreated RRMM and triple-class–refractory MM. This analysis examines pts with EMD in HORIZON.

Methods: Pts with RRMM (≥2 lines of prior therapy; refractory to pomalidomide and/or an anti-CD38 monoclonal antibody) received melflufen 40 mg (intravenous; d1 of each 28-d cycle) and dex 40 mg/wk. The primary endpoint was overall response rate (ORR; ≥ partial response; investigator-assessed per International Myeloma Working Group [IMWG] criteria). Secondary endpoints included progression-free survival (PFS), overall survival (OS), duration of response (DOR), and safety. For pts with known or suspected EMD (isolated soft-tissue and/or bone-related extramedullary plasmacytomas with soft-tissue extension) an assessment was required at screening and to confirm a response per IMWG criteria.

Results: Of 157 pts enrolled and treated (data cutoff, Jan 14, 2020), 55 (35%) had EMD (49% soft-tissue and 51% bone-related soft-tissue plasmacytoma). In pts with EMD (median age 64 y [range, 43-82]), 60% had disease that was refractory to prior alkylator therapy and 91% had triple-class–refractory MM. ORR (95% CI) was 24% (13-37) in pts with EMD and 29% (22-37) overall (N=157). In the overall population, median (95% CI) DOR was 5.5 mo (3.9-7.6), PFS was 4.2 mo (3.4-4.9), and median OS was 11.6 mo (9.3-15.4). Efficacy results of pts with EMD are shown in the Table 1. Grade 3/4 treatment-emergent adverse events (TEAEs) occurred in 89% of the overall population and 78% of pts with EMD. In pts with EMD, the most common grade 3/4 TEAEs were anemia (42%), thrombocytopenia (40%), and neutropenia (38%). Overall, no treatment-related deaths were reported.

Conclusions: Melflufen + dex showed activity in advanced RRMM with EMD and a consistent safety profile with previous reports. To date, this study provides the largest cohort of pts with EMD, demonstrating benefit in this population with a high unmet medical need and supporting further evaluation of melflufen + dex in RRMM with EMD.

Table 1. Efficacy Summary for Patients With EMD.

Response Category in EMD Subgroup[a]	Best Response[a] (95% CI), %	Median Duration of Best Response (95% CI), mo	Median PFS (95% CI), mo	Median OS (95% CI), mo
All (N=55)	N/A	5.5 (1.8-NE)	2.9 (2.0-3.8)	6.5 (5.1-9.7)
≥ PR[b] (n=13)	23.6 (13.2-37.0)	5.5 (1.8-NE)	17.3 (5.3-NE)	18.5 (8.7-NE)
≥ MR (n=17)	30.9 (19.1-44.8)	5.5 (3.1-16.4)	8.5 (4.1-18.2)	18.5 (8.7-NE)
≥ SD (n=28)	50.9 (37.1-64.5)	5.3 (3.9-8.5)	5.3 (3.9-8.5)	13.6 (8.1-24.4)

EMD, extramedullary disease; MR, minimal response; N/A, not applicable; NE, not evaluable; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease.

[a]Best response assessed by the investigator per International Myeloma Working Group criteria.

[b]Due to low event numbers, the results in pts with ≥PR may be overestimated.

Acute Leukemia 1

C011

ABSTRACT WITHDRAWN

C012

UPDATED RESULTS OF THE GIMEMA LAL2116, D-ALBA TRIAL, FOR NEWLY DIAGNOSED ADULTS WITH PH+ ALL

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The outcome of Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) has dramatically changed since the advent of TKIs. To further improve the outcome of these cases, we designed an induction/consolidation chemo-free trial (GIMEMA LAL2116, D-ALBA) based on the administration of dasatinib plus steroids followed by at least two cycles of blinatumomab (maximum 5) and central nervous system (CNS) prophylaxis. The first results have been published (Foà *et al.*, NEJM 2020): after the 2nd cycle of blinatumomab, a molecular response was achieved in 60% of cases, which increased after additional cycles of blinatumomab, translating into an overall survival (OS) and disease-free survival (DFS) of 95% and 88%, respectively. To provide an update of the study, patients were followed for 12 months and data on subsequent treatment and survival are being collected in the ancillary study GIMEMA LAL2217. As reported, 63 patients were enrolled (median age 54 years, range 24-82; no upper age limit); the median follow-up is now 27.2 months (range 0.9-45.2). 3 additional relapses were observed (total= 9): 4 hematologic, 4 at the CNS and 1 nodal; 3additional deaths in complete hematologic remission (CHR) were recorded (total=6). Of the 58 evaluable patients who started blinatumomab, 29 were allografted. Prior to transplant, 8 patients received 2 and 3 cycles of blinatumomab, respectively, 5 received 4 cycles and 6 5 cycles (2 patient were allografted after 1 cycle, for medical decision). Five deaths occurred, 2 in patients transplanted in 2nd CHR. Within not-transplanted patients, 29 continued treatment with a TKI: 21 continued with dasatinib alone, 3 switched to imatinib due to intolerance and 5 switched to ponatinib for a molecular increase or medical decision: in the latter group, 1 CNS relapse was observed. In the update, the 24 months estimated OS is 87.8% and DFS is 79.8%; DFS was significantly better in patients achieving a molecular response (both complete molecular remission and positive non-quantifiable response) than in those who did not (100% *vs* 75.9%, p=0.028). Furthermore, we confirm the inferior DFS for patients carrying an IKZF1-plus genotype compared to cases with no IKZF1 deletions or with IKZF1 deletions alone (84.5% *vs* 54.5%, p=0.026). Finally, a low transplant-related mortality rate was recorded. Notably, among the few relapses, we observed a rather high incidence of CNS involvement; thus, in the upcoming trial, CNS prophylaxis will be increased.

C013

COMBINING THE EXPRESSION OF CD33.CAR AND CXCR4 TO AUGMENT CAR-CIKS HOMING TO BONE MARROW NICHE AND LEUKEMIC STEM CELL ERADICATION IN ACUTE MYELOID LEUKEMIA

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Chimeric Antigen Receptor (CAR) cytokine-induced killer (CIK) cell therapy is a promising treatment for acute myeloid leukemia (AML). Specifically, it is crucial to improve CAR CIK-cells infiltration ability into the bone marrow (BM) niche to eradicate leukemia stem cells (LSCs) at their location. Actually, BM mesenchymal stromal cells (MSCs) interact with LSCs, residing in the niche, releasing different chemokines and soluble factors. The chemokine ligand 12 (CXCL12), produced by MSCs, and its chemokine receptor 4 (CXCR4) regulate leukocytes trafficking to the BM. In AML, CXCL12 binds CXCR4 overexpressed on blasts, promoting their homing in the niche. On the contrary, CXCR4 expression is drastically downregulated on CIKs during culture. Combining the expression of CD33.CAR and CXCR4 might facilitate CAR-CIKs homing to the BM and subsequent leukemia eradication. We designed two bicistronic Sleeping Beauty transposon vectors: CXCR4(IRES)CD33.CAR and CD33.CAR(2A)CXCR4. The monocistronic CD33.CAR was used as control. We observed both CD33.CAR(2A)CXCR4-CIKs (n=22, P<0.0001) and CXCR4 (IRES)CD33. CAR-CIKs (n=9, P<0.0001) maintained CXCR4 overexpression during culture, whereas in CD33.CAR-CIKs was drastically downregulated (n=22). However, CD33.CAR expression was lower in CXCR4(IRES)CD33.CAR-CIKs (n=8, P<0.0001) compared with CD33.CAR-CIKs, while CD33.CAR(2A)CXCR4-CIKs (n=11) exhibit a significant co-expression of both proteins against control (P=0.001). Chemotaxis assays toward recombinant CXCL12 confirmed both CXCR4(IRES)CD33.CAR-CIKs (n=7, P=0.01) and CD33.CAR(2A) CXCR4-CIKs (n=8, P=0.0006) displayed a migration advantage over CD33.CAR-CIKs (n=12) with a mean percentage of migration of 58.5% and 67.2% respectively, compared to 40.1%. Interestingly, CD33.CAR(2A)CXCR4-CIKs (n=2) showed an increased specific chemotactic response toward HD- (n=3) and AML-MSCs (n=2) supernatants, as demonstrated by the use of CXCR4 antagonist Plerixafor. Moreover, CXCR4(IRES)CD33.CAR-CIKs and CD33.CAR (2A)CXCR4-CIKs retained killing of CD33+ KG1 target cell line, maintained their capacity to produce IL-2 and IFN-y and to proliferate after CD33 antigen exposure. However, CXCR4(IRES) CD33.CAR-CIKs exhibited lower effector responses against control, due to inferior CAR expression. Taking together, these data demonstrating enhanced migration while maintaining CAR functionalities, make CD33.CAR(2A)CXCR4 the best candidate for further *in vivo* homing and anti-leukemic evaluation.

C014

OUTCOMES OF RELAPSED OR REFRACTORY AND NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA AFTER HYPOMETHYLATING AGENT AND VENETOCLAX. THE ITALIAN REAL-LIFE EXPERIENCE BEFORE PUBLIC HEALTH REIMBURSEMENT (AVALON STUDY)

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P. Salutari[22], M. Gottardi[23], A. Molteni[24], V. Martini[25], M. Lunghi[26], L. Fianchi[27], D. Cilloni[28], F. Lanza[29], E. Abruzzese[30], N. Cascavilla[31], F. Rivellini[32], F. Ferrara[33], L. Maurillo[34], J. Nanni[2], A. Romano[5], V. Cardinali[6], F. Gigli[1], F. Vincenzo[8], R. Volpi[4], M. Sciumè[3], C. Tarella[1], G. Rossi[20], G. Martinelli[4] & AVALON cooperative Group[#]

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AVALON (NCT 04070807) is a cooperative retrospective observational study, co-sponsored by IRST IRCCS and Rete Ematologica Lombarda and endorsed by GIMEMA, aimed at collecting real-life data of the off-label use of Venetoclax (Ven) for acute myeloid leukemia (AML) in Italy from Jan 2015 to March 2020, before Ven became available under the Italian Law No.648/96. A total of 218 patients have been enrolled in 32 Italian centers. Preliminary data from 155 patients treated with the combination of Ven and Hypomethylating agents (HMA) are reported on Table 1. 82/98 (83,7%) patients obtained Ven through a nominal request to the 5% AIFA fund (law no. 326 of 2003). Median time to get approval was 27 days (IQR 34). For this reason, 50 (32%) patients started HMA within 3 months before Ven. Ven rump up was performed in 88% patients and various dosage have been reported during treatment (data not shown). 60/155 patients (38,7%) received antifungal prophylaxis mainly posaconazole (41/60) and 51/155 (33%) antibacterial. Median time to first disease revaluation was 64 days (IQR 80.5) and in 38/144 patients (26,4%) was performed after 4 months. Response data were available in 123 patients and are shown on Table 1. Of the 12 patients relapsing after allotransplant, 4 (30%) achieved CR. Overall median OS was 8,1 months (5,8-10,2), for newly diagnosed patients median OS (95%CI) was 12,7 months (5,1-15,7), 9,7 months (5,7-16) for refractory (R) and 6,4 months (3,6-10,2) for relapsed (Re). Median follow-up of alive patients was 13,2 months (1,7-29,7) and 16,9 (13,4-17,6) using the reverse K-M approach. Serious Adverse Events (SAEs) occurred in 49/155 (31,6%) patients, for 33/49 (67.3%) occurred within 60 days from start of Ven. Tumor lysis syndrome (TLS) occurred in one patient and was resolved. In conclusion, this is the largest experience reported in the literature on R/Re AML treated with Ven and HMA. The combination is confirmed to be an effective treatment for both newly diagnosed and R/Re patients with acceptable toxicity suggesting that even in heavily pre-treated setting, can efficiently debulk leukemia "bridging" patients to allotransplant.

Results can be definitively improved by a more appropriate use of the combination: simultaneous start of Ven and HMA after rump up, uniform the Ven dosage, reduce SAEs incidence by hospitalization during first cycle, administration of antifungal-bacterial prophylaxis and an earlier disease revaluation.

Table 1. Patient characteristics and response evaluation.

Patients characteristics	N=155
Gender	
F	67 (43.2 %)
M	88 (56.8 %)
Age at diagnosis (yrs)	
Median [IQ range]	67 [18]
Min-max	22-84
ELN risk *‡	
Low	4 (14.8 %)
Intermediate	16 (59.3 %)
Adverse	7 (25.9 %)
Type of HMA	
5-azacytidine	105 (67.7 %)
Decitabine	50 (33.3 %)
Disease status before treatment‡	
Newly diagnosed	31 (21.1 %)
Primary chemorefractory	56 (38.1 %)
Relapsed	60 (40.8 %)
N. of previous treatment lines of intensive CT¥	
Median [IQ range]	2 [1]
Min-max	1-7
N. of patients receiving previous treatment with HMA¥	24 (22.0 %)
N. of patients receiving previous treatment with intensive CT and HMA¥	16 (14.7 %)
N. of patients receiving previous Transplantation°	12 (20 %)
Single transplantation	9 (15 %)
Double transplantation	3 (5 %)
Response evaluation	**N=123**
All patients	
CR§	52 (42.3 %)
PR	14 (11.4%)
SD/refractoriness	57 (46.3%)
Newly diagnosed	***N=28***
CR§	14 (50%)
PR	5 (17.9%)
SD/refractoriness	9 (32.1%)
Primary chemorefractory	***N=47***
CR§	18 (38.3%)
PR	6 (12.8%)
SD/refractoriness	23 (48.9%)
Relapsed	***N= 48***
CR§	20 (41.7%)
PR	3 (6.3%)
SD/refractoriness	25 (52.1%)

*only for newly diagnosed patients
‡ Sum does not add up to the total due to missing values
¥ Only for primary chemorefractory and relapsed patients; data available for 109/116 patients
° Only for relapsed patients (n=60)
§ include also CRi, CRp
IQ: interquartile range; CT: chemotherapy

C015

LABNET AML: AN EFFICIENT NETWORK THAT CONNECTS HEMATOLOGY CENTERS AND LABORATORIES FOR A HIGH-LEVEL DIAGNOSTIC/PROGNOSTIC WORKUP OF AML

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Di Rilievo Nazionale Antonio Cardarelli, UOC Ematologia Con Trapianto di Midollo; [17]AO Ospedali Riuniti "Papardo Piemonte" - Po Papardo, SC Ematologia; [18]Ospedale Mauriziano Umberto I, SCDU Ematologia; [19]AO di Perugia, Ospedale S. Maria Della Misericordia - Ematologia e Trapianto Midollo Osseo, Italy

In 2016 GIMEMA – with an unconditional grant from Novartis - launched the LabNet AML project, with the aim of guaranteeing all AML patients, regardless of the treating institution, a high-level diagnostic characterization, as well as disease monitoring. To this end, GIMEMA created a network connecting Italian hematology centers with reference laboratories, able to accurately diagnose and monitor AML by performing cytogenetics and molecular biology tests, in accordance to WHO and ELN recommendations.

Table 1.

Clinical Center	Coordinators	No of patients total N=1.994
Barletta - SC Ematologia con Trapianto	Giuseppe Tarantini,Caterina Buquicchio	213
Roma - Università Campus Bio-Medico	Giuseppe Avvisati, Alessandra Scardocci, Chiara Sarlo, Elisabetta Cerchiara	207
Bari - Università degli Studi di Bari	Francesco Albano, Paola Carluccio, Mario Delia	171
Palermo - Università degli Studi di Palermo	Sergio Siragusa,Maria Enza Mitra	117
Modena - Centro Oncologico Modenese	Fabio Forgieri,Mario Luppi,Monica Morselli,Leonardo Potenza,Ambra Paolini,Roberto Marasca,Deborah Grisolia	107
Milano - Ospedale Niguarda " Ca' Granda"	Roberto Cairoli,Valentina Mancini,Giovanni Grillo,Alfredo Molteni,Laura Marbello	103
Parma - Università degli Studi di Parma	Monica Crugnola,Lucia Prezioso,Benedetta Cambò,Federica Galaverna,Francesca Re	90
Piacenza - AUSL Ospedale di Piacenza	Daniele Vallisa, Lara Pochintesta, Vanessa Valenti	83
Roma - Tor Vergata	Adriano Venditti, Maria Teresa Voso, Francesco Buccisano, Luca Maurillo, Maria Ilaria Del Principe	67
Napoli - A. Cardarelli	Felicello Ferrara,Olimpia Finizio	58
Messina - Papardo	Donato Mannina,Pietro Terrizzi,Barbara Loteta,Santo Neri,Silvana Biondi	56
Orbassano - S. Luigi Gonzaga	Daniela Cilloni	50
Sassari - Serv. di Ematologia Ist. di Ematologia ed Endocrinologia	Fausto Dore,Claudio Fozza,Francesco Longu	48
Trieste - Ospedale Maggiore	Gianluca Festini, Cristina Skert, Gabriele Pozzato, Giovanni De Sabbata, Manuela Caizzi	48
Roma - "Sant' Andrea"	Agostino Tafuri, Sabrina Mariani	47
Taranto - "SS Annunziata" - P.O. S.G. Moscati	Patrizio Mazza, Claudia Ingrosso	43
Messina - Policlinico G. Martino	Caterina Musolino,Sabina Russo,Demetrio Gerace	39
Brindisi - A. Perrino	Domenico Pastore, Maria Rosaria Coppi	36
Roma - S. Giovanni Addolorata	Laura Cudillo,Anna Lia Piccioni,	33
Roma - S.Eugenio	Paolo De Fabritiis, Pasquale Niscola, Benedetta Neri, Teresa Dentamaro, Luciana Morino	31
Como - Ospedale Valduce	Mauro Turrini, Erika Ravelli	26
Reggio Calabria - "Bianchi-Melacrino-Morelli"	Bruno Martino	25
Bergamo - Papa Giovanni XXIII	Alessandro Rambaldi,Orietta Spinelli,Giulia Quaresimi,Tamara Intermesoli,Paola Stefanoni,Chiara Caprioli	24
Taormina - P.O. S.Vincenzo	Giuseppe Longo,Giuseppe Mineo	23
Bologna - S. Orsola Malpighi	Cristina Papayannidis,Stefania Paolini	21
Verona - G.B. Rossi	Mauro Krampera	19
Catania - Ferrarotto	Francesco Di Raimondo	18
Novara - Amedeo Avogadro	Gianluca Gaidano,Monia Lunghi,Clara Deambrogi	17
Padova - Università degli Studi di Padova	Giampietro Semenzato, Carmela Gurrieri, Federica Lessi, Silvia Imbergamo	17
Perugia - S.Maria della Misericordia	Brunangelo Falini,Maria Paola Martelli	16
Siena - A.O. Senese Policlinico " Le Scotte"	Monica Bocchia,	16
Catania - Garibaldi	Ugo Consoli,Stefania Impera,Marilena Salerno,Fabrizio Lo Presti	14
Roma - Università "Sapienza"	Maurizio Martelli, Massimo Breccia, Gioia Colafigli, Sara Pepe, Emilia Scalzulli	13
Frosinone - F.Spaziani	Roberta Sala,Vincenza Martini	12
Pisa - A.O. Pisana	Francesco Caracciolo, Serena Baliducci	11
Palermo - Villa Sofia Cervello	Francesco Fabbiano	8
Tricase - Pia Fondazione di Culto e di Religione Card. G.Panico	Vincenzo Pavone	8
Bari - Giovanni Paolo II	Attilio Guarini,Angela Iacobazzi,Antonello Rana	7
Brescia - Spedali Civili	Giuseppe Rossi,Erika Borlenghi,Chiara Cattaneo	7
Campobasso - Fondazione Giovanni Paolo II	Sergio Storti,Vincenzo Fraticelli,Annalisa De Vellis,Cristiana Gasbarrino	5
Ragusa - Azienda Sanitaria Provinciale 7	Giovanni Garozzo,Agostino Antolino,Sergio Cabibbo,Giovanna Oriella Manenti,Massimo Poidomani	5
Aviano - Centro di Riferimento Oncologico	Mariagrazia Michieli, Barbara Montante, Rosanna Ciancia, Maurizio Rupolo, Silvia Crestani, Pietro Bulian	4
Genova - Azienda Ospedale Università San Martino	Germana Beltrami	4
Lecce - Vito Fazzi	Nicola Di Renzo,Rossella Matera,Michelina Dargenio,Claudia Quintavalle,Giuseppina Loglisci	4
Palermo - La Maddalena	Maurizio Musso, Ferdinando Porretto	4
Mestre - Ospedale dell'Angelo	Anna Maria Scattolin, Elena Maino	3
Reggio Emilia - Arcispedale S. Maria Nuova	Francesco Merli,Annalisa Imovilli,Katia Codeluppi	3
Roma - Regina Elena	Andrea Mengarelli,Antonio Spadea,Francesco Marchesi,Atelda Romano	3
Napoli - Federico II	Fabrizio Pane, Francesco Grimaldi	2
Roma - S. Camillo	Luca Pierelli, Stefano Mancini	2
Torino - A.O. Mauriziano	Giuseppe Saglio, Carmen Fava, Alessandro Cignetti	2
Foggia - Ospedali Riuniti Foggia	Giuseppina Spinosa	1
Matera - Ospedale di Matera	Alberto Fragasso,Clara Mannarella	1
Palermo - ARNAS "Civico - Di Cristina - Benfratelli"	Carmela Tomaselli	1
Salerno - San Giovanni di Dio e Ruggi D'Aragona	Carmine Selleri,Bianca Serio,Luca Pezzullo,Raffaele Fontana,Roberto Guariglia	1

Laboratory	Coordinator	No of affiliated centers
Palermo - Cervello	Alessandra Santoro	11
Roma - Tor Vergata	Maria Teresa Voso	8
Bari - Policlinico Universitario	Francesco Albano	7
Bologna - S. Orsola	Emanuela Ottaviani	5
Padova - Istituto Oncologico Veneto	Roberta Bertorelle	4
Orbassano (TO)	Angelo Guerrasio	4
Napoli - Federico II	Barbara Izzo	4
Milano - Niguarda	Silvio Veronese	3
Perugia - Misericordia	Brunangelo Falini	2
Siena - Policlinico 'Le Scotte'	Monica Bocchia	1
Roma - Sapienza	Daniela Diverio	1
Pisa - AO Pisana	Sara Galimberti	1
Piacenza - Ospedale 'G. da Saliceto'	Angela Rossi	1
Nuoro - San Francesco	Maria Monne	1
Brescia - Spedali Civili	Silvana Archetti	1
Bergamo - Papa Giovanni XXIII	Orietta Spinelli	1

These laboratories fulfill quality controls and regularly undergo standardization procedures. Genetic aberrations included in the basic panel are: *PML/RARA*, *BCR/ABL1*, R*UNX1/RUNX1T1*, *CBFB/MYH11*, *NPM1*, *FLT3*-ITD and *FLT3*-D835 mutations. Besides providing a valuable healthcare service, this effort allows to perform an epidemiologic analysis of Italian patients with AML, deriving both from real-life and clinical trials. The connection between the hematology centers and laboratories is managed by a web-based GDPR compliant platform that allows to: i) request cytogenetics and/or molecular tests, ii) share results, iii) archive the results Data were exported on the 1st of March 2021 and those concerning diagnosis were examined. At the time of data export, the network included 18 active reference laboratories - covering the entire Italian territory - and 55 hematology centers (Table 1). Overall, 1994 patients were registered in the platform, 60% of them were older than 60 years old. A total of 2435 tests were requested: 1742 (72%) at diagnosis, 478 (29%) during follow-up, 145 (6%) at relapse and 44 (1.8%) at refractoriness. At diagnosis, 26.1% of samples were *NPM1*-mutated, 20.2% *FLT3*-ITD+, and 5.6% *FLT*3-D835+. The incidence of *NPM1* mutations and *FLT3*-ITD increased with age (p=0.001 and p=0.029 respectively). As expected, *FLT3*-ITD mutations prevailed in AML with a normal karyotype (NK) (26.7% *vs* 9.3%, p<0.001), while only 10% of AML with an altered karyotype were *NPM1*-mutated (p<0.001). The distribution of main fusion genes was: *PML/RARA*: 7.1%, *CBFB/MYH11*: 5.4%, *RUNX1/RUNX1T1*: 3.9% and *BCR/ABL1*: 3%. The highest incidence of *FLT3* mutations was in *PML/RARA*+ APL (38.4%), followed by *BCR/ABL1*+ AML. *NPM1* mutations were rarely detected in *BCR/ABL1*+ and *CBFB/MYH11*+ AML (1 case each). The LabNet AML project allowed to collect data on roughly 2000 AML patients in the whole Italian territory, and represents a remarkable resource for future research project.

Allogeneic and Autologous Transplantation 1

C016

HIGH DOSE THERAPY AND STEM CELL RESCUE IN MANTLE CELL LYMPHOMA: THE MCL0208 TRIAL FROM FONDAZIONE ITALIANA LINFOMI

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Introduction: High dose therapy (HDT) and autologous stem cell transplantation (ASCT) dramatically improved outcome of younger mantle cell lymphoma (MCL) patients (pts), despite a variable grade of toxicity and incomplete hematological recovery (HR). Unfortunately, there are still no clear correlations between the amount of collected peripheral blood stem cells (PBSC) and toxic events. FIL□MCL0208 phase III trial (NCT02354313, HDT, ASCT and randomized lenalidomide maintenance [LEN] for <65 years MCL) showed a 21% of early treatment interruptions not due to PD. Therefore, we analyzed the PBSC rescue process in order to monitor its eventual impact on hematological toxicity and LEN dose reduction.

Methods: Pts received 3 R-CHOP, R-cyclophosphamide 4 g/sqm, 2 R-cytarabine 3 g/sqm x2/die days 1-3 (R-HD-ARA-C) and BEAM conditioned ASCT: only responding pts with complete HR were randomized. G-CSF was administered from day 5 after I R-HD-ARA-C until PBSC collection (LK1). A LK2 was performed after the II R-HD-ARA-C in pts who collected < $3.5x10^6$ CD34+/kg, did not achieve MRD negativity in LK1 or lacked a MRD marker.

Results. Of the 300 enrolled pts 273 proceeded with LK1: 245 (90%) collected >$3.5x10^6$ CD34+/kg (median 11: "good mobilizers", GM), 7 (3%) mobilized <3.5 (median 3: "poor mobilizers", PM) and 21 (8%) failed LK1 (due either to no mobilization [n=6], adverse events or other reasons). 73 pts proceeded with LK2: 61 (83%) were GM (median 6), 7 (10%) PM (median 2) and 5 (7%) failures; only 1 patient failed both LK. Overall, 251 pts received ASCT, with a median reinfusion of $5x10^6$ CD34+/kg (IQR 4-7), namely 5 (IQR 4-7) for GM and 3 (IQR 3-6) for PM. The median HR time to 0.5 and $1x10^9$/L ANC after ASCT was 10 (IQR 10-11) and 11 (IQR 10-13) days, respectively, and to 20 and $50x10^9$/L PLTs was 13 (IQR 10-16) and 19 days (IQR 15-25). Notably, 24 pts (10%) did not achieve complete HR after ASCT. Interestingly, no significant difference of HR trends was seen neither per mobilizing subtype nor per quantity of PBSC reinfused. No PFS advantage was recorded for GM *vs* PM (Figure 1). Finally, 27/52 (52%) of pts who completed LEN treatment, experienced a dose reduction due to toxicity: again, no impact of any harvest feature was demonstrated.

Conclusions: Despite high rates of PBSC collection and adequate reinfusions, incomplete HR after ASCT is still an issue in MCL. This risk should be considered, as it might hamper the delivery of effective maintenance therapies.

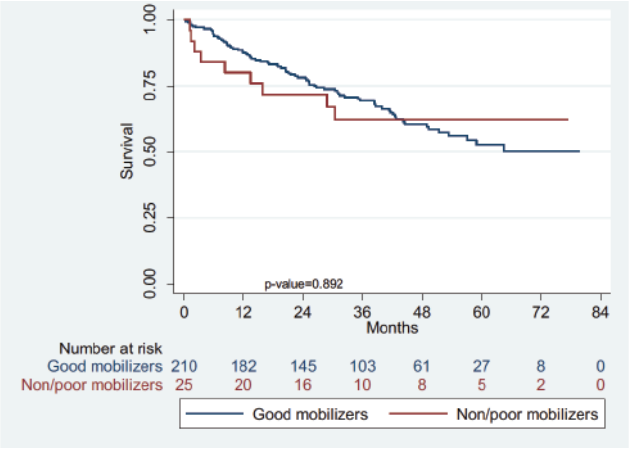

Figure 1. Progression-free survival (PFS) in good vs non/poor mobilizers. Landmark analysis starting from the date of first leukapheresis.

C017

ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS OLDER THAN 60 YEARS: A REGISTRY STUDY OF THE TRANSPLANT ACTIVITY FROM 2000 TO 2017 ON BEHALF OF THE GRUPPO ITALIANO TRAPIANTO DI MIDOLLO OSSEO (GITMO)

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Nowdays, allogeneic stem cell transplantation (Allo-SCT) can ben offered up to the age of 70- 72 years and represents one of the most effective curative treatments for hematological malignancies. Here we report the results of a registry-based retrospective study on behalf of GITMO (GITMO AlloEld), aimed to describe the transplant activity among elderly patients in Italy, between 2000 and 2017. Thirty GITMO Centers participated to the Study. 2061 allo-SCTs in patients older than 60 years were exported from PROMISE database. The present analysis focuses on 1996 first transplants of the series. The median age of the patients at transplant was 63,5 years (59,5-77,8). The most commonly transplanted disease was acute leukemias and myelodysplastic syndromes (67,5%). 28% and 27% of the patients showed a HCT-CI of 1-2 or greater than 3, respectively. The KPS was 100% in 27,2% and 90% in 42,9% of the cases. With a median follow up of 10,4 years, the NRM at 2 and 5 years was 31% and 35%, and the RI at 2 and 5 years was 30% and 35%, respectively. The projected OS at 2 and 5 years was 46% and 34%, respectively. The OS at 5 years significantly improved over the time, moving from 28% (2000-2005) to 37% (2012-2017) (p=0,012). The improvement of survival was related to a significant reduction in RI (45% *vs* 30%, p<0,0001), whereas NRM remained stable over time (33% *vs* 35%, p=0,5). The cumulative incidence of aGVHD did not significantly changed over time, whereas the incidence of extensive cGVHD at 1 year was significantly reduced (15,3% *vs* 10,6%, p=0,004). These differences can be partially explained considering that increasing proportion of patients were transplanted in CR (29% -2000-2012 - *vs* 54% -2012-2017- p<0,0001), underwent a myeloablative conditioning (14% *vs* 42%, p<0,001), and intensified GVHD prevention with *in vivo* T-cell depletion (17% *vs* 43%, p<0,0001). By multivariate analysis factors negatively associated with OS were MUD or UCB, non-response at the time of SCT and male recipient, whereas HCT-CI <1, KPS 90-100 and transplant between 2011-2017 significantly improved the outcome. The HCT-CI significantly affected the 5-year OS and NRM between 2000-2011, whereas it lost its impact in more recent years (2012-2017). These retrospective data showed that the allo SCT for elderly patients became safer and more effective over the time, because of a significant reduction of RI. The HCT-CI score is nowadays probably less efficient to estimate OS and NRM in the elderly population.

C018

ABSTRACT WITHDRAWN

C019

TRIPLE PTCY BASED GVHD PROPHYLAXIS: HLA MATCHED VERSUS HLA HAPLOIDENTICAL TRANSPLANTS

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We report a retrospective analysis of 198 patients who underwent an allogeneic stem cell transplant (HSCT) from 2016 to 2020. All patients received the same triple GVHD prophylaxis, namely post-transplant cyclophosphamide (PTCy), cyclosporine and mycophenolate mofetil. We compared 78 HLA matched transplants (32 siblings and 46 MUD), versus 120 HLA haploidentical related transplants. Patients in matched-HLA group were younger (median age 49 *vs* 56.5), and were transplanted more recently (median 2020 *vs* 2018); no other significant difference was found among the two groups. The diagnosis was mainly acute leukemia (57%), myelofibrosis (21%) or lymphoma (12%). Conditioning was myeloablative in 77% and 73% respectively (p=0.57). Overall, 40 (20%) patients developed acute GVHD grade II-IV: 10% and 27% in the matched and haplo-HLA group, respectively (p=0.005). Also moderate to severe chronic GVHD was more frequent in the haplo-HLA group (4% *vs* 23%, p<0.001).

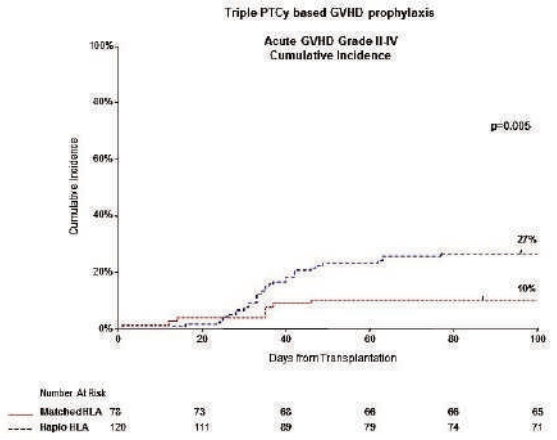

Figure 1.

The Cumulative Incidence (CI) of transplant related mortality (TRM) at 1 year for matched-HLA *vs* haplo-HLA was 10% *vs* 21% (p=0.04). In a Cox multivariate analysis, age over 60 years alone predicted TRM (HR 3.41, p<0.001) while haplo-HLA transplants only gave a trend for more TRM (HR 2.07, p=0.09). The CI of relapse at 1 year for matched-HLA *vs* haplo-HLA was 24% *vs* 10% (p=0.05). In a Cox univariate analysis matched-HLA had only a trend as risk factor for relapse (p=0.088) when compared to haplo-HLA. In patients with myeloproliferative or lymphoproliferative diseases, identical HLA was associated with less TRM (10%

vs 27%, p=0.04) and no difference in relapses. When selecting for patients with acute leukemia or with early disease in CR1/CR2, there was no difference in TRM nor in relapses by HLA matching. Disease free survival (DFS) at 1 year was 65% and 68% in matched and haplo HLA group, respectively (p=0.85). The only predictive variable for DFS was age over 60 years (HR 1.73, p=0.03).

In conclusion: GVHD is reduced in HLA-matched transplants when receiving PTCy+CSA+MMF, as compared to haploidentical grafts, with 10% grade II-IV acute GVHD and 4% moderate-severe chronic GVHD in HLA-matched. This translates in significantly reduced TRM. However, there is a trend of increased relapse which is not apparent in early diseases, leading to identical disease free survival. One may therefore consider tailored GVHD prophylaxis strategies according to disease burden and patients characteristics.

C020

THE IMPACT OF GRAFT CD3/TREGS RATIO ON POST-TRANSPLANT AGVHD INCIDENCE RATE: A PROSPECTIVE, MULTICENTER, OBSERVATIONAL STUDY ON PATIENTS WITH ACUTE LEUKEMIA UNDERGOING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: While it is well known that tumor site- or bone marrow-infiltrating Tregs might be correlated with the worst outcome in solid tumors and acute leukemias by promoting immune surveillance escape; new evidence is emerging in regard to their contribution to the immediate post allotransplant phase by peripheral blood (PB) allo-graft. In fact, Tregs content in stem cells harvested from PB has been suggested to be correlated with aGvHD and immunological recovery after PB stem cell transplant (PBSCT).

Aim: The aim of our study was to investigate the impact of graft content Tregs, as the gCD3/Tregs ratio (gCD3/TregsR), on acute GvHD and post-PBSCT outcome. Patients. We prospectively enrolled 94 consecutive patients at 9 italian centers belonging to the Gruppo Italiano Trapianto di Midollo Osseo (GITMO) affected by AML or ALL in complete remission (CR) who underwent MRD (n=35, 37%) or MUD PBSCT (n=59, 63%). Patient characteristics are summarized in Table 1.

Results: Any grade and grade II or greater aGVHD occurred in 24 (26%) and 17 (18%) allotransplanted patients, respectively. The median graft CD3+, Tregs and CD3/TregsR values were 196x10^6/kg of body weight (range (r), 17-666x10^6/kg), 3x10^6/kg (r, 0.1-35x10^6/kg) and 71 (r, 1-1883), respectively. According to gCD3/TregsR-ROC value associated with the apperance of grade II-IV aGvHD, patients were subdivided into a high gCD3/TregsR (≥70) group (HR; n=48) and a low gCD3/TregsR (<70) group (LR; n=46). The incidence of grade II-IV aGvHD was lower in the LR compared with the HR group [4/46 (9%) *vs* 13/48 (27%)] both in univariate (OR 4.8; CI95%:1.44-16.17; p=0.015) and in multivariate (OR 5.0; CI95%:1.34-18.93; p=0.017) analysis, while no differences were documented taking into account any grade aGvHD events. The OS, DFS, NRM, and relapse rates at 2 and 3 years were 61 and 54%, 62 and 55%, 15 and 23%; 27 and 30%, respectively. By multivariate analysis, LR did not significantly predict better OS, DFS, NRM and relapse.

Conclusions: Our data seem to confirm the value of Tregs in preventing aGvHD, while maintaining the graft versus leukemia effect. Larger studies should be performed to investigate the possible additional impact on post-allotransplant survival outcomes.

Table 1.

Patient characteristics	n	%
Patients, n	94	100
Median age, range	49, [18-68]	
Sex		
M/F	41/53	44/56
Karnofsky performance status (<80%)	11	12
Interval from diagnosis to allotransplant		
days median value, range	226 [86-8134]	
Disease status at allotransplant		
CR1	61	65
CR2	21	22
CR>2	12	13
Type of disease		
AML	71	75
ALL	23	25
Type of myeloablative regimen		
BuCY	15	16
BuFlu	31	33
TBF	31	33
TBI based	10	10
others	7	8
CMV risk		
low	27	29
high	54	57
very high	13	14
Sex match		
Donor female/recipient male	11	12
Other combinations	83	88
Type of donor		
MRD	35	37
MUD	59	63
GvHD prophylaxis strategy		
ATG based	75	80
not ATG based	19	20
Associated immunosuppressive agents		
Cyclosporine alone	0	0
Cyclosporine + methotrexate	89	95
Cyclosporine + mycophenolate	5	5
HLA disparity: 'antigenic' mismatch		
10/10	85	90
not 10/10	9	10
graftCD3+/Tregs ratio		
median value, range	71 [1-1883]	

CR indicates complete remission; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; BuCy, busulfan + cyclophosphamide; BuFlu, busulfan + fludarabine; TBI, total body irradiation; TBF, thiothepa + busulfan + fludarabine; CMV cytomegalovirus; MRD, matched related donor; MUD, matched unrelated donor; GvHD, graft versus host disease; ATG, anti-tymoglobulin; HLA, human leukocyte antigen.

Non Hodgkin Lymphoma 2

C021

THE ELDERLY PROGNOSTIC INDEX (EPI) PREDICTS EARLY MORTALITY IN OLDER PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL). A SUBSTUDY OF THE ELDERLY PROJECT BY THE FONDAZIONE ITALIANA LINFOMI

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Introduction: The Elderly Prognostic Index (EPI) is based on the integration of a simplified geriatric assessment (sGA), haemoglobin levels, and International Prognostic Index (IPI) (figure 1a) and has been validated to predict overall survival in older patients with DLBCL *(Merli et al, JCO 2021)*. In this study we evaluated the ability of EPI to predict the risk of early mortality in older DLBCL patients.

Methods: This analysis was conducted starting from the dataset of the Elderly Project (EP) study. The main endpoint was early mortality rate defined as death occurring within 90 days from the date of diagnosis. Starting from EP we only excluded alive pts with a follow-up shorter than 90 days. Treatment was classified in three groups: Full Dose (FD; >70% of theoretical dose of anthracycline), Reduced Dose (RD; <70%), and Palliative Therapy (PT; no anthracyclines).

Results: This study was conducted on 1150 out of 1163 pts; median age was 76 years (65 to 94). Thirty-one percent were older than 80 years; 55%, 28% and 17% were FIT, UNFIT, and FRAIL based on sGA. EPI score was low, intermediate, and high in 24%, 48% and 28%, respectively. Time to Therapy (TTT) was shorter than 15 days in 24%; a prephase therapy was administered in 14% of pts but details were lacking. Overall, 69 early deaths were observed being 19% of all reported deaths. The cumulative incidence of early death at 90 days was 6.0%. Comparing the causes of the deaths occurring earlier or later than 90 days we observed lower frequency of deaths due to lymphoma progression for early events (42% *vs* 75%) and higher frequency due to toxicity and to infections (32% *vs* 4% and 22% *vs* 3%, respectively). In univariable analysis factors associated with higher risk of early deaths were age >80 years, sGA, anemia, high risk IPI, TTT <15 days, bulky disease, EPI (intermediate and high) and the use of PT. A multivariable analysis on 931 patients (excluding PT) confirmed an independent prognostic role to predict early death for high risk EPI (OR 3.45; 95% CI 1.07–11.2) (Figure 1b) and for bulky disease (OR 2.09; 95% CI 1.09–3.98).

Conclusions: The cumulative incidence of early death for older pts with DLBCL is not negligible (6%), is mainly associated with non-lymphoma related events and suggests the adoption of adequate preventive measures. For patients treated with an anthracycline containing regimen, high risk EPI and bulky disease are independent factors to predict the risk of dying early during treatment.

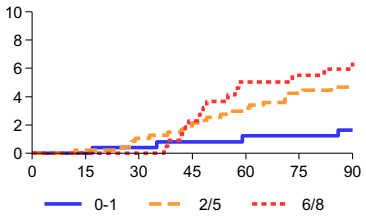
Figure 1a. EPI model

EPI model parameters	
Factors	*Weight*
FIT *	0
UNFIT *	3
FRAIL *	4
IPI 1	0
IPI 2	1
IPI 3-5	3
Hb <12 g/dL	1
EPI Risk Groups Score **	
Low	0-1
Intermediate	2-5
High	6-8

** FIT, UNFIT and FRAIL according to sGA (Merli F et al, JCO 2021)*
*** EPI is available at: http://www.filinf.it/epi*

Abbreviations: EPI, Elderly Prognostic Index; IPI, International Prognostic Index

Figure 1b. Cumulative incidence (%) of early deaths by EPI group



Abbreviations: EPI, Elderly Prognostic Index.
EPI risk groups: 0-1: Low Risk; 2-5: Intermediate Risk; 6-8: High Risk

Figure 1.

C022

RESPONSE ADAPTED POST INDUCTION THERAPY IN FOLLICULAR LYMPHOMA: UPDATED RESULTS OF THE FOLL12 TRIAL BY THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Two years of rituximab maintenance (RM) after first-line rituximab-based chemotherapy significantly improved progression-free survival (PFS) in patients with follicular lymphoma (FL). However, one important question is whether this approach is really suitable for all patients. Here, we report the results from the FOLL12 study, comparing RM with a response adapted post-induction approach.

Methods: We randomly assigned treatment naïve, advanced stage, high tumor burden FL patients to receive standard RM or a response-oriented post-induction approach based on metabolic response and molecular assessment of minimal residual disease (MRD). End of Induction (EOI) metabolic response was centrally defined applying the Deauville scale (DS) that defined Complete metabolic response (CMR) in case of DS 1-3. MRD was defined according to nested PCR assessment of Bcl2/IgH rearrangement on bone marrow and peripheral blood, only for patients with a molecular marker (MM) at baseline. Post induction therapy in the experimental arm consisted of: CMR and MRD- patients, observation; CMR and MRD+, 4 weekly rituximab until MRD- for up to 3 courses; no CMR, one dose of ibritumomab tiuxetan followed by RM. The primary endpoint was 3-year PFS.

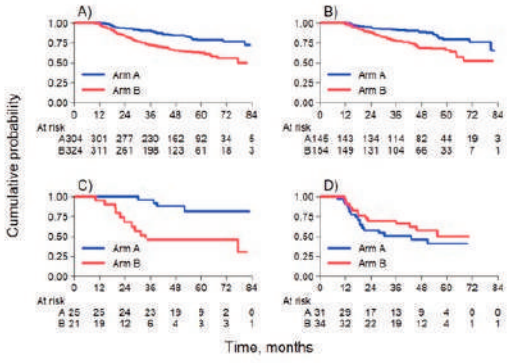

Figure 1) Progression free survival by study arm for patients' sugroups: A) Patients with complete metabolic response (CMR) after induction therapy; B) Patients with both CMR and complete molecular response (MRD-); C) patients with CMR and MRD+; D) Patients without CMR
Legend to figure: A: reference arm; B: experimental arm

Figure 1.

Results: This analysis was conducted on 712 patients who achieved at least a Partial response at EOI. After a median follow-up of 53 months (range 1 to 92), patients in the standard arm had a significantly better PFS than the experimental approach (3-year PFS 86% *vs* 72%, P <0.001). The improved PFS of the standard *vs* experimental arm was confirmed in the study subgroups (Figure 1); CMR patients (A) (N=628: 3-yr PFS 90% and 72% for standard and experimental arm, respectively (P=< 0.001); CMR and MRD- (B) (N=299: 92% *vs* 78%; P<0.001); CMR and MRD+ (C) (N=46: 96% and 45%; p=0.004). In the group of 65 patients without CMR no difference in PFS was observed between reference and experimental arm (P = 0.274) (D). At time of last update 30 deaths were reported, of which 15 associated with disease progression or recurrence. The 3-yr OS was 98% (95%CI 96-99) and 97% (95%CI 95-99) in the reference and experimental arm, respectively (p=0.238).

Conclusions: A metabolic and a molecular response adapted therapy as assessed in the FOLL12 study was associated with a significantly inferior PFS compared to 2-year RM. The better efficacy of standard RM was confirmed in the subgroup analysis and in particular for patients achieving both CMR and MRD-.

C023

UPDATED RESULTS OF THE ASPEN TRIAL FROM A COHORT OF PATIENTS WITH *MYD88* WILD-TYPE (*MYD88*^{WT}) WALDENSTRÖM MACROGLOBULINEMIA (WM)

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Background: Inhibitors of Bruton tyrosine kinase (BTK) have shown significant activity in patients with WM harboring a mutation in the *MYD88* gene. However, lower response rates and shorter progression-free survival have been reported in patients with WM who lack such mutations.

Aim/Objective: The ASPEN trial (NCT03053440) evaluated zanubrutinib (ZANU), a potent and selective BTK inhibitor, in patients with *MYD88* mutation and wildtype *MYD88* (*MYD88*^{WT}) WM. The objective of this abstract is to detail the safety and efficacy of ZANU in patients with *MYD88*^{WT} WM.

Methods: In the ASPEN trial, bone marrow *MYD88* mutations were assessed at study entry by a central laboratory (NeoGenomics). Based on the results of the *MYD88* mutation assay, patients were assigned to cohort 1 (*MYD88* mutation) or cohort 2 (*MYD88*^{WT} or mutation unknown). All cohort 2 patients received ZANU 160 mg twice daily until disease progression.

Results: In total, 28 patients with WM were enrolled in cohort 2; of which, 26 had *MYD88*^{WT}. The median age of patients in cohort 2 was 72

years; five patients were treatment-naïve and 23 patients had relapsed/refractory (≥1 prior therapy) WM. Most patients had intermediate- (39.3%) or high-risk (42.9%) disease (defined by the International Prognostic Scoring System for WM). At median follow-up of 17.9 months, two patients discontinued ZANU due to adverse events (AEs) and six experienced disease progression; there were no cases of disease transformation. In patients with confirmed *MYD88^{WT}*, overall response rate by independent review committee (IRC) was 80.8%, with a major response rate of 50.0% including a very good partial response rate of 26.9% (Table). Progression-free survival event-free rate at 12 months was 72.4%. The most frequently reported AEs were diarrhea, anemia, contusion, pyrexia, and upper respiratory tract infection. Major hemorrhage was reported in two patients, and atrial fibrillation was reported in one patient. There were no fatal AEs.

Conclusions: ZANU showed clinically meaningful antitumor activity, including achieving major responses and durability of responses, and was considered well tolerated with a low discontinuation rate due to AEs, in patients with *MYD88^{WT}* WM.

Table 1. Best Overall Response by Independent Central Review in Patients with MYD88WT WM.

	Treatment-naïve WM (n=5)	Relapsed/refractory WM (n=21)	Overall (N=26)
Median follow-up, mo	19.3	17.1	17.9
Best overall response by IRC, n (%)			
Complete response	0	0	0
Very good partial response	1 (20.0)	6 (28.6)	7 (26.9)
Partial response	1 (20.0)	5 (23.8)	6 (23.1)
Minor response	2 (40.0)	6 (28.6)	8 (30.8)
Stable disease	1 (20.0)	3 (14.3)	4 (15.4)
Progressive disease	0	1 (4.8)	1 (3.8)

IRC, independent review committee; WM, Waldenström macroglobulinemia.

C024

PROGNOSTIC FACTORS, MANAGEMENT AND OUTCOME OF AN INTERNATIONAL SERIES OF 41 PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL) AND CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT

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Introduction: CNS dissemination is an uncommon, poorly-investigated event in PMLBCL. International cooperation is needed to define its management.

Methods: Data from PMLBCL pts with CNS disease at presentation or relapse treated at 24 Centers from 6 countries were analyzed.

Table 1.

Treatment Line for CNS disease	Induction	N°	Consolidation ASCT ± WBRT	CRs
1st (n=41)	RCHOP + HD-MTX#	1 (2%)	0	1
	HD-MTX ± rituximab	2 (5%)	2	2
	HD-MTX + cytarabine ± rituximab	13 (32%)	5	3
	MATRIX (MTX, cytarabine, thiotepa, rituximab)	14 (34%)	7	6
	R-ICE regimen	3 (7%)	2	1
	Supportive care	8 (12%)	0	0
2nd (n=24)	WBRT	7 (24%)	5	5
	WBRT + ibrutinib	1 (4%)	0	1
	WBRT + pembrolizumab/nivolumab	2 (8%)	1*	2
	High-dose-ifosfamide-based therapy	6 (24%)	2	1
	Pembrolizumab	1 (4%)	0	0
	Supportive care	7 (24%)	0	0

#At initial diagnosis

*Consolidated by CAR-T

Results: 41 pts (median age 32, range 14-52; 22 males) were considered. At PMLBCL diagnosis, 49% had advanced stage, 51% B symptoms, 95% bulky disease, 90% raised LDH, 44% extranodal disease (89% in abdomen), 63% an aaIPI ≥2. First-line treatment was CHOP14/21 in 20 pts, daEPOCH in 6, M/VACOP-B in 15; with rituximab in 39 pts, and mediastinal irradiation in 14. CNS prophylaxis was administered in 6 pts. CNS involvement was recorded at initial diagnosis in one (2%) pt, at first relapse in 34 (83%), at late relapse in 6 (15%). The median time to CNS relapse was 7 (0-24) months. CNS was the only site of relapse in 24 (59%) pts, all at first failure. CNS relapse sites were brain or cerebellum in 38 (93%) pts, associated with meningeal infiltration in 6; spinal cord in 1; meninges in 2 (5%). Treatment for CNS disease and responses are reported in the Table: 13 pts (32%; 95%CI=18-46) achieved a CR, all of them were treated at presentation or first relapse, and, all except one received high-dose-methotrexate (HD-MTX)-based therapy plus ASCT ± WBRT. 24 pts experienced further failure (Table), invariably in the CNS, with concomitant systemic disease in 8; 10 pts with progressive disease limited to the CNS received WBRT, combined with ASCT and/or other drugs, 8 achieving a CR lasting 16-84 mo. Pts with CNS involvement at 3rd-4th relapse also had systemic, uncontrolled disease, and did not benefit from treatment. At a median follow-up of 61 (10-173) months, 9 pts remain relapse-free, with a 5-yr PFS after CNS relapse of 21%; 17 pts are alive, with a 5-yr survival after CNS relapse of 42%. Systemic disease and meningeal infiltration were not associated

with outcome. The 5-yr survival after CNS relapse of the 26 pts treated with HD-MTX-based combinations was 52%.

Conclusions: Advanced stage, abdominal extranodal disease and high LDH levels often precede CNS recurrence in PMLBCL pts. Unlike other aggressive lymphomas, CNS involvement at initial diagnosis and meningeal disease are rare in PMLBCL. Prognosis is poor, but HD-MTX-based therapy + ASCT are associated with encouraging *Results:* WBRT contributes to the achievement of long-lasting remission even in pts with chemorefractory disease.

C025

DIRECT-ACTING ANTIVIRALS AS PRIMARY TREATMENT FOR HCV-ASSOCIATED INDOLENT NON-HODGKIN LYMPHOMAS: THE PROSPECTIVE BART STUDY OF THE FONDAZIONE ITALIANA LINFOMI

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The most convincing argument for the role of hepatitis C virus (HCV) in the pathogenesis of some indolent non-Hodgkin lymphoma (iNHL) subtypes, especially marginal-zone lymphomas (MZL), is represented by retrospective observations of tumor regression after viral eradication by interferon (IFN)-free direct-acting antivirals (DAAs). However, no prospective studies in this setting have been performed so far. In 2016 the Fondazione Italiana Linfomi started the prospective, multicenter, phase 2, BArT study, evaluating IFN-free DAAs regimens in untreated, HCV-RNA+, non-cirrhotic, iNHL patients (pts) without criteria for immediate conventional treatment. Pts with genotypes (GT) 1 and 4 received ledipasvir (LDV)/sofosbuvir (SOF) for 12 (naïve) or 24 weeks (IFN experienced), GT2 pts SOF + ribavirin (RBV) for 12 weeks and GT3 pts LDV/SOF+RBV for 24 weeks. After amendment (Jul 2017), GT2 and 3 pts received the novel SOF/velpatasvir (VEL) regimen for 12 weeks. The primary objective was sustained virological response (SVR) while the main secondary objectives were overall response rate (ORR) of lymphoma, progression-free survival (PFS) and toxicity. Forty pts (17 males, 23 females) were enrolled, including 27 MZL (14 MALT, 9 nodal and 4 splenic), 6 lymphoplasmacytic lymphoma, 4 CD5-negative iNHL NOS, 2 small lymphocytic lymphoma and 1 follicular lymphoma. Median age was 68 years (yrs) (45-83). Stage was III/IV in 34 pts (85%). GT was 1 in 17 (43%), 2 in 21 (52%), 3 in 2 pts (5%). Four pts (10%) previously failed an IFN-based regimen. All pts received GT-appropriate DAAs: 17 LDV/SOF, 8 SOF+RBV, 15 SOF/VEL. The primary endpoint was met as all pts achieved SVR (100%). DAAs were well tolerated, with 17 pts (43%) experiencing 30 g1-2 (including 2 RBV-related g1 anemia) and 2 g3-4 adverse events (g4 lipase increase; g3 breast cancer). ORR of lymphoma was 45%, including 8 pts (20%) achieving complete response (CR) and 10 (25%) partial response (PR), while 16 (40%) exhibited stable disease (SD) and 6 (15%) progressed. No significant difference in ORR was recorded between MZL and non-MZL cases (48 *vs* 38%, p=0.74). At a median follow-up of 33 months, no pt died, 3 additional pts progressed, with a 3-yr PFS of 80% (95%CI 64-89%, Figure 1). This is the first prospective trial investigating the role of DAAs in HCV+ iNHL pts not requiring immediate conventional treatment. Our results suggest that HCV eradication with DAAs should be considered as first-line therapy in this setting.

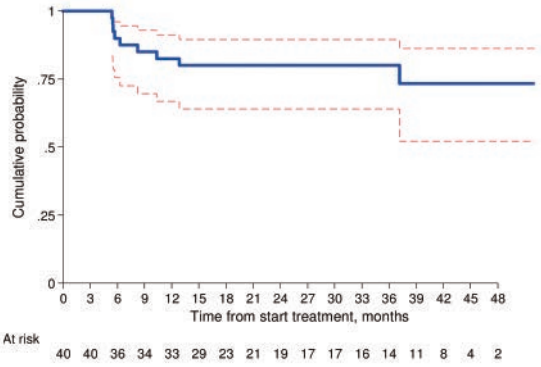

Figure 1.

Cytogenetic and Quality of Life

C026

NEXT GENERATION SEQUENCING PROVIDES NOVEL MOLECULAR MARKERS FOR MINIMAL RESIDUAL DISEASE MONITORING IN FOLLICULAR LYMPHOMA: BIOLOGICAL RESULTS FROM FONDAZIONE ITALIANA LINFOMI (FIL) FOLL12 TRIAL

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Background: Despite immunochemotherapy provides durable responses in follicular lymphoma (FL) patients, the majority of them eventually relapse. Minimal residual disease (MRD) analysis, based on the detection of Bcl-2/IGH rearrangement, by highly standardized and sensitive PCR approach is able to early identify patients at high risk of relapse. Nevertheless, this tool fails in almost 40% of FL cases. Next-generation sequencing (NGS) is going to overcome this bias. *Aims*. We screened by NGS a cohort of 120 FL lacking Bcl-2/IGH marker, enrolled in the FIL "FOLL12" prospective clinical trial (EudraCT: 2012-003170-60), in order to increase the number of patients eligible for MRD.

Methods: Baseline gDNA of "no marker" patients with documented BM infiltration were screened by IGH-NGS using EuroClonality-NGS approach. In parallel, targeted locus amplification (TLA) was used to identify novel Bcl-2/IGH rearrangements in a preliminary cohort of 15 patients. Finally, to test the reliability of newly identified markers we carried out MRD analysis by ASO qPCR, following EuroMRD guidelines.

Results: Overall, 111/120 (93%) sequenced samples passed the quality control (>10000 raw reads). A clonotype was identified in 56% (63/111): 61 monoclonal and 2 biclonal, 54 productive and 9 unproductive IGH rearrangements (Figure 1). Moreover, in 8/15 (53%) patients screened by TLA a novel Bcl-2/IGH rearrangement was found; interestingly, TLA was able to identify a marker in 2 out of 5 cases failed by IGH-NGS. Finally, the reliability of these markers for MRD detection was preliminary assessed in three patients by ASO qPCR (targeting one Bcl2/IGH and two IGH rearrangements). The assays reached high level of sensitivity (from 1E-04 to 5E-05) and MRD kinetics well described patients outcome.

Conclusions: Preliminary data on wide NGS-based marker screening in FL patients from a clinical trial lacking a conventional MRD marker suggested that: 1) EuroClonality-NGS IGH approach was able to provide a new marker in more than half of the patients with BM infiltration; 2) TLA approach showed similar success rates in a small patients group; 3) the two techniques are not alternative and should rather be considered as complementary; 4) the new markers allowed to perform a "proof of concept" MRD monitoring by ASO qPCR. These data are highly promising to provide an MRD marker in the majority of FL patients: next steps of the present project will evaluate MRD by NGS assessing its clinical impact.

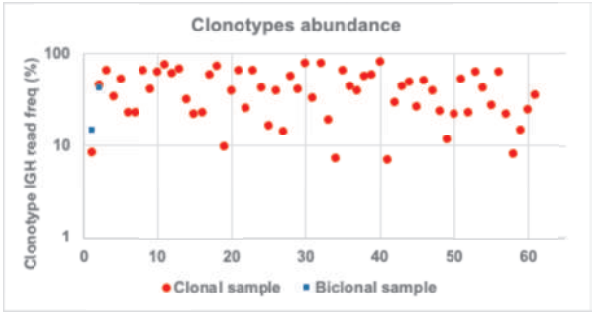

Figure 1.

C027

AZACYTIDINE TREATMENT IN PATIENTS WITH ACUTE MYELOID LEUKEMIA/HIGH-RISK MYELODYSPLASTIC SYNDROME: DAY-HOSPITAL MANAGEMENT COMPARED TO HOME CARE SETTING

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Treatment with Hypometilating Agents (HMA) of unfit patients (pts) with Acute Myelogenous Leukemia (AML) and High-Risk Myelodysplastic Syndromes (HR-MDS) is often difficult in the standard Day-Hospital (DH) setting, due to the number of hospital admissions required and the frail clinical conditions of pts. In the Viterbo province, accounting for 3612 Km^2 divided into 60 municipalities, is operative an Unit of Domiciliary Hematologic Care (UDHC) for clinical assistance to frail pts with hemopathies. To evaluate the role of the UDHC compared to standard DH setting in the active frontline treatment with HMA, all pts with AML/HR-MDS unfit for intensive care and treated frontline with HMA from 1/2010 to 12/2020 were analysed. In this study period, 93 patients (51 AML/42 HR-MDS) received HMA (azacytidine in 89 cases and decitabine in 4 cases): of them, 59 (63.4%) were treated in a standard DH setting and 34 (36.6%) were followed by UDHC: pts were allocated to DH or home care setting by responsible physician based on clinical conditions, comorbidities, caregiver availability and distance from hospital. The main features at baseline of HMA in the whole cohort and according to management are reported in the Table 1. Median interval from diagnosis to HMA initiation was 0.9 months (IQR 0.5 – 2.8). Median number of HMA cycles administered was 8 (IQR 4 – 16). The overall response rate (ORR), including complete response, partial response and hematologic improvement, was 40.9% (38/93 pts) in the whole cohort, without differences according to management [25/59 (42.3%) in DH *vs* 13/34 (38.2%) in home care, p=0.347]. Infections were also equally reported [39/59 pts (66.1%) in DH *vs* 24/34 (70.5%) in home care setting had at least 1 infection, p=0.362]. Median response duration of the whole cohort was 8.7 months (95%CI 2.9 – 14.4), without differences according

to management (8.7 months in DH *vs* 13.8 months in home care, p=0.569). Median Overall Survival (OS) of the whole cohort was 12.5 months (95%CI 8.4 – 16.5): median OS of pts treated in DH was 13.0 months (95%CI 8.1 – 17.8) compared to 12.5 months (95%CI 6.4 – 18.5) of pts managed by UDHC (p=0.546). Home care management of HMA for unfit AML/HR-MDS pts is feasible and effective, with results similar to those achievable in a standard DH setting: this approach is thus adequate to offer active therapies in a fraction of frail pts considered up to now ineligible.

Table 1. Clinical features at AZA baseline of the whole cohort and according to management.

	All patients N° 93	Day hospital setting N° 59	Home care setting N° 34	p
Diagnosis, AML/HR-MDS, n° (%):	51/42 (54.8/45.2)	30/29 (50.8/49.2)	21/13 (61.8/38.2)	0.308
MDS – RCMD	1 (1.1)	1 (1.7)	/	
MDS – RAEB 1	18 (19.4)	13 (22.0)	5 (14.7)	
MDS – RAEB 2	23 (24.6)	15 (25.4)	8 (23.5)	
De novo AML	30 (32.3)	21 (35.6)	9 (26.5)	
AML post MDS	6 (6.5)	2 (3.4)	4 (11.8)	
AML post MPN	12 (12.9)	5 (8.5)	7 (20.6)	
Therapy-related AML	3 (3.2)	2 (3.4)	1 (2.9)	
Gender, M/F (%)	53/40 (57.0/43.0)	36/23 (61.0 – 39.0)	17/17 (50.0/50.0)	0.301
Median age (years) (IQR)	74.1 (69.2 – 79.6)	73.4 (66.8 – 79.5)	75.0 (71.8 – 79.8)	0.248
Hb, g/dl (IQR)	8.6 (7.8 – 9.4)	8.6 (7.8 – 9.4)	8,6 (7.4 – 9.6)	0.877
WBC, x 10^9/l (IQR)	3.28 (1.98 – 6.94)	3.17 (1.97 – 7.32)	3.65 (1.90 – 6.83)	0.965
PLTS, x 10^9/l (IQR)	80 (31 – 161)	93 (36 – 176)	54 (22 – 101)	0.174
Marrow blasts, n° (%):				
< 20%	45 (48.4)	31 (52.5)	14 (41.2)	
≥ 20 – 40%	22 (23.6)	16 (27.2)	6 (17.6)	0.093
≥ 40%	26 (28.0)	12 (20.3)	14 (41.2)	
Karyotype, n° (%):				
46 XY/46 XX	19 (20.4)	13 (22.0)	6 (17.6)	
Isolated del 5q-/+ another anomaly	8 (8.6)	4 (6.8)	4 (11.8)	
Del20q	2 (2.2)	1 (1.7)	1 (2.9)	
Trisomy 8	4 (4.3)	3 (5.1)	1 (2.9)	0.423
Other single anomaly	15 (16.1)	10 (16.9)	5 (14.7)	
Double anomalies	1 (1.1)	1 (1.7)	/	
Monosomy 7/del7q	7 (7.5)	6 (10.2)	1 (2.9)	
Complex karyotype	16 (17.2)	12 (20.3)	4 (11.8)	
Failed/not done	21 (22.6)	9 (15.3)	12 (35.3)	
Charlson Comorbidity Index, n° evaluable (%):	88	58	30	
< 5	59 (67.0)	39 (67.2)	20 (66.7)	0.957
≥ 5	29 (33.0)	19 (32.8)	10 (33.3)	

C028

NURSING MANAGEMENT DURING COVID-19 PANDEMIA IN THE VITERBO DOMICILIARY CARE UNIT: DATA ANALYSIS FROM MARCH 2020 TO MARCH 2021

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In the Viterbo province (3612 Km^2 divided into 60 municipalities) is operative a Domiciliary Hematologic Care Unit (DHCU) for clinical assistance to frail patients (pts) with hemopathies: DHCU nursing activity is done by 4 units who were employed during Covid-19 pandemia to avoid as possible risks of viral contagium due to hospital admissions of our pts. To evaluate the entity of nursing management for frail pts followed by DHCU during Covid-19 pandemia, all activities from 3/2020 to 3/2021 in the lockdown framework were analysed. On the whole, 107 pts in 43 municipalities of Viterbo province were followed by DHCU nurses in the study period. Main features of the pts at baseline of domiciliary assistance are reported in the Table. At beginning of the study period (08/03/2020), 37 pts (34.5%) were already followed by DHCU, while 70 pts (65.5%) entered domiciliary assistance during the year of study. Median distance from DHCU central site to pts house was 25 Km [Interquartile range (IQR) 16 – 34]: distance from DHCU was < 20 Km in 32 cases (29.9%), ≥ 20 < 40 Km in 57 (53.2%) and ≥ 40 Km in 18 (16.9%). A total number of 2609 nursing accesses was done in the whole period. According to different procedures, 1152 blood samples were performed, with a median number of 7 (IQR 3 – 15) for each pts: in addition, there were 1040 accesses for chemotherapy (CHT) administration (108 cycles of azacytidine in 15 pts, 87 bortezomib-based cycles in 30 pts, 16 administrations of other CHTs in 2 pts) and 417 accesses for other procedures (260 venous catheter medications, 125 therapy other than CHT, 32 nursing assistances of transfusions or marrow aspirates). Finally, 20 pts were vaccinated at home with respective caregivers. During the entire study period, 2 pts (1.8%) developed Covid-19 infection while in home care. At the last follow-up (31/03/2021), 59 pts (55.1%) were alive and still followed by DHCU, 20 pts (18.6%) were alive and returned to sDay-Hospital (DH) setting due to improvement of clinical conditions and 28 pts (26.3%) died while in domiciliary assistance. Domiciliary nurse assistance during Covid-19 pandemia allowed to follow in a safer way compared to standard DH/ordinary admission settings > 100 frail pts with hemopathies, most of them in 1st or subsequent active lines of therapy, in a wide geographic area. In our opinion, this approach should represent the best type of assistance for a high rate of hematologic pts even beyond Covid-19 period of pandemia.

Table 1. Patient clinical features at baseline of domiciliary nursing management

N° of patients	107
M/F, n° (%)	61/46 (57.0/43.0)
Median age, years (IQR)	74.5 (67.4 – 80.6)
Diagnosis, n° (%):	
Acute myeloid leukemia	17 (15.8)
Acute lymphoid leukemia	3 (2.8)
Myelodysplastic syndromes	11 (10.3)
Multiple myeloma	44 (41.1)
Non-Hodgkin lymphoma	17 (15.8)
Chronic lymphocytic leukemia	3 (2.8)
Other hemopoietic diseases	12 (11.4)
Phase of disease, n° (%):	
1st line treatment	41 (38.3)
Resistant to 1st line treatment	6 (5.6)
1st relapse	16 (14.9)
2nd or following relapse	11 (10.3)
Chronic phase/maintenance/untreated	33 (30.9)
Reason for domiciliary management, n° (%):	
Age only	22 (20.6)
Symptoms burden	21 (19.6)
Performance status ≥ 2 (ECOG)	6 (5.6)
Motility impairment	33 (30.9)
Social/familiar disability	10 (9.3)
Prevention of Covid-19 infection	15 (14.0)

C029

INFLAMMATORY BOWEL DISEASES AND HEMATOLOGICAL MALIGNANCIES: COULD CLONAL HEMATOPOIESIS BE THE BIOLOGICAL LINK?

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Inflammatory bowel diseases (IBDs) are a group of chronic relapsing conditions of the gastrointestinal tract. Nationwide studies have revealed a higher risk of hematological malignancies (HMs) but not colorectal cancer in IBD patients. Clonal hematopoiesis (CH) is a premalignant condition defined by the presence of an acquired somatic mutation characterized by a variant allele frequency (VAF) of ≥2% in a gene frequently associated with HMs. A growing body of evidence suggests a correlation between inflammation and CH. To assess CH possible co-occurrence in patients with an IBD associated with HMs, we performed a targeted next-generation sequencing analysis in a cohort of seven patients who were referred to our center from February 2011 to December 2019 with IBD associated with HMs. A customized panel, encompassing 26 target genes frequently mutated in myeloid malignancies, was performed on genomic

DNA from bone marrow (cases #3, #4, #5, #6, #7) or peripheral blood (cases #1, #2) samples. Only variants (non-intronic, non-synonymous, with >1% global MAF in the healthy population) affecting the CH-associated genes, with ≥2% VAF, and with a depth of coverage >500x were considered. Overall, 11 variants affecting six CH-associated genes (ASXL1, DNMT3A, ETV6, EZH2, GATA2, JAK2) were detected; all patients showed one or more mutations, with a VAF ranging from 2.6% to 53.0%. The median age of our patient series (59 years, range 47 – 70) was lower compared to that expected in healthy individuals with CH. In accordance with previously published data in the IBD context, DNMT3A was the most frequently mutated gene (4/11, 36.4%), followed by ASXL1 and ETV6 (2/11, 18.2% each), EZH2, JAK2, and GATA2 (1/11, 9.1% each). All mutations are single nucleotide variants (SNV) with different functions: missense variants (6/11, 54.5%), nonsense variants (3/11, 27.3%), and unknown variants affecting a splice site or an untranslated region (2/11, 18.2%). It is worth noting that 4 (57%) cases in our series bore DNMT3A gene mutations. Our report suggests that CH may be the biological link between the IBD and the onset of HM. Recent works showed as chronic infection and ulcerative colitis may promote the selection of the DNMT3A gene mutation associated with CH by the IFNγ signaling induced in the course of these disorders. If these data are confirmed, IBD patients screened and positive for CH should undergo hematologic follow-up to assess the risk of developing HM.

C030

A NEW TOOL TO DETECT SF3B1 K700E MUTATION IN MYELODYSPLASTIC SYNDROMES WITH RING SIDEROBLASTS AND MYELOFIBROSIS

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Most human genes encode for several mRNA isoforms by the alternative splicing process, whose regulation depends on the spliceosome. Spliceosome mutations have recently sparked significant interest in hematological malignancies. Among the spliceosome mutations, those in the SF3B1 gene were correlated with the presence of ring sideroblasts (RS) in both myelodysplastic syndromes (MDS) and myelofibrosis (MF). The SF3B1 mutations in MDS-RS and MF were associated with a shorter median duration of response to erythropoiesis-stimulating agents (ESA) and fibrosis. Nowadays, the SF3B1 mutational status is considered important for both the diagnosis and therapy decision. Indeed, Luspatercept, a TGF-β superfamily inhibitor, has been proven to be effective in low-risk MDS patients, particularly in SF3B1-mutated patients, and in MF-associated anemia patients. Sanger sequencing and Next Generation Sequencing (NGS) are the currently available methods to identify the SF3B1 mutations, but both are time-consuming and expensive techniques that are not practicable in most small/medium laboratories. This can often result in a slow and rough characterization of patients, reducing their therapeutic choices. Using peptide nucleic acid (PNA)-PCR clamping technology, we designed and validated a new molecular assay able to recognize the SF3B1 K700E mutation, which is the most frequent of all the SF3B1 mutations. A total of 91 DNA samples were collected and double-blind tested by both PNA-PCR Clamping and Sanger sequencing. The samples are divided as follows: 67 MDS and 24 MF patients. We found 11/67 (16,4%) SF3B1 K700E MDS mutated patients and only 1/24 (4,2%) MF patient. All the mutated MDS patients showed RS>5%, while 3 MDS-RS patients were SF3B1 wild type. The only one SF3B1-mutated MF was a patient refractory to ESA, who evolved from essential thrombocythemia. Our data demonstrated that PNA-PCR Clamping and Sanger sequencing results were perfectly concordant. In contrast, the PNA-PCR Clamping showed many advantages: it is faster, cheaper, and showed a lower limit of detection than Sanger sequencing. In conclusion, considering the relevance of SF3B1 K700E mutation as a biomarker in MDS and MF patients, PNA-PCR clamping could be considered as a valid alternative to Sanger sequencing in routine tests. Further, our assay could be used for a fast and massive screening able to identify the largest number of patients with the K700E mutation who are candidates for Luspatercept treatment.

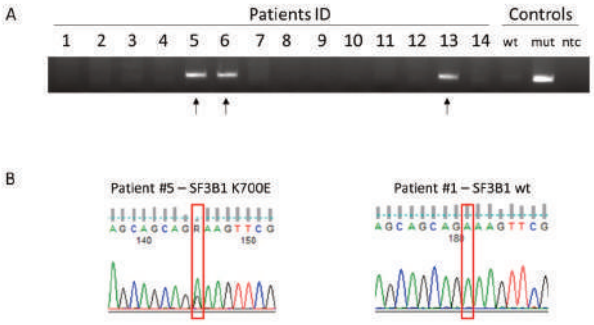

Figure 1: A. PNA-PCR Clamping example: if the DNA is amplified, the patient is SF3B1 K700E mutated (indicated by arrows); if the DNA is not amplified, the patient is wild type for the SF3B1 K700E mutation. Abbreviations: wt, wild type; mut, mutated; ntc, no template control. **B.** Confirmation of PNA-PCR Clamping results by Sanger sequencing for patient #5, SF3B1 K700E mutated, and patient #1, wild type for SF3B1 K700E mutation.

Figure 1.

Myelodysplastic Syndromes

C031

TRAJECTORIES OF CLONAL EVOLUTION AFTER APLASTIC ANEMIA AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

C. Gurnari[1,2], S. Pagliuca[1], B. Patel[1], H. Awada[1], W. Shen[1], S. Kongkiatkamon[1], L. Terkawi[1], M. Zawit[1], V. Visconte[1], S. Corey[1], M.T. Voso[2], H.E. Carraway[1], J.P. Maciejewski[1]

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Up to 20% of aplastic anemia (AA) patients treated with immunosuppression will evolve to myeloid neoplasia (MN) over a median time of 10 years (Figure 1A).The pathogenesis of MN post-AA (sMN) is diverse and will often include antecedent clonal facilitating events that herald progression. Progression to MN may also reflect an immune escape due to selection pressure e.g., through acquisition of HLA mutations. Here, we studied the molecular landscape of sMN, to better understand their pathogenesis and to develop measures of early detection, prevention, and therapeutic strategies. An integrative mutational analysis of myeloid/germline (GL) and HLA genes was performed to comprehensively evaluate their role within the scenario of AA/paroxysmal nocturnal hemoglobinuria (PNH) clonal evolution. Among 350 AA/PNH patients, 11% (median age 61 years) developed a sMN. Evolution was less common in patients with moderate AA or in the presence of a PNH clone (p=.0003). Cytogenetics at evolution revealed abnormalities in 83% of patients, with chromosome 7 alterations in 47% of cases. By comparison, -7/del(7q) were present in 7.5% of patients with primary MN (p=.0001; Figure 1B). GL alterations were classified as Tier1 (9/38 patients) and Tier2 (11/38 patients). Tier1 variants included NF1, CBLC, SBDS and SAMD9L and were enriched in progressors (p=.008) as well as del(7q) patients (p=.0001).A total of 148 somatic variants were found at evolution in 34/38 sMN patients with no differences in cases with or without chromosome 7 abnormalities (Figure 1C).

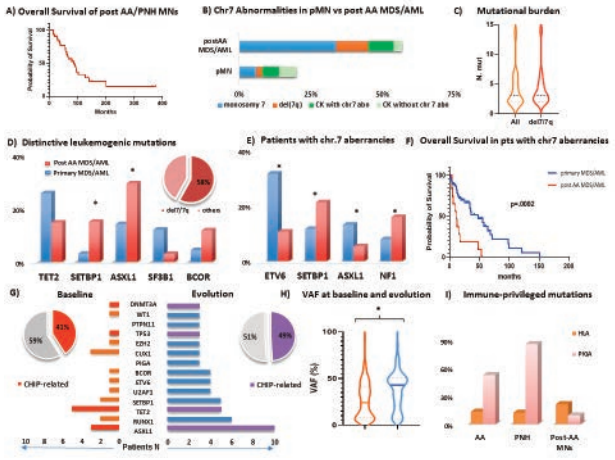

Figure 1.

ASXL1 (p=.02) and SETBP1 (p=.005) were more frequent in sMN (Figure 1D). When comparing patients with chromosome 7 abnormalities with de novo counterpart, sMN appeared most commonly mutated in ASXL1 (p=.02), SETBP1 (p=.0007), ETV6 (p=.02) and NF1 (p=.02) and had a shorter median survival time (p=.0002; Figure 1 E-F).In a cross-sectional analysis, hits in TET2, DNMT3A and ASXL1 were found in 9 cases at baseline, with a higher clonal burden at sMN onset (p=.0001; Figure 1 G-H).The HLA mutational analysis showed the presence of somatic class I/II loci variants in 21% of sMN, found in only 13% of non-progressing AA cases (Figure 1L).We demonstrate that AA progression to sMN has distinct molecular characteristics. The presence of HLA mutations suggests that immune escape/selection may play a role, while the presence of GL predisposition variants shows that they not only may facilitate AA but also clonal evolution as described from classic congenital BMF.

C032

MECHANISMS OF RESISTANCE TO THE HYPOMETHYLATING AGENT AZACITIDINE IN MYELODYSPLASTIC SYNDROMES: FOCUS ON METHYLATION

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Hypomethylating agents (HMA) are standard of care for Myelodysplastic syndromes (MDS) but, still only ~50% of patients with MDS have a clinical response to HMAs and all of them will lose response after variable length of time. In this scenario, we aimed to investigate how and why some patients are primary resistant to AZA therapy while others initially respond and then relapse and develop a secondary resistance. We evaluated 22 cases of high-risk MDS, treated with the HMA azacitidine (75 mg/m^2/d for 7 days every 28 days). Bone marrow aspirates were collected before and after treatment with the drug. For some cases, samples at baseline, at remission and at relapse were available. DNA methylation in CD34+ cells were investigated by ERRBS (Enhanced Reduced Representation Bisulfite Sequencing). Thirteen cases were classified as primary resistant to AZA, 6 as responders and 3 as secondary resistant cases (patents who initially responded to the therapy and, after a variable length of time, relapsed). At present, complete methylation analysis is available for three sets of paired samples. 25.538 (Baseline *vs* Post treatment), 4.010 (Post treatment *vs* relapse), 127 (Baseline *vs* relapse) differentially methylated regions (DMRs) were identified. The majority of DMRs localize in intergenic and intronic regions. After treatment with AZA, global genome DNA methylation decreased due to the widespread hypomethylating effect of the drug, while at relapse methylation increased in specific genomic regions. 127 DMRs were identified between baseline and relapse samples, 96 were hypermethylated at baseline, while 31 at relapse.

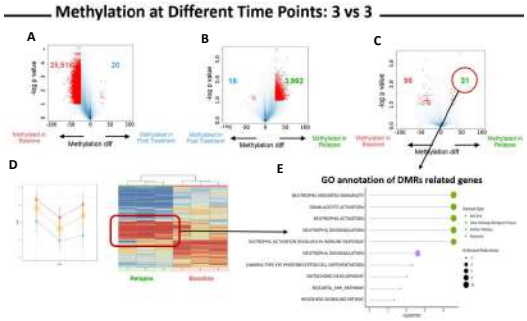

Fig. 1: (A) *Volcano plot illustrating methylation differences between baseline and post treatment only in paired samples. Mean methylation difference between the two groups is represented on the x axis and statistical significance (-log$_{10}$P value) on the y axis. Beta-binomial test* identified 25 538 *DMRs represented with red dots (FDR ≤ 0.1 and absolute methylation difference ≥ 25%).* **(B)** *Volcano plot illustrating methylation differences between post treatment and relapse in the three paired samples.* **(C)** *Volcano plot illustrating methylation differences between post treatment and relapse in the three paired samples.* **(D)** *Heatmap representing DMRs between baseline and relapse in the three paired samples. Samples at baseline are shown in red, after treatment in blue and at relapse in green. Representation of DMRs in heatmap with specific loci associated with different level of methylation (from low methylation in blue to high methylation in red).* **(E)** *Go annotation of DMR related genes with top enrichment numbers covering domains of higher methylated regions in the relapse group.*

Figure 1.

These 31 DMRs that gained methylation at relapse were different from

the baseline ones indicating a reprogramming of DNA methylation in CD34+ cells. Gene Ontology annotation of DMR-related genes between baseline and relapse, reveals an enrichment in biological process related to neutrophil and granulocyte pathways. An increased methylation in these pathways is correlated with a block in maturation and differentiation of neutrophils. This finding is very important because for the first time a methylation analysis has identified in relapsed cases that loss of response to AZA could be caused by emerging reprogrammed clones. Further analysis of these regions will help in understanding HMA resistance and relapse mechanisms.

C033

ABSTRACT WITHDRAWN

C034

A MULTI-CENTER EXPERIENCE OF HYPOCELLULAR MYELODYSPLASTIC SYNDROMES (H-MDS): FROM CLINICAL DESCRIPTION TO IMMUNOLOGICAL CHARACTERIZATION

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Background: Hypocellular Myelodysplastic Syndromes (h-MDS) are a rare subgroup of MDS, defined by a reduced age-adjusted bone marrow (BM) cellularity, irrespective of WHO classification. The distinctive features of h-MDS patients suggest a unique underlying pathogenesis and response to treatment, likely related to an immune-mediated damage, to the point that they are starting to be considered in a spectrum between aplastic anemia and normo/hypercellular MDS (n-MDS).

Aim: We aimed at comparing the clinical features, overall survival (OS) and treatment of h-MDS with respect to n-MDS. In a restricted number of cases, T and NK cell populations were investigated in h-MDS patients at diagnosis.

Methods: We compared 336 h-MDS and 1609 n-MDS enrolled in the national registry of the Italian Foundation of MDS (FISiM). T and NK cell characterization was performed by immunophenotypic and molecular analyses in both BM and peripheral blood (PB) samples of 12 h-MDS patients recruited within FISiM NK-hMDS protocol.

Results: No significant difference in median age, gender and R-IPSS distribution was observed. According to R-IPSS groups, lower-risk h-MDS had a median OS of 125months (m) *versus* 74m of n-MDS ($p< 0.001$). Differently, higher-risk h-MDS had a median OS of 19m, similar to 20m of n-MDS. Interestingly, immunosuppressive therapy was administered in a low proportion of cases of Lower Risk MDS, irrespective of BM cellularity. Prospective flow cytometry and T cell Receptor (TCR) rearrangement analyses revealed a clonal $CD3^+/CD8^+/CD57^+$ T cell expansion in 6/12 (50%) h-MDS patients; according to the R-IPSS, 5/6 (83%) belong to the Higher Risk. Moreover, 2/6 (33%) Higher Risk patients harbored a *STAT3* mutation. According to the pattern of Killer Immunoglobulin-like Receptors (KIR) expression, a restricted $CD3^-/CD16^{bright}/CD56^{dim\text{-}neg}$ NK cell expansion was found in 4/12 (33%) cases, 75% of them belonging to the Lower Risk.

Conclusion: Our analysis showed an advantage in OS in Lower Risk h-MDS, compared to n-MDS. Despite various guidelines recommend immunosuppressive agent for h-MDS, we revealed that the choice of therapy is not influenced by BM cellularity. Our data also revealed a peculiar association of T cell clonal expansions with Higher Risk and of NK cell expansion with Lower Risk R-IPSS h-MDS groups. This evidence highlights a potential prognostic role of T and NK cell clones in pathogenesis and controlling clonal HSC outgrowth, respectively.

C035

MARROW BLOOD EVALUATION OF T-LARGE GRANULAR LYMPHOCYTES (T-LGL) AND NK CELLS MAY HELP TO BETTER CHARACTERIZE MYELODYSPLASTIC SYNDROMES

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Several biological features are included among prognostic markers (PM) of Myelodysplatic syndromes (MDS), but a clear-cut definition of a personalized prognosis is still challenging. While T-Large Granular Lymphocyte Leukemia (T-LGLL) might be associated to MDS, a concurrent Chronic Lymphoproliferative Disorder of NK cells (CLPD-NK) has never been reported, NK cells (NKc) being usually scantly present in MDS bone marrow (BM). We aimed to characterize putative T-LGL and NKc expansions to identify potential new MDS PM. BM of 122 MDS patients collected at our Institution were analysed by flow-cytometry (F). T-LGL clonality was assessed by TCR gene rearrangement and NKc restriction was used as a surrogate of clonality by F. The presence of STAT3/STAT5b mutations was investigated by Sanger sequencing or allele-specific PCR. All results were correlated with clinical and lab data. Ogata score was calculated as reported (Leuk Res. 2018, 71:75-81). According to the Ogata score, MDS patients were classified as score 0 (8.3%), 1 (41.5%), 2 (68%), 3 (66.6%) or 4 (100%). Increased T-LGL or NKc were homogeneously distributed among the 5 Ogata groups. More specifically, an increase of T-LGL or NKc was detected in 31% and 20% of MDS cases, respectively and equally represented in high or low risk MDS according to IPSS and R-IPSS. Patients requiring 5-Azacytidine had T-LGL or NKc increases in 45% of cases; this proportion raised to 63% in untreated subjects. The two patients treated with immunosuppressive agents (IST) were also affected by T-LGLL. Clonal T-LGL expansions were found in 13% of MDS cases and were associated with lymphocytosis ($p<0.05$). Five % of STAT3 and no STAT5b mutations were identified. A Killer Immunoglobulin Receptors (KIR)-restricted NKc increase was identified in 8% of MDS patients, with one case satisfying criteria for the CLPD-NK diagnosis. These cells displayed a dominant memory phenotype ($CD56^{Dim}/CD16^{High}/CD57^+/CD62L^-$). Of note, the presence of restricted NKc correlated with excess of blasts (EB, $p<0.05$). For the first time we found that restricted NKc expansions might contribute to early identify MDS-EB1/2. In our cohort we also confirmed the concurrence of MDS and T-LGLL, these cases often needing IST therapy. Moreover, the high incidence of MDS diagnosis in the Ogata 1 group further underlines the need to define new PM. Altogether, a discrete characterization of T-LGL and NKc may help in the MDS diagnostic workflow.

Anemia and Erythrocyte Disorders

C036

ACTIVATE-T: A PHASE 3, OPEN-LABEL, MULTICENTER STUDY OF MITAPIVAT IN ADULTS WITH PYRUVATE KINASE DEFICIENCY WHO ARE REGULARLY TRANSFUSED

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Pyruvate kinase (PK) deficiency is a rare hereditary disease caused by reduced red blood cell PK (PKR) enzyme activity leading to defective glycolysis and decreased red blood cell (RBC) lifespan, resulting in lifelong hemolytic anemia. ACTIVATE-T (NCT03559699) was a global, multicenter, open-label, phase 3 study that evaluated the efficacy and safety of mitapivat, an investigational, first-in-class, oral, allosteric activator of PKR, in adult patients (pts) with PK deficiency who were regularly transfused. A 16-week (wk) dose escalation (5, 20, 50 mg BID) period (Part 1) was followed by a 24-wk fixed-dose period (Part 2). Key inclusion criteria: age ≥18 years (yrs), documented presence of ≥2 mutant alleles in PKLR (of which ≥1 was a missense mutation) and ≥6 transfusion episodes in the past 52 wks. Primary endpoint: reduction in transfusion burden, defined as ≥33% reduction in number of RBC units transfused during Part 2 compared with the pt's individual historical transfusion burden standardized to 24 wks. Pts who discontinued the study before completing 12 wks of treatment in Part 2 were considered non-responders. Secondary endpoints included transfusion-free responders (defined as no transfusions during Part 2) and annualized RBC units transfused.

Table 1.

Table: Primary and secondary efficacy endpoint outcomes with mitapivat (N=27)

Primary endpoint	Responders, n[d]	Responders, %	95% CI	1-sided p-value[e]
Reduction in transfusion burden[a]	10	37%	19–58%	0.0002
Secondary endpoint	**Responders, n[d]**	**Responders, %**	**95% CI**	
Transfusion-free[b]	6	22%	9–42%	
Secondary endpoint	**Historical mean, n**	**During study mean, n**	**Relative reduction**	
Annualized RBC units transfused[c]	16.63	11.38	39%	

[a]Defined as ≥33% reduction in number of RBC units transfused during Part 2, standardized to 24 wks, compared with historical transfusion burden standardized to 24 wks; [b]Defined as no transfusions during Part 2; [c]Defined as total number of RBC units standardized to 52 wks; [d]Pts who discontinued before completing 12 wks of treatment in Part 2 were considered non-responders; [e]Based on binomial exact test of H0: transfusion reduction response rate ≤10% vs. H1: transfusion reduction response rate >10% at a 1-sided α=0.025.
CI=confidence interval; pts=patients; RBC=red blood cell; wks=weeks

Twenty-seven pts were enrolled: mean age 36.6 yrs, 26% male, mean baseline Hb 9.2 g/dL. Of the 27 pts treated in the study, 20 (74%) completed Part 2, 6 (22%) discontinued treatment, and 1 was lost to follow-up. Ten (37%) pts treated with mitapivat achieved a ≥33% reduction in transfusion burden (1-sided p=0.0002; Table 1). Mean number of RBC units transfused (annualized) was 11.38 compared with 16.63 historically; relative reduction=39%. Six (22%) pts were transfusion-free during Part 2. Treatment-emergent adverse events (TEAEs) occurred in all pts. TEAEs grade ≥3 occurred in 8 (30%) pts. The most common TEAEs (any grade) were increased alanine aminotransferase (n=10; 37%) and headache (n=10; 37%). No TEAEs led to discontinuation of treatment. ACTIVATE-T is the first phase 3 study in pts with PK deficiency who are regularly transfused. The study demonstrated a significant decrease in transfusion burden, and 22% of pts were transfusion-free. No new safety signals were identified. These results support that mitapivat provides meaningful benefit to regularly transfused pts with PK deficiency and has the potential to become the first disease-modifying drug therapy approved for this condition.

C037

CTX001 FOR SICKLE CELL DISEASE (SCD): SAFETY AND EFFICACY RESULTS FROM THE ONGOING CLIMB SCD-121 STUDY OF AUTOLOGOUS CRISPR-CAS9-MODIFIED CD34+ HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS)

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Introduction: Elevated fetal hemoglobin (HbF) is associated with improved outcomes in patients (pts) with SCD. CTX001™ is a novel cell therapy using non-viral, *ex vivo* CRISPR-Cas9 gene editing in autologous HSPCs at the erythroid-enhancer region of *BCL11A* to reactivate HbF by reducing expression of BCL11A. Early data from pts with SCD infused with CTX001 showed increased total Hb, HbF, and F-cell pancellularity. No vaso-occlusive crises (VOCs) occurred after infusion. The safety profile was generally consistent with myeloablative conditioning. We present an interim data cut (Jan 28, 2021) of pts with SCD in CLIMB SCD-121 (NCT03745287) infused with CTX001 with ≥3 months (mo) of follow-up (f/u) (N=4).

Methods: Pts aged 12–35 years (ys) with severe SCD (≥2 VOCs/y requiring medical care in the prior 2 ys) were eligible. After mobilization with plerixafor, peripheral $CD34^+$ HSPCs were collected by apheresis and edited via a specific single-guide RNA and Cas9 nuclease. Before infusion, pts received busulfan myeloablation. We monitored for engraftment, hematopoietic recovery, adverse events (AEs), total Hb, HbF, hemolysis, F-cells, and VOCs.

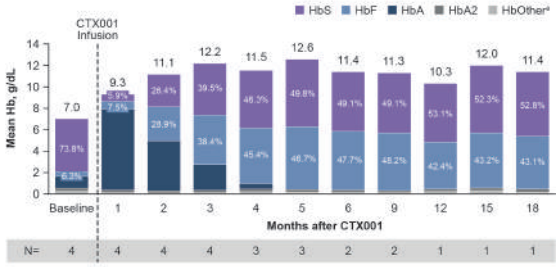
Results: Mean f/u was 10.1 mo (range 4.3–19.2). At baseline, pts had mean (SD) total Hb of 7.0 (1.6) g/dL, HbF of 6.3% (2.2%) of total Hb, and a mean of 5.3 severe VOCs/y in the prior 2 ys. After CTX001 infusion, median neutrophil engraftment occurred on Day 25.5 (range 17–30) and median platelet engraftment occurred on Day 31.5 (range 30–40). All 4 pts reported AEs, mostly mild/moderate in severity. The CTX001 safety profile was generally consistent with busulfan myeloablation. No serious AEs related or possibly related to CTX001 were reported. All pts showed increases in total Hb, HbF (Figure), and F-cell pancellularity. Mean %HbF rose to >30% by mo 3; all pts had %HbF >30% at the data cut. No pts had VOCs (up to 19.2 mo). Hemolysis markers (haptoglobin, lactate dehydrogenase, and total bilirubin) improved in all pts and normalized by mo 6.

Conclusions: These data confirm reports showing CTX001 increases total Hb and %HbF with F-cell pancellularity in pts with SCD, with efficacy up to 19.2 months of f/u. All pts were VOC-free from infusion to

analysis. The safety profile remained generally consistent with myeloablative conditioning and autologous hematopoietic stem cell transplantation. Results support continued investigation of CTX001 as a potential functional cure for pts with SCD.

Previously presented: EHA 2021

Figure. Mean Hb fractionation and total Hb in patients with SCD (N=4)



Hb, hemoglobin; HbA, adult hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin; SCD, sickle cell disease
Mean %HbF and %HbS of total mean Hb at each visit is indicated within the bars and mean total Hb (g/dL) is shown above each bar
Number of patients (N) with data available at each time point is noted
[a]Hb adducts and other variants

Figure 1.

C038

CTX001 FOR TRANSFUSION-DEPENDENT -THALASSEMIA (TDT): SAFETY AND EFFICACY RESULTS FROM THE ONGOING CLIMB THAL-111 STUDY OF AUTOLOGOUS CRISPR-CAS9-MODIFIED CD34+ HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS)

F. Locatelli,[1] S. Ailinca-Luchian,[2] Y. Bobruff,[2] M. Domenica Cappellini,[3] S. Corbacioglu,[4] J. Domm,[5] J. Foell,[4] J. de la Fuente,[6] R. Handgretinger,[7] T.W. Ho,[2] W. Hobbs,[8] A. Kattamis,[9] A. Li,[10] Y. Lu,[8] L. Palomino,[2] N. Shanbhag,[8] A. Sharma,[2] S. Sheth,[11] P. Sripakdeevong,[2] D. Wall,[12] H. Frangoul[5]

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Introduction: Elevated fetal hemoglobin (HbF) leads to improved outcomes in patients (pts) with TDT. CTX001™ is a novel cell therapy using non-viral, *ex vivo* CRISPR-Cas9 gene editing in autologous HSPCs at the erythroid-enhancer region of *BCL11A* to reactivate HbF by reducing expression of BCL11A. We present an interim data cut (Jan 21, 2021) of pts in CLIMB THAL-111 (NCT03655678) infused with CTX001 with ≥3 months (mo) follow-up (f/u) (N=10).

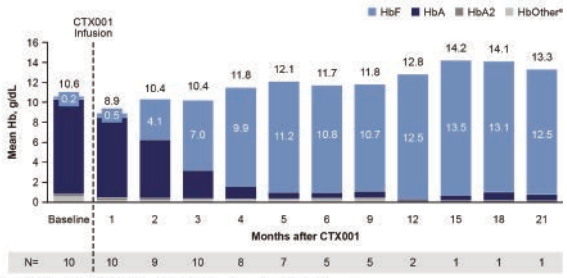
Methods: Pts aged 12–35 years (ys) with TDT receiving packed red blood cell (pRBC) transfusions of ≥100 mL/kg/y or ≥10 units/y in the prior 2 ys were eligible. After mobilization with G-CSF (filgrastim) and plerixafor, peripheral CD34+ HSPCs were collected by apheresis and edited via a specific single-guide RNA and Cas9 nuclease. Before infusion, pts received busulfan myeloablation. We monitored engraftment, hematopoietic recovery, adverse events (AEs), total Hb, HbF, F-cells, and pRBC transfusion.

Results: Mean f/u was 9.8 mo (range 4.3–23.8). 4/10 pts had severe genotypes. Mean (SD) baseline total Hb and HbF was 10.6 (2.2) and 0.2 (0.2) g/dL, respectively; pts received a mean of 14.7 pRBC transfusions/y. After CTX001 infusion, median neutrophil engraftment occurred on Day 30 (range 19–39); median platelet engraftment occurred on Day 38.5 (range 29–52). All pts reported AEs, mostly mild/moderate in severity. The CTX001 safety profile was generally consistent with busulfan myeloablation. 1 pt had 4 serious AEs related or possibly related to CTX001: headache, hemophagocytic lymphohistiocytosis (HLH), ARDS, and idiopathic pneumonia syndrome, all in the context of HLH; all resolved. Pts showed increases in Hb (Figure) and F-cell pancellularity. Pts stopped pRBC transfusions ≤2 mo after infusion and all were transfusion-free at analysis (≤23.8 mo total f/u).

Conclusion: We confirm that CTX001 increases total Hb and HbF in pts with TDT, with durability up to 23.8 mo f/u. Pts were transfusion-free ≤2 mo after infusion through analysis. Safety profile was generally consistent with myeloablative conditioning. Results support continued investigation of CTX001 as a potential functional cure for pts with TDT.

Previously presented: EHA 2021

Figure. Mean Hb fractionation and total Hb in patients with TDT (N=10)



Hb, hemoglobin; HbA, adult hemoglobin; HbF, fetal hemoglobin; TDT, transfusion-dependent β-thalassemia
Mean HbF (g/dL) is indicated within the light blue bars and mean total Hb (g/dL) is shown above each bar
Number of patients (N) with data available at each time point is noted
[a]Hb adducts and other variants

Figure 1.

C039

ELTROMBOPAG ADDED TO STANDARD IMMUNOSUPPRESSION IMPROVES RESPONSE RATE IN SEVERE APLASTIC ANEMIA: RESULTS OF THE MULTICENTER PHASE III PROSPECTIVE RANDOMIZED RACE TRIAL

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Introduction: A phase II study suggested that the addition of eltrombopag (EPAG) to standard immunosuppressive therapy (IST) by horse antithymocyte globulin (hATG) and cyclosporin (CsA) may improve out-

comes in severe aplastic anemia (SAA). We have compared hATG and CsA with or without EPAG in an open-label, phase III, investigator-driven, randomized trial enrolling treatment-naive SAA patients (clinicaltrials.gov, NCT02009747).

Methods: From July 2015 to April 2019, 197 patients stratified by disease severity and age were randomized to receive standard IST (hATG 40 mg/kg x4d and CsA 5 mg/kg/d) with (arm B) or without (arm A) EPAG at the dose of 150 mg/d from day +14. The primary endpoint was hematological complete response (CR) at 3m. Secondary endpoints included hematological response at 6m, clonal evolution, overall survival (OS), event free survival, and somatic myeloid mutations. The study was powered to detect an increase in CR from 7% in arm A to 21% in arm B at 3m.

Results: One-hundred-one and 96 patients were randomized to arm A and arm B, respectively. Baseline characteristics were comparable between the 2 arms, including median age (52 and 55 years in arms A and B), age stratum (age <40 was 35.6% in arm A and 30.2% in arm B), disease severity (vSAA was 33.7% in arm A and 35.4% in arm B), and presence of a PNH clone (59.2% in arm A and 45.2% in arm B). Median follow-up was 24 months. The primary endpoint was reached with 3m CR rates of 9.9% and 21.9% in arms A and B (pooled Odds Ratio 3.2, p=0.012). Overall response rates were 31.7% and 59.4%, respectively (p<0.0001). At 6 months, overall response rate was 40.6% in arm A *vs* 68.4% in arm B (OR:3.8; p<0.0001). SAE were comparable in both arms. Eight patients came off study prematurely in arm A and 7 in arm B requiring second-line transplantation. One patient in arm A and 2 patients in arm B experienced clonal evolution. High sensitivity NGS analysis was performed using a 31 gene target molecular bar coded panel. At baseline, samples from 121 patients showed no difference in term of somatic myeloid mutations (VAF >1% 38.2% in arm A *vs* 36.6% in arm B). Follow up samples were analyzed from 121 and 53 patients at 6 and 24 months, respectively. During the study, 22 patients died (14 in arm A, OS of 83.2% at 24m and 8 in arm B, OS 86.3% at 24m) (p=0.142).

Conclusions: Standard IST+EPAG improves rate, speed and quality of hematological response in treatment-naïve SAA patients with no additional toxicity.

C040

FINAL RESULTS OF THE PHASE 3 STUDIES NORTHSTAR-2 AND NORTHSTAR-3 IN PATIENTS WITH TRANSFUSION-DEPENDENT β-THALASSEMIA (TDT): SUBGROUP ANALYSIS OF ITALIAN PATIENTS TREATED WITH BETIBEGLOGENE AUTOTEMCEL (BETI-CEL), LENTIGLOBIN FOR THALASSEMIA

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After beti-cel gene therapy in the phase 3 Northstar-2 (NCT02906202; non-β^0/β^0 genotypes) and Northstar-3 (NCT03207009; β^0/β^0, $\beta^0/\beta^{+IVS\text{-}I\text{-}110}$ or $\beta^{+IVS\text{-}I\text{-}110}/\beta^{+IVS\text{-}I\text{-}110}$ genotypes) studies, transfusion independence (TI) was achieved by 30/34 (88.2%) evaluable patients, including 6/7 (85.7%) patients with β^0/β^0 genotypes and 24/27 (88.9%) patients with non-β^0/β^0 genotypes (IVS-I-110 genotype, n=8). Here, we present pooled results of the Italian patients (n=10) who were enrolled in these studies. CD34+ hematopoietic stem cells collected via mobilization/apheresis were transduced with BB305 lentiviral vector, using a refinement of the phase 1/2 study manufacturing process. Patients were infused with transduced cells after PK-adjusted, single-agent busulfan myeloablation administered over 4 days (in 4 refracted doses/day) and followed for 24 months. Data presented as median (min–max). The Table 1 shows baseline patient and treatment characteristics. All 10 patients achieved and maintained TI (TI=weighted average haemoglobin [Hb] >9 g/dL without pRBC transfusions for 12 months). Weighted average Hb during TI was 11.8 (9.7–13.0) g/dL. Last pRBC transfusion post-infusion was at 1.1 (0.7–1.9) months. Duration of ongoing TI is 21.1 (19.4–32.0) months. At study end (Month 24), total unsupported Hb was 12.3 g/dL (9.6–13.2) (n=10), mainly driven by HbA^{T87Q} at 9.4 g/dL (5.0–10.8) (n=10). Markers of erythropoiesis, such as levels of soluble transferrin receptor, serum ferritin, and hepcidin improved after treatment with beti-cel, tending towards normal levels. Bone marrow evaluations for myeloid:erythroid ratio showed improved 12-month outcomes versus baseline in all evaluable patients, indicating improvement in erythropoiesis. Post-infusion non-hematologic grade ≥3 adverse events (AEs) were stomatitis (n=7), pyrexia (n=1), hypertransaminasaemia (n=1) and epistaxis (n=1). Neither veno-occlusive liver disease nor drug product-related AEs were reported. All patients are alive and had polyclonal vector integration; no integration site contributed >3% of all integration sites at last assessment (n=10). Beti-cel gene therapy is a potentially curative treatment option in TDT: all Italian patients across a wide range of severity and age achieved and maintained transfusion independence with median Hb levels of 12.3 at study end (24 months). The treatment regimen had a safety profile consistent with busulfan myeloablation.

Table 1.

Baseline patient and treatment characteristics (median [min-max], unless otherwise noted)

Parameters			N = 10
Age at consent, median (min—max) years			**15 [7–23]**
< 12 years, n			2
≥ 12 — < 18 years, n			6
≥ 18 years, n			2
Male, n (%)			7 (70)
Liver iron concentration†			6.1
Median (min—max), mg Fe/g dw			[1.2–16.8]
Cardiac T2*			36.2
Median (min—max), msec			[23.3–42.4]
Splenectomy, n (%)			1 (10)
Genotypes			
Genotypes, n	β^+/β^0	4	IVS-1-6 / Codon 44 (-C) (n=2) IVS1-NT6 / IVS1-NT1 (n=1) IVS-I-110 / Codon 39 (C>T) (n=1)
	β^+/β^+	4	IVS-I-110 / IVS-II-745 (n=2) IVS-I-110 / IVS-I-110 (n=1) IVS-I-6 / IVS-II-745 (n=1)
	β^E/β^0	1	Glu>Lys CAG>AAG / IVS-1-1
	β^0/β^0	1	Codon 39 (C>T)
Pre-study pRBC transfusion history Retrospective data 2 years prior to enrollment			**N = 10** Median (min–max)
Volume, mL/kg/yr			192.5 [165.6–240.5]
Number, transfusion episodes/yr			20.5 [17.5–37.0]
Pre-transfusion Hb, g/dL			9.5 [8.8–9.9]
Conditioning and engraftment characteristics			
Estimated daily average AUC over 4 days, µM*min			5695 [3708–8947]
Neutrophil engraftment, ANC ≥ 500 cells/µL x 3 days, days			28 [19–38]
Platelet engraftment, ≥ 20,000 platelets/µL x 3 days, days			50 [33–80]

†5/10 patients had LIC > 7.0 mg Fe/g dw
Hb, hemoglobin; LIC, liver iron concentration; pRBC, packed red blood cells
Data as of 30 November 2020

Non Hodgkin Lymphoma 3

C041

RESULTS OF THE IELSG32 TRIAL AT A MEDIAN FOLLOW-UP OF 88 MONTHS DEMONSTRATE THAT MATRIX FOLLOWED BY AUTOLOGOUS TRANSPLANT IS ASSOCIATED WITH EXCELLENT SURVIVAL AND NEUROTOLERABILITY IN PATIENTS WITH PRIMARY CNS LYMPHOMA

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Introduction: The MATRix regimen (methotrexate, cytarabine, thiotepa, rituximab) significantly improved outcome of pts with primary CNS lymphoma (PCNSL) enrolled in the IELSG32 trial. At a median follow-up of 40 months, both whole-brain irradiation (WBRT) and autologous transplantation (ASCT) were safe and equally effective. However, sound assessment of OS, late complications, incidence of secondary tumors, and cognitive impairment requires longer follow-up. Herein, we report the results of IELSG32 trial at a median follow-up of 88 (IQR 77-99) months.

Methods: pts with untreated PCNSL (18-70 years) were randomly assigned to methotrexate-cytarabine (arm A), or arm A + rituximab (arm B), or arm B + thiotepa (MATRix; arm C). A second randomization assigned pts with responsive/stable disease after induction to WBRT (arm D) or BCNU-Thiotepa-conditioning ASCT (arm E). Treatment effect on cognitive functions and quality of life (QoL) were addressed by IPCG tests panel and EORTC-QLQ.

Results: 219 pts were randomized (arm A 75; B 69; C 75). After induction, 167 had responsive/stable disease: 118 were assigned to WBRT (59) or ASCT (59) while 49 were excluded from 2nd randomization. Fifteen pts died of iatrogenic toxicity; 87 (40%) pts remain relapse-free (A 17; B 28; C 42), 14 of them died of unrelated causes (Table 1). Among 117 relapsing pts, 96 died of PCNSL, 7 of salvage therapy complications. Eight pts developed second cancers at 48-96 months from WBRT (5) or ASCT (3). Second tumors and deaths in relapse-free pts or during salvage were not significantly related to treatments (Table 1). Neuropsychological tests showed a significant impairment in attentiveness and executive functions in the WBRT arm, while transplanted pts had a significant improvement in these functions as well as memory and QoL. Pts treated with MATRix showed significantly better PFS (7-year: 20% arm A; 29% arm B; 52% arm C) and OS (7-year: 26% arm A; 37% arm B; 56% arm C). No significant differences were seen between the consolidation arms for either PFS (7-yr: 55% arm D; 50% arm E) or OS (7-yr: 63% *vs* 57%). Pts treated with MATRix and consolidation had a 7-yr OS of 70%, without a difference between WBRT and ASCT.

Conclusions: MATRix was linked to excellent long-lasting outcome. WBRT and ASCT have comparable efficacy. MATRix and ASCT did not result in higher non-relapse mortality or second tumors onset. WBRT led to impairment of specific cognitive functions.

Table 1.

IELSG 32 study of PCNSL. Late complications by arm in patients who completed the planned treatment

	Arm A (N=42)	Arm B (N=50)	Arm C (N=63)	WBRT* (N=70)	ASCT* (N=60)
Second tumors[a] (n=8)	1 (2%)	2 (4%)	5 (8%)	5 (7%)	3 (5%)
Deaths in relapse-free patients[b] (n= 14)	2 (5%)	6 (12%)	6 (9%)	9 (13%)	3 (5%)
Deaths during salvage treatment (n=7)	4 (9%)	0 (0%)	3 (5%)	2 (3%)	2 (3%)

*Actually delivered consolidation regardless of random allocation.
[a]Acute erythroid leukemia, high-grade glioma, melanoma (2), Paget's disease of the breast, prostate cancer, colon cancer, basal cell carcinoma; the first two were lethal, the other six remained relapse-free after surgical resection.
[b] Causes of deaths in relapse-free patients were: infections (4), sudden death (4), cognitive decline (3), second tumor (2), and car accident (1).

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C042

AN ITALIAN MULTICENTER RETROSPECTIVE OBSERVATIONAL STUDY TO ASSESS THE CLINICAL CHARACTERISTICS AND THE OUTCOME OF PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA TREATED WITH IDELALISIB IN EVERYDAY CLINICAL PRACTICE

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Although current approaches to relapsed/refractory (RR) follicular lymphoma (FL), including newer agents as the PI3K inhibitor idelalisib, have greatly improved the outcomes of affected patients, this disease is still characterized by high relapse rates, with most patients eventually dying of lymphoma progression. Observational studies are essential for the continuing monitoring of drugs. Data from patients treated with idelalisib outside a controlled clinical trial could give additional information about the clinical use, treatment duration, effectiveness, and toxicity of the drug given to RR FL patients in a real life context. To this aim, an Italian multicentric observational retrospective study was conducted in 19 hematology centers. We herein report the results of a multicentric, retrospective observational study conducted on 72 heavily pre-treated FL patients who received idelalisib after a median of 3 previous lines of therapy (range 1-10). Fifteen patients (20.8%) were refractory to the last treatment before idelalisib. After a median of 4 months of treatment (range 1-40), 30 patients (41.7%) obtained an objective response with 20.8% of complete responses (CR, 15/72 patients); 31 patients experienced a progression of disease (PD, 43%). Median progression-free survival and overall survival (OS) were reached at 8.4 months and 4 years, respectively; OS was 37.6% after 5 years of follow-up. Drug-related toxicities were observed in 32 patients (44.4%), 24 of which (33.3%) had to discontinue idelalisib for this reason. Twenty-six adverse events (36.1%) were grade 3 or higher; one patient died due to severe pneumonia. Seven patients (2 CR, 2 partial responses, 2 PD, 1 stable disease) underwent stem cell transplantation (SCT), 3 of them receiving an autologous SCT (ASCT) while the remaining 4 patients proceeded to allogeneic SCT. The 3 patients who underwent ASCT are still in CR after 24,1, 15,4 and 7 months, respectively, since the first documentation of response. These results confirm that idelalisib represents a valid treatment alternative for patients with pre-treated FL, even following multiple lines of therapy. The drug can also have a role as a bridge to transplantation in young and fit patients who fail standard salvage chemotherapy approaches.

C043

A COMPLETELY GENETIC PROGNOSTIC MODEL OVERCOMES CLINICAL PROGNOSTICATORS IN MANTLE CELL LYMPHOMA: RESULTS FROM THE MCL0208 TRIAL FROM THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction: TP53 and KMT2D disruptions, as well as high risk MIPI-c class were independent prognosticators for younger mantle cell lymphoma (MCL) patients enrolled into the FIL MCL0208 trial and were thus integrated in the MIPI-g prognostic model. Furthermore, MCL is characterized by many copy number variations (CNVs), whose clinical impact is not clearly understood. This study aimed at refining the MIPI-g model by incorporating CNVs.

Methods: DNA from bone marrow CD19+ sorted cells was profiled with the Illumina HumanOmni2.5 array in 165 patients. Minimal common regions were identified by a bioinformatic pipeline and smaller regions with the GISTIC algorithm.

Results: 351 CNVs were identified in at least one patient. Besides TP53 deletion 10 further CNVs showed predictive by univariate analysis and were selected for multivariate Cox modelling. Actually, 4 CNVs maintained independent association with PFS: Loss@chr22 in 10/165 (6%) patients with a HR of 4.14 (p=0.028), LOH@chr17 in 5 (3%) with a HR of 4.79 (p=0.010), HDEL@chr9 in 3 (2%) with a HR of 18.1 (p=0.001) and CDKN2A loss in 30 (18%) with a HR of 2.6 (p=0.002). By using the same approach employed for the MIPI-g, we assigned a score to each single predic-tor based on the Cox regression analysis. KMT2D mutations (HR 2.43, 95% CI 1.21-4.87, p=0.012) TP53 disruptions (HR 2.63, 95% CI 1.38-5.02, p=0.003) and 4CNVs (HR 2.56, 95% CI 1.43-4.61, p=0.002) had superimposable HRs for PFS and thus scored 1 point. Interestingly, MIPI-c high risk class lost its independent prognostic value (HR 1.42, 95% CI 0.65-3.12, p=0.382), thus scoring 0 points. Consequently, a novel "genetics-only" model was devel-oped, with patients grouped into 3 risk classes: i) 0 points, low risk group (LR=97, 60%); ii) 1 point, intermediate risk (IR=41, 26%); iii) >2 points, high risk (HR=22, 14%). 3-years PFS for LR, IR and HR was 86%, 50% and 24%, re-spectively (p<0.0001) (Figure 1A). The novel genetic score improved the model discrimination ability, with a C-statistics of 0.715 as compared to 0.675 for MIPI-g. Interestingly, this model was highly promising in terms of OS, too: actually, 3-years OS for LR, IR and HR was 92%, 74% and 59%, respectively (Figure 1B).

Discussion: The inclusion of 4CNVs into the MIPI-g allowed the development of a completely molecular model that improved the stratification in MCL and identified addi-tional primary refractory patients or destined to an early relapse after high dose chemotherapy and ASCT.

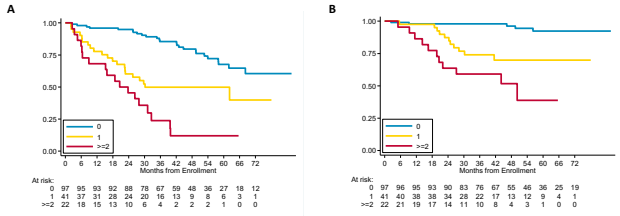

Figure 1.

C044

SAFETY AND EFFICACY OF ZANUBRUTINIB IN PATIENTS WITH RELAPSED/REFRACTORY MARGINAL ZONE LYMPHOMA (MAGNOLIA PHASE 2 STUDY)

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Background: Zanubrutinib is a potent, specific next-generation BTK inhibitor with higher selectivity for BTK compared with TEC- and EGFR-family kinases, which may be related to off-target toxicities.

Aim/Objective: The objective of this abstract is to present initial efficacy and safety results of zanubrutinib in patients (pts) with relapsed/refractory marginal zone lymphoma (R/R MZL) enrolled in the MAGNOLIA study (BGB-3111-214; NCT03846427).

Methods: In this single-arm, multicenter study, adults with R/R MZL who had received ≥1 prior therapy including at least one CD20 antibody regimen were treated with zanubrutinib 160 mg twice daily until disease progression or unacceptable toxicity. The primary endpoint was overall response rate (ORR) by independent review committee (IRC). Secondary endpoints included investigator-assessed (INV) ORR, duration of response (DOR), progression-free survival (PFS), and safety.

Table 1. Efficacy and Safety Outcomes in R/R MZL.

Efficacy (investigator assessment)	**(N=66)[a]**
ORR, n (%) [95% CI]	49 (74) [62, 84]
Complete response	16 (24)
Partial response	33 (50)
Stable disease[b]	11 (17)
Progressive disease	5 (8)
Discontinued study before first assessment	1 (2)
Time to response (months), median (range)	2.8 (1.7, 8.5)
Safety[c]	**(N=68)[d]**
Any AE, n (%)	65 (96)
Grade ≥3 AE, n (%)	26 (38)
Serious AE, n (%)	25 (37)

[a] Efficacy-evaluable set: pts who received at least one dose of study drug and with centrally-confirmed diagnosis of MZL (two pts were excluded due to MZL transformation to diffuse large B-cell lymphoma).
[b] Three pts with stable disease were continuing on study treatment.
[c] Treatment-emergent AEs.
[d] Safety analysis set: all pts who received at least one dose of study drug.
Abbreviations: AE, adverse event; MZL, marginal zone lymphoma; ORR, overall response rate; pts, patients; R/R, relapsed/refractory.

Results: As of January 11, 2021, 68 pts were enrolled and treated. Median age was 70 years (range, 37-95), with 28% aged ≥75 years. MZL subtypes included extranodal (38% of pts), nodal (38%), splenic (18%), and indeterminate (6%). Median number of prior therapies was 2 (range, 1-6), and 32% of pts had disease refractory to last therapy. Median duration of drug exposure was 59.1 weeks (range, 3.7-84.1). At a median follow-up of 15.5 months (range, 1.6-21.7), INV ORR was 74% with a complete response rate of 24%. Responses were observed in all subtypes, with an ORR of 68%, 84%, 75%, and 50% in extranodal, nodal, splenic, and indeterminate subtypes, respectively. Median DOR and PFS were not reached. IRC review is ongoing. Twenty-eight (41%) pts discontinued treatment (20 due to disease progression; 4 due to adverse events [AEs]). The most common treatment-emergent AEs reported in ≥10% of pts were diarrhea (22%), bruising (21%), and constipation (15%). Neutropenia was the most common grade ≥3 AE (10%). All-grade AEs of interest included neutropenia (13%), thrombocytopenia (13%), atrial fibrillation/flutter (3%), and hypertension (3%). AEs leading to treatment discontinuation included fatal COVID-19 pneumonia (n=2), fatal myocardial infarction in one pt with pre-existing coronary artery disease, and pyrexia attributed to disease transformation. No major/serious hemorrhage was reported. No AEs led to dose reductions.

Conclusions: Zanubrutinib demonstrated high response rates and durable disease control with a favorable safety profile in pts with R/R MZL.

C045

LONG TERM CYTOPENIA AND INFECTIONS IN PATIENTS TREATED WITH ANTI-CD19 CAR T-CELLS: AN ANALYSIS OF BONE MARROW AND CLINICAL RISK FACTORS

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During follow-up after the administration of Chimeric antigen receptor (CAR) T-cells therapy targeting CD19, patients can have long-lasting cytopenias and infections, the causes of this late toxicity have not yet been clearly explained. We investigated the frequency of cytopenias and infections after CAR T-cells infusion and whether clinical characteristics, laboratory data and bone marrow (BM) function could be associated to the risk of long term toxicity. Consecutive patients receiving CAR T-cells at Fondazione IRCCS Istituto Nazionale dei Tumori in Milano between 2019 and 2020 with at least 3 months of follow-up were included. Clinical and laboratory data were collected from electronic medical records; patients with persistent severe cytopenia (*i.e.* ANC < 1000/ul and/or PLT < 50000/ul) at 3 months of follow-up underwent BM biopsy, cytogenetic, FISH analysis and NGS studies to investigate clonal hematopoiesis. Forty-six patients receiving anti-CD19 CAR T-cells affected by DLBCL (n=34) and PMBCL (n=12) were included. Median age was 49 years (range, 21-72) and median follow-up was 9 months (range, 1-21). Four patients died before 3 months of follow-up. In the first 3 months we observed 21 infectious events in 15 of 42 patients (33%). Cytokine Release Syndrome (CRS) of any grade, the use of steroids and tocilizumab were significantly associated with an increased risk of infections at 3 months. Severe cytopenia was observed in 11 of 42 (26%) of patients at 3 months and in 9/31 (29%) at 6 months. Neutropenia before lymphodepletion, a peak of ferritin after CAR T-cells > 5 times upper value (*i.e* > 1375 ng/ml) and female sex were significantly associated with a higher incidence of cytopenia at 3 months whereas neutropenia before lymphodepletion was associated with cytopenia at 6 months. Three months after CAR T-cells BM examination was performed in 6/11 of patients with severe cytopenia and showed dysmyelopoiesis (n=1), hypocellular marrow (n=3) and normal morphology (n=2), cytogenetics and FISH were normal. NGS study was performed in 4 patients showing a clonal hematopoiesis of indeterminate potential (CHIP) due to DNMT3A mutations with a VAF of 2% and 9% in two of them. CRS and consequent treatment seemed to be factors predisposing to infections whereas cytopenia seemed to be associated to pre CAR T-cells neutropenia or high ferritin peak. Neither age >65 years nor number of chemotherapy regimens before CAR T-cells were associated with cytopenia or infections.

Hemostasis, Thrombosis, Thrombocytopenia and Platelet Diseases

C046

FREQUENCY AND RISK FACTORS OF THROMBOSIS IN ACUTE MYELOID LEUKEMIA TREATED WITH INTENSIVE CHEMOTHERAPY. AN OBSERVATIONAL STUDY

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Thrombosis is common in cancer but its frequency in acute myeloid leukemia (AML) has been evaluated in a few reports only and no validated predictive model is currently available. We performed a retrospective observational study in newly diagnosed adult AML patients (pts) treated with intensive chemotherapy between January 2013 and February 2020, to evaluate the frequency and the potential predictive factors of thrombosis. 222 pts were included, with a median age of 60 years, 21% in the ELN2010 adverse risk category. With a median follow-up of 44 months, we observed 50 thrombotic events (90% were venous, VTE). Twenty-eight pts (62% of VTE) had catheter-related thrombosis (CRT), 93% of which were peripherally inserted central catheter; in 6 and 5 case VTE occurred in lower and upper extremity, respectively; 3 pts experienced pulmonary embolism. Among arterial thromboses, we observed 2 myocardial infarctions and 3 cerebral vascular accidents. The prevalence of thrombosis was 23% and 6-months, 1-year and 2-year cumulative incidence was 10%, 22% and 25%, respectively. The median time to thrombosis was 84 days and 52% of the events occurred within 100 days from AML diagnosis, during induction/consolidation chemotherapy or just before starting treatment. History of VTE (p=0.005) and baseline platelet count higher than $100x10^9$/L (p=0.036) significantly increased the risk of thrombosis, as confirmed by multivariate analysis (OR=5.5, 95%IC 1.2 -2 4.5, p=0.026 and OR=2.2, 95%IC 1.1 – 4.2, p=0.02, respectively). AML genetic profile did not affect thrombotic risk. Khorana and DIC score failed to stratify pts according to their thrombotic risk. Results were confirmed considering only thromboses occurring within day 100 from diagnosis. In a subgroup analysis excluding CRT, ELN Intermediate-1 risk group was significantly associated with thromboses (p=0.039), especially FLT3-ITD/NPM1 mutated pts. No impact of thrombosis on survival was observed, while DIC score at diagnosis was independently associated with reduced survival (p=0.004) by multivariate analysis. Thromboses are a frequent complication in AML, but we did not show an impact on survival. Previous VTE and baseline platelet count could predict thrombotic risk, while AML genetic profile did not. Khorana score is not a robust tool in this setting, and we could not validate the association of DIC score with thromboses, warranting further studies on the subject to better predict thrombosis occurrence.

C047

CLINICAL AND HISTOLOGICAL DATA MAY PREDICT RESPONSE TO SPLENECTOMY IN ITP PATIENTS

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Introduction: Primary immune thrombocytopenia (ITP) is an acquired immune-mediated disorder characterized by isolated thrombocytopenia. Only a minority of patients will experience remission after the first episode with up to 70% of patients developing chronic ITP. Splenectomy remains the most effective therapy for ITP, inducing long-lasting remissions in up to 70% of patients. However, alternative effective treatments, the potential complications of surgery and the inability to predict response are factors limiting splenectomy as therapeutical approach to refractory cases.

Aim: The aim of this study was to: (i) define histological characteristics and the immunological microenvironment of the spleens of ITP patients; (ii) identify clinical-pathological predictors of response to splenectomy.

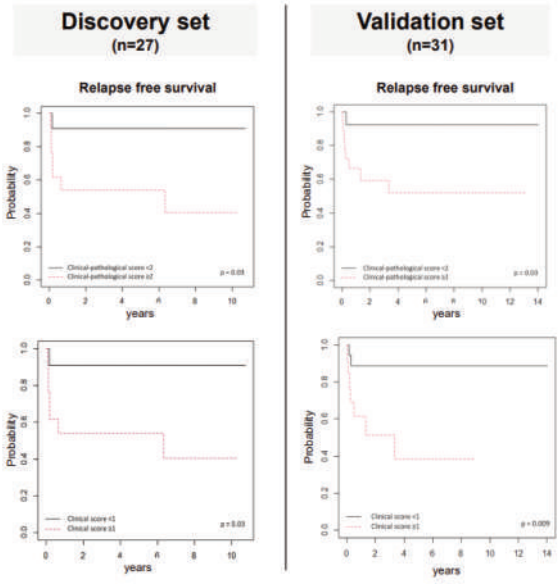

Figure 1. Disease free survival curves of the discovery set and validation set. The curves stratifying patients according to clinical-pathological risk factors included the following variables: (i) age at surgery >45 years; (ii) concurrent autoimmune diseases; (iii) low splenic T follicular help cell density; (iv) low splenic marginal zone width.

Material and Methods: Fifty-eight adults with ITP who underwent splenectomy were considered for the study. Clinical and laboratory data were available for all patients. With regard to histological and immunological features the following parameters were considered: (i) density of lymphoid follicles (FL), (ii) presence of reactive germ centers (GCs), (iii) density of marginal zones (MZ), (iv) follicular helper T cell density, (v) thickness of T-cell perivascular covers, (vi) cytotoxic T cell density and (vii) red pulp characteristics (*i.e.* sine and histiocyte density; presence of extramedullary hematopoiesis). Clinical (autoimmune diseases; age of diagnosis) and histological data were correlated with the post-splenectomy response to assess its role as predictors of surgical outcome.

Results: We identified 3 histological patterns: (i) presence of white pulp hyperplasia (many secondary FL with well-developed MZ), (ii) pattern of non-activated white pulp (no GC and secondary FL), and (iii) presence of white pulp lymphoid depletion (marked FL atrophy and T perivascular covers). Post-splenectomy ITP recurrence was significantly

associated with: (i) age at diagnosis; (ii) presence of autoimmune comorbidities; (iii) low density of follicular helper T cells in FL; (iv) marginal zone expansion. (Figure 1). The combination of these parameters identified groups of patients with different risk of post-splenectomy recurrence.

Conclusions: Specific clinical and histological parameters, including microenvironment immunological features, may help to predict the response to splenectomy.

C048

EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN EVANS SYNDROME: A SINGLE CENTER SERIES

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Evans syndrome (ES) is defined as the presence of two autoimmune cytopenias, including autoimmune hemolytic anemia and immune thrombo-cytopenia (ITP). Thrombopoietin receptors agonists (TPO-RA), romiplostim (ROMI) and eltrombopag (EPAG), are effective in primary ITP but have never been systematically studied in ES. We therefore assessed the efficacy and safety of TPO-RA in patients with ES treated in our centre. Base-line hematologic parameters, associated conditions, previous/concomitant treatments were registered. The time from diagnosis to first TPO-RA was collected. Response rates were evaluated at 1, 3, 6, and 12 months and classified as partial (PR) or complete (CR), for platelets >30x10^9 or >100x10^9/L, respectively. Treatment emergent adverse events (TEAE) were registered and graded according to CTCAE. Eleven patients have been evaluated (table): 4 males and 6 females, median age at the start of TPO-RA was 55.6 years (24-85); six suffered from secondary ES (2 primary immune deficiencies, 2 antiphospholipid syndromes, 1 post allogenic stem cells transplant, and 1 lymphoproliferative disease). All pa-tients had received steroids +/- IVIG, and the majority (N=7) at least one further line. The median time from diagnosis to TPO-RA was 25.8 months (0.6-360.4).

Table 1.

Pt	Gender	Age at TPO-RA (years)	Associated condition	Time to TPO-RA (months)	Previous therapy lines	TPO-RA type	Concurrent Therapy	PLT pre-TPO-RA	R-M1	R-M3	R-M6	R-M12	AE Grade (type)	STOP (reason)
1	M	24	PID	16.6	steroid, rituximab, IVIG	Eltrombopag	-	29	PR	CR	PR	PR	-	NO
2	F	50	PID	1.5	Steroid, IVIG	Eltrombopag	Danazol	41	CR	NR	CR	CR	-	YES (CR)
3	F	78	LPD	10.2	Steroid, rituximab	Eltrombopag	Danazol	11	NR	NR	NR	CR	-	NO
4	F	62	-	86.3	Steroid, rituximab, IVIG, splenectomy	Romiplostim	Steroid, rituximab	7	NR	NR	-	-	-	YES (NR)
						Eltrombopag	Steroid	5	NR	CR	CR	CR	II (DVT)	YES (thrombosis)
5	F	67	-	39	Steroid, rituximab, danazol, IVIG	Eltrombopag	Steroid	31	PR	PR	NR	PR	II (BMF)	YES (BMF)
6	F	58	-	360.4	Steroid, IVIG	Eltrombopag	Steroid	45	PR	CR	CR	CR	-	YES (CR)
7	M	85	-	25.8	Steroid, IVIG	Eltrombopag	Steroid	31	CR	CR	CR	-	-	NO
8	F	62	-	0.6	Steroid, IVIG	Eltrombopag	Steroid, IVIG	2	CR	CR	CR	CR	-	YES (CR)
9	M	35	HSCT	1.9	Steroid, rituximab, IVIG	Eltrombopag	Steroid, IVIG	3	NR	NR	NR	-	-	YES (NR)
						Romiplostim	Steroid, IVIG, splenectomy	12	NR	-	-	-	-	NO
10	M	54	APS	156.2	Steroid, IVIG, splenectomy, rituximab, azathioprine, danazol	Romiplostim	Steroid	8	CR	-	-	-	-	YES (CR)
						Eltrombopag	IVIG, danazol		CR	CR	CR	PR	IV (AMI,CVT)	YES (thrombosis)
11	F	37	APS	204	Steroid, IVIG, splenectomy, azathioprine	Eltrombopag	Steroid, azathioprine	23	CR	CR	CR	CR	III (splancnic thrombosis)	YES (thrombosis)

R-M1-12, response at month 1-12; PR, partial response; CR, complete response; NR, non response; DVT, deep venous thrombosis; BMF, bone marrow fibrosis; AMI, acute myocardial infarction; CVT cerebral vein thrombosis.

Response rates to the first TPO-RA (9 EPAG and 2 ROMI) were 72% at month 1 (45% CR, 27% PR, N=11), 60% at month 3 (50% CR, 10% PR, N=10); 66% at month 6 (55% CR 11% PR, N=9); 100% at 12 months (71% CR, 28% PR, N=7). Notably, 91% of patients required concomitant therapies including steroids +/- IVIG (N=10), danazol (N=3), rituximab, splenectomy and azathioprine (N=1 each). Three patients switched to the alternative TPO-RA (2 ROMI to EPAG and 1 vice versa) mainly because of no response (NR) and 2 responded. Four patients (36%) developed at least one TEAE: 3 venous thromboses (1 deep venous thrombosis, 1 cerebral vein thrombosis CVT, and 1 splanchnic thrombosis) and 1 acute myocardial infarction (in the same patients experiencing CVT). Seven patients (63%) stopped TPO-RA for persistent CR (N=3), thrombosis (N=3), or increased bone marrow reticulin fibrosis (N=1). TPO-RA were effective in about 70% of ES patients even if the majority required concur-rent therapies. Moreover, TPO-RA use was complicated by a high occurrence of thrombotic events (possibly favored by underlying conditions). These findings highlight that in ES bone marrow stimulation alone may be not enough to control disease.

C049

GLOBAL HAEMOSTASIS ASSAYS AND ACUTE LEUKEMIA: RESULTS OF AN OBSERVATIONAL STUDY

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Introduction: Acute leukemia (AL) is characterized by a complex spectrum of coagulopathy ranging from bleeding to thrombotic risk, varying according to disease phases and treatments. To date platelet count (PC) and conventional coagulation tests (CCTs) cannot predict and/or guide therapy in acute hemorrhages and are unable to predict thrombotic risk in AL. Thromboelastography (TEG) is a global haemostatic test that measures the viscoelastic properties of the clot, thus providing information on the entire process of blood coagulation. This was a prospective study of newly diagnosed AL adult (> 18 years) patients treated with first line of chemotherapy (CHT). Primary aim was to asses with TEG haemostatic balance from diagnosis to the end of CHT.

Table 1. Patient Demographics and Clinical Characteristics.

N° patients	40
Age — yr mean (range)	62 (18-80)
Sex F/M — no. (%)	18 (53)/22(55)
Time of follow-up –days (mean,range)	31 (7-69)
Diagnosis — no. (%)	
• AML-NOS	27 (67)
• FLT3 mutated	3 (11)
• AML-NMP1 mutated	2 (5)
• AML post-PV	1 (2)
• AML with MDS changes	1 (2)
• ALL	8 (20)
• ALL Ph+	1(2)
Comorbidities—no. (%)	
• DM	6 (15)
• Hypertension	20(50)
• Obesity	3 (7)
• COPD	8 (20)
• Hypothyroidism	4(10)
• Prostatic hypertrophy	4(10)
• Hypercholesterolemia	5 (12)
• No comorbidity	5 (12)
N° patients	29
Treatment plan — no. (%)	
• TKI	1 (3)
• Hydroxyurea	1 (3)
• Cytarabine+daunorubicine (7+3)	13 (44)
• FLAG	1 (3)
• Vincristine,prednisone,cyclophosphamide,doxorubicin	4 (13)
• L-Asparaginase	4 (13)
• Midostaurin	3 (10)
• Azacytidine	5 (17)
• Decitabina	4 (13)

Abbreviation: AML: Acute Myeloid Leukemia; ALL Acute Lymphoid Leukemia; COPD: Chronic obstructive pulmonary disease; DM: Diabetes Mellitus; FLAG: fludarabine, cytarabine, and granulocyte colony-stimulating factor; MDS: Myelodisplastic syndrome; NOS: Not otherwise specified; Ph: chromosome Philadelphia; PV: Polycythemia Vera; TKI: tyrosine kinase inhibitor

Methods: Patients were enrolled at Unit of Hematology of University Hospital of Palermo from May 2008 until December 2019. None of the patients had a known past medical history of thrombosis or bleeding or clotting disorders and none were taking any medications that could affect

coagulation. Patients had complete blood counts (CBC), TEG and CCTs (INR/PT, PTT, fibrinogen and D-dimers) performed at 3 time points: 1) Diagnosis of AL (T0); 2) during first cycle of CHT (T1); 3) At the end of CHT (T2). An algorithm of the instrument indirectly calculated thrombin generation (TG). Patients were followed-up for bleeding and thrombotic episodes daily up to the time of hospital discharge or death.

Results: Forty consecutive patients were included (see Table 1 for demographics). TEG results were compared using repeated measures analysis of variances (Manova). At T1, maximum amplitude (MA), TG and K time were significantly ($p<0.05$) shifted toward a hypocoagulability state when compared to T0, in presence of mild thrombocytopenia. In addition, a hypercoagulable in T2 was showed by changes of alpha-angle, MA and TG values ($p<0.05$). TEG results showed no differences between the group with and without hemostatic complications (thrombosis or hemorrhages). Additionally, there were no statistically significant differences in CCTs between 3 time points and no relationship with any TEG variables.

Conclusions: Cumulative, we demonstrate the capacity of TEG revealing complex and dynamic abnormalities in patients with AL according to course of disease and treatment, respect CCTs. Further studies will investigate the role of TEG in defining hemostatic profile and in individualizing anticoagulant therapy/prophylaxis in patients with AL.

C050

TREATMENT FREE REMISSION AFTER THROMBOPOIETIN RECEPTOR AGONIST DISCONTINUATION IN NEWLY DIAGNOSED PERSISTENT, CHRONIC ITP PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Recent evidence suggests that in patients with immune thrombocytopenia(ITP) with a stable response on TPO- RAs Eltrombopag (E) and Romiplostim (R), treatment may be tapered and discontinued. Aim: To observe the rate of discontinuation of TPO- RAs in patients (pts) with chronic, persistent and newly diagnosed ITP in our center and to identify predictive factors of treatment free response.

Patients and Methods: We retrospectively evaluated 59 pts(25F, 34M)treated with TPO RAs from June 2010 to April 2021. The median age at the start of TPO was 65 years(y). 58 pts were treated with E , 14 pts received E and R , 1 patient was treated with R, (73 total treatments), 11 pts were splenectomized, 31 pts were allocated to chronic ITP, 17 to persistent ITP, 11 to newly diagnosed ITP. The median follow up from the start of TPO- RAs was 18 months(m) (range 1- 129), the median of previous lines of therapy was 2. The median time of treatment was 8 m (range 1-95) for E treatments and 8 m (range 1-40) for R treatments.

Results: In R group 8 pts achived a CR, 3 pts achieved a R, 4 pts were refractory, 6 pts discontinuated the treatment: 4 for no response, 1 for adverse event, 1 for loss of response. In E treatments we observed 46 CR, 6 R, while 6 pts were no responders, 31 pts stopped the drug : 6 for no response, 2 for adverse event, 5 for loss of response, 5 died for other causes, 1 died for acute myocardial infarction, 9 pts discontinued for sustained CR. These pts were pretreated with a median of 2 therapy lines(range 1-4) including splenectomy in 4 pts. At starting TPO- RAs 5 pts were male, their median age was 43 y (range 16-85), the ITP stage was newly in 2 pts, persistent in 2 pts, chronic in 5 pts, the median duration of E treatment was 15 m(range 2-95). These pts achieved a CR in a median time of 28 days, started the tapering (if they required <25 mg/daily dose) after of a median of 16,7 m of treatment , the median duration of tapering was 10 m (range 1-20), the pts were tapered at 4 week intervals in 10-20% dose increments until 25 mg every 10 days (in 2 pts TPO-RAs exposure was shorter: 2 and 5 m). None pts experienced disease relapse after therapy discontinuation in a median follow of 23,6 m , 4 pts showed a durable response respectively for 44,42,41 and 38 m.

Conclusion: In our court of pts the only predictive factor of free treatment response were age (43 y in the discontinuation group *vs* 65 y in the whole court) and the achievement of CR (100% *vs* 73%).

Chronic Lymphatic Leukemias and other Chronic Lymphoproliferative Syndromes 1

C051

Tγδ LARGE GRANULAR LYMPHOCYTE LEUKEMIA: CLINICAL AND BIOLOGICAL FEATURES OF AN INTERNATIONAL COHORT OF 137 PATIENTS

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T-cell Large Granular Lymphocyte Leukemia (T-LGLL) is a rare lymphoproliferative disorder in which a Tαβ and a Tγδ subsets can be recognized. As compared to the more frequent Tαβ LGLL, Tγδ LGLL has been less investigated, especially in term of the frequency of novel biological LGLL markers *i.e. STAT3* and *STAT5b* mutations. The aim of this collaborative study is to characterize the clinical, phenotypical and biological features of Tγδ LGLL patients. Clinical, phenotypical and molecular features, including *STAT3* and *STAT5b* mutation status, of 137 patients affected by Tγδ LGLL and followed at eight referral centers across the world, were retrospectively collected. By phenotype, CD16 and CD57 were the main antigens expressed in 72.3% and 78.4% of cases, respectively. Neutropenia -Absolute Neutrophil Count (ANC) $<1.5x10^9$/L- and anemia -hemoglobin, Hb <120g/L- were the major clinical features, being present in 54.2% and 49.6% of cases, respectively, including severe neutropenia (ANC$<0.5x10^9$/L- and severe anemia (Hb<90g/L) in around 20% of cases each. DNA samples of 97 and 94 patients were available for *STAT3* and *STAT5b* mutations analysis, respectively. *STAT3* mutations were found in 37/97 cases (38.1%), with Y640F and D661Y being the most common as in Tαβ LGLL, while only 4 patients were *STAT5b* mutated (4.2%). *STAT3* mutated patients were characterized by higher frequency of neutropenia (p=0.0288), anemia (p=0.0726), autoimmune/autoinflammatory disorders (p=0.0139), and treatment requirement (p=0.0169). On the opposite, *STAT5b* mutated patients were mostly asymptomatic. Overall, 53.7% of patients required therapy with the majority (59.6%) having received methotrexate (MTX) while 26.3% cyclosporine A (CyA). Of notice, overall response rates (ORR) and complete response (CR) rates were lower in MTX treated patients as compared to CyA treated patients (ORR: 26.9% and 53.9% respectively, CR: 7.7% and 23.1% respectively). Considering the first line therapy and irrespectively from the subsequent lines of treatments, responders were characterized by a significantly longer progression free survival (PFS) with respect to non-responders (p=0.0029) and, most importantly, by significantly better overall survival (OS) (p=0.048). In conclusion, our results showed that *STAT3* mutation identifies a subset of Tγδ LGLL patients characterized by more symptomatic disease. In addition, CyA treatment results in higher response rates, translating in improved patients' survival.

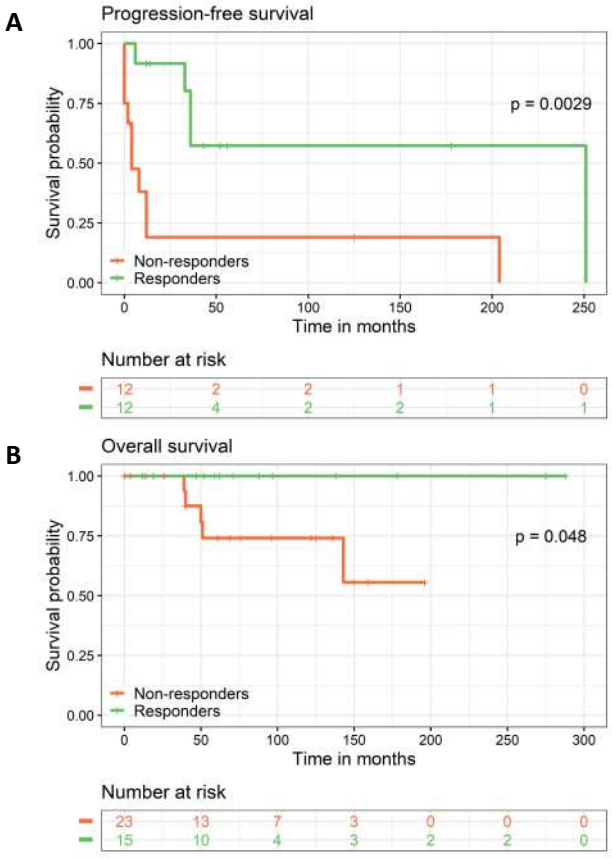

Figure 1.

C052

IBRUTINIB INTERFERES WITH INNATE IMMUNE RESPONSE DURING COVID-19 INFECTION

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that from December 2019 is spreading throughout the world causing a pandemic of Coronavirus Disease 2019 (COVID-19). COVID-19 is posing several challenges to the management of patients with hematologic malignancies. Chronic lymphocytic leukemia (CLL) is characterized by perturbations of the immune system. For this reason, CLL patients might have increased risk of COVID-19 infection. Ibrutinib, a potent BTK/ITK inhibitor, has shown potential protective effects against lung injury in COVID-19-infected patients. We examined the impact of ibrutinib treatment on the cytokine release by immune cells in CLL during stimulation with SARS-CoV-2 overlapping peptides.

Methods: Two experimental settings were performed. Untreated CLL PBMC were treated *in vitro* with ibrutinib and stimulated with SARS-CoV-2 Protein S, S1, S+, N and M. In the second, PBMC isolated from patients before and after 3 months of ibrutinib therapy were stimulated with SARS-CoV-2 peptides. Secretion of TNF-α and IFN-γ was determined by flow cytometry gating CD3+ and CD14+ populations.

Results: We mimic COVID-19 infection *in vitro* by pulsing CLL PBMC with SARS CoV 2 peptides. Following stimulation, we measured a significant release of pro-inflammatory cytokines by both CD3+ and CD14+ cells characterized by increased of TNF-α and IFN-γ. Ibrutinib did not modify TNF-α secretion either in presence or not of stimulation with SARS-CoV-2 in CD3+ population with a slight increase in IFN-γ secretion. BTK inhibition affected a productive inflammatory response of monocytes impairing the release of TNF-α and IFN-γ induced by SARS-CoV-2. We planned to analyze samples from CLL patients before and after 3 months of treatment with ibrutinib. Our data show no significant modifications in pro-inflammatory release by CD3+ cells during treatment with ibrutinib. On the contrary, the secretion of TNF-α and IFN-γ by monocytes observed in pre-treatment samples was significantly reduced during the first 3 months of therapy.

Conclusions: Our results demonstrate how ibrutinib reduces the cytokine response in monocytes stimulated by SARS-CoV-2, supporting the hyphothesis of a protective effects against major clinical complications induced by COVID-19 in CLL patients. Ibrutinib skews monocytes towards an immunosuppressive profile confining the cytokine storm with the possibility to reduce the inflammation status and prevent lung injury.

C053

"MOLTO", A MULTICENTER, OPEN LABEL, UNCONTROLLED, PHASE II CLINICAL TRIAL ON VENETOCLAX, ATEZOLIZUMAB, OBINUTUZUMAB (AVO) COMBINATION IN RICHTER TRANSFORMATION: SAFETY INTERIM ANALYSIS

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Diffuse large B-cell (DLBCL) transformation of chronic lymphocytic leukemia/small lymphocytic lymphoma, Richter Syndrome (RS), has poor prognosis. Both atezolizumab (A)+/-obinutuzumab (O) and Venetoclax (V), are active in DLBCL. The multicenter international ph II MOLTO study (EUDRACT2018-005028-40), evaluates AVO combination in 28 RS. Treatment scheme: 35 cycles (c) with A (1200 mg c1-18) + V (400 mg/d c1-35) + O (1000 mg c1-8), in untreated DLBCL-RS. At initial safety run phase 9 patients (pts) received at least 3 c with possible premature enrollment stop in case of ≥3 non-infective/non-hematologic (hema) therapy-related G≥4 adverse events (AEs). Remaining pts received same safety cohort schedule. From Oct 2019, in the absence of safety warning enrollment went on and, as of Feb 2021, 14 pts received at least 1 c. Table 1 shows pts characteristics. Overall, 110 c have been administered. 5 serious AE (SAEs) were recorded in 4 pts: 1 fever of unknown origin (FUO) and 1 autoimmune encephalitis, both G3, resolved and not leading to discontinuation, and 1 G5 pneumonia. The remaining 2 SAEs were disease progression (PD)-related hospitalizations. 12/14 pts (85.7%) developed G≥1 AE of any grade. Severe (G3-5) hema toxicity occurred in 7 pts, while 5 pts (including 2 developing SAEs) experienced severe non-hema toxicity. The most common hema toxicity was thrombocytopenia: 20 episodes/110 c (18.2%), 9 being G≥3. Other G≥3 hema toxicities: 10% neutropenia and 0.9% anemia. Three severe non-hema toxicities were recorded: 1 hyperamylasemia; 1 hypercalcemia; 1 purpura. Seven pts (50%) showed G2 FUO and only 1 major infection was observed. 2 were the AE of special interest: 1 autoimmune myositis and 1 autoimmune encephalitis both successfully managed with steroids.

No G≥3 infusion related reactions (IRR) occurred and all G1/2 were O-related. All pts completed V ramp-up, in 2 cases with accelerated dose-escalation (Koening et al. 2020) due to rapidly PD. No tumor lysis syndrome (TLS) was recorded. Overall, 9 pts are still on treatment with a median time on study of 4.4 (range 1-16.7) m, only 1/5 pts discontinued due to toxicity. As AVO was well tolerated even in elderly, Independent Data Monitoring Committee allowed the accomplishment of safety run phase. O+A combination did not result in an enhanced IRR rate or severity. In this highly proliferating disease, V did not lead to TLS even with the accelerated ramp-up. Accrual is ongoing and updated results will be presented.

Table 1. Patients' characteristics.

	Number (%)
Age Median (range)	69.5 yrs (range: 51-81)
Pts with age ≥65 years	11 (78.6)
Male/Female	6/8
N° of pts pretreated for CLL	9 (64.3)
Median number of prior CLL Tx (range)	1 (0-3)
N° of pts pretreated with BTKi	5 (35.7)
N° of pts pretreated with CIT	5 (35.7)
Bulky disease (≥ 5 cm)	11 (78.6)
Presence of del17p and/or *TP53mut*	6 (42.9)
Ann Arbor Stage	
I	1 (7.1)
II	2 (14.3)
III	6 (42.9)
IV	5 (35.7)

C054

CLADRIBINE AS FRONTLINE TREATMENT OF HAIRY CELL LEUKEMIA: A MULTICENTER EUROPEAN EXPERIENCE OF MORE THAN 30 YEARS ON 384 PATIENTS

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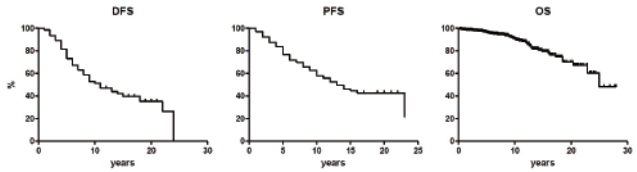
Background: Cladribine is regarded as the first treatment of choice of symptomatic hairy cell leukemia (HCL): it provides high rates of response and very long duration of remission in some cases.

Methods: Disease-specific patients records have been reviewed at four European centers of excellence (Bologna, Italy; Caen, France; London, United Kingdom, Lodz, Poland) and all patients requiring treatment who received frontline cladribine have been extrapolated. Responses have been classified according to Consensus Resolution Criteria. The main study objective were long-term overall survival (OS), disease-free survival (DFS) and progression-free survival (PFS) rates. PFS calculation involved all patients obtaining at least a partial response; DFS was determined only in patients with a complete response (CR) after treatment. Determining events for DFS and PFS were disease progression (decline in hematologic parameters, reappearance of marrow infiltration and/or organomegaly), initiation of a subsequent treatment, death for any cause.

Results: Three hundred and eighty-four HCL patients (including 3 patients with HCL variant) have been diagnosed and followed between 1969 and 2018, and all of them received frontline cladribine (either subcutaneously or intravenously, according to era- and site-specific guidelines and experience). A CR was obtained in 150 cases (39.1%), a partial response in 50 (13.0%) and a minor response in 7 (1.8%). Two hundred and eight patients (54.2%) received no further therapy besides cladribine as they did not require further treatment for their disease. A continuous CR was documented in 76 patients (19.8%), at a median follow-up period of 8.5 years (range, 1-22 years). Median OS was reached at 25.0 years, with 48.3% of patients being alive at 28 years. Median PFS was 13.0 years, with 43% of patients being free of progression at 22 years. DFS was 26.5% at 22 years, with median reached at 11 years (Figure). Retreatment with cladribine in relapsed patients occurred in 106 cases.

Conclusions: Cladribine is effective as frontline treatment of HCL and may determine deep disease control in a significant proportion of cases, given that more than 50% of treated patients require no further therapy. Good quality responses may be maintained for more than 20 years in nearly 40% of patients. Data obtained from this large international cohort of patients recapitulate the results obtained from smaller single-center clinical experiences with purine analogs.

Figure 1.



C055

PROGNOSTIC IMPACT OF SOMATIC MUTATIONS ON TIME TO FIRST TREATMENT: RESULTS OF TARGETED NEXT-GENERATION SEQUENCING IN 211 PATIENTS WITH EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The clinical course of patients with CLL is highly heterogeneous reflecting an underlying biologic heterogeneity of the disease. Next generation sequencing (NGS) may have a role for the identification of outcome predictors in early stage, treatment-naïve patients.

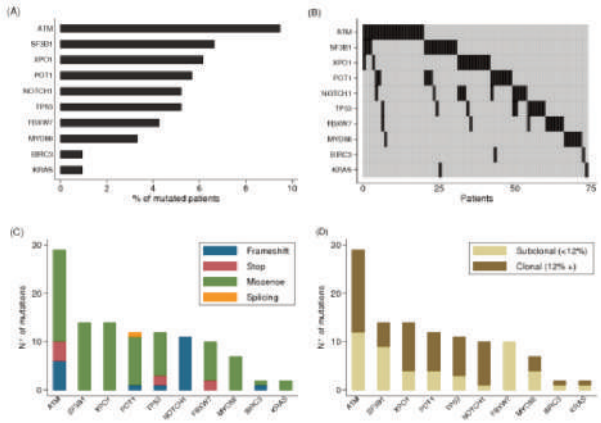
Methods: In a retrospective study we analyzed by NGS the mutational status of 10 target genes in a cohort of patients with Binet A stage CLL. All patients were diagnosed and treated according to the iWCLL 2008 criteria. Mononuclear cells were isolated from peripheral blood in 182 cases or from bone marrow in 29 cases. Targeted mutation analysis for 10 genes (ATM, BIRC3, FBXW7, KRAS, MYD88, NOTCH1, POT1, SF3B1, TP53, XPO1) was performed using a Truseq Custom Amplicon Sequencing Panel (Illumina, San Diego, CA, USA). We correlated the mutational status with time-to-first-treatment (TTFT) and overall survival (OS). In order to estimate the additional role of NGS mutational status in the prediction of TTFT, we calculate a new integrated prognostic model.

Results: We analyzed 211 patients with stage A CLL. A total of 113 mutations were found in 74/211 (35%) patients and co-occurence of mutations in > 2 genes was observed in 24/74 (32%) of mutated cases. Frequency, type and VAF of mutations are reported in Figure 1. With a

median follow-up of 8 years from diagnosis, 108 patients (51%) were treated. The median TTFT was 6 years (95% CI: 5-12) with 10-year treatment free survival (TFS) of 45% (95%CI: 37-52). In univariate analysis, the presence of one or more mutations by NGS was associated with shorter TTFT (p<0.001). Mutations in POT1, ATM, FBXW7 and MYD88 were independently associated with shorter TTFT. Integration of IPS-E and somatic mutations with p<0.1 in univariable analysis identified 4 groups of patients with different TFS (10-yrs TFS: 74%, 53%, 32% and not reached for patients with score 0, 1, 2 and≥ 3 respectively). Median OS was 15 years with 10-years OS of 72%. Patients with 2 or more mutations by NGS had a significant shorter survival as compared with those with no or one mutation (p<0.001). POT1 mutation and SF3B1 mutations were independently associated with shorter OS.

Conclusions: One third of patients with Binet stage A CLL harbor somatic mutations with prognostic relevance. The presence and number of somatic mutations by NGS was predictive of significantly shorter TTFT and OS, the former emerging as an important end-point for untreated CLL patients.

Figure 1.



Allogeneic and Autologous Transplantation 2

C056

IMMUNE RECONSTITUTION AND CLINICAL OUTCOMES IN THE SETTING OF HLA-IDENTICAL ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION

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Introduction: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) can lead to prolonged immunodeficiency, albeit its curative role in diverse settings. Deficits both in innate and adaptative immunity can contribute to treatment-related mortality (TRM). We investigate thymus-dependent and independent role in immune reconstitution (IR) kinetics and long-term clinical outcomes.

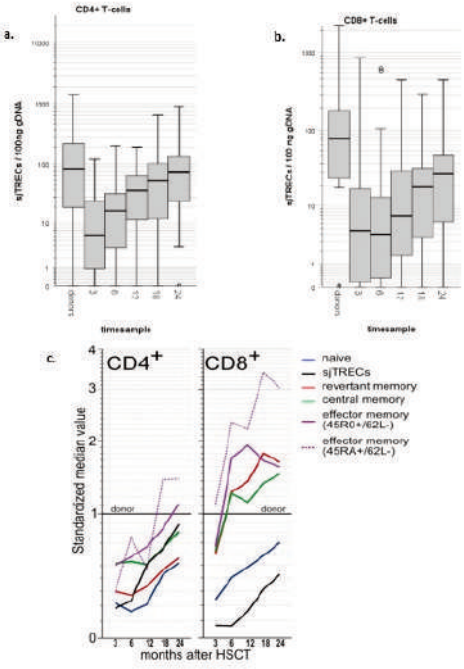

Figure 1. a) sjTRECs copy number variation in CD4+ T cells. (dashed line=median healthy donor levels). b) sjTRECs copy number variation in CD8+ T cells. (dashed line=median healthy donor levels). c) standardized values of CD4+ and CD8+ T-cell subsets and sjTRECs reconstitution at different time-points after allo-HSCT. (1=donor median values).

Figure 1.

Methods: Sixty-four patients (median age 56) undergoing HLA-identical sibling or unrelated donor allo-HSCT after a reduced intensity con-

ditioning (RIC) were enrolled. Peripheral blood samples were collected before conditioning and at 1, 3, 6, 12, 18, 24 months after allo-HSCT from patients and healthy donors as controls. Evaluation of IR was conducted by flow-cytometry analyses of CD4+ and CD8+ T-cell subsets [naïve, central memory (CM), effector memory (EM), CD45RA-expressing terminal effector memory (EMRA) and revertant] and Real-Time PCR quantification of signal joint T-cell receptor excision DNA circles (sjTRECs), performed on genomic DNA extracted from sorted CD4+ and CD8+ T cells.

Results: A constant gradual increase in absolute numbers of T-cell subsets and sjTRECs from the first month up to 2 years post-transplant was observed. Overall, median CD4+ and CD8+ T-cell and sjTRECs levels were lower than those observed in healthy donors at +2 years. sjTRECs kinetics was associated with CD4+ naïve T cells increase ($p < 0,001$), clearly suggesting that most of CD4+ naïve T cells derived from thymic re-education of donor precursor stem cells, whereas CD8+ naive T cells underwent peripheral expansion. By contrast, CM and EM T cells showed a faster thymic-independent expansion. By multivariate analysis, gr.II-III chronic GVHD (p 0.004 in CD4+, p 0.032 in CD8+) and age >60 (p <0.001 in CD4+, p 0.015 in CD8+) were significantly associated with low thymic output at +1 year. We also observed a significant effect of 3-month post allo-HSCT CD4+ sjTRECs levels on the risk of CMV reactivation: cumulative incidence within 2 years post-alloHSCT was 69.6% in patients with CD4+ sjTRECs levels below the median *vs* 40.2% in those with levels above the median (p 0.008).

Conclusions: Active thymic function despite age-dependent involution substantially contributes to T-cell reconstitution after allo-HSCT. Chronic GVHD and older age are significantly correlated with thymic activity. Correlation between IR and clinical outcomes need further investigations and prospective analyses to be confirmed.

C057

IMMUNOGENETIC LANDSCAPE OF LEUKEMIA RELAPSE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: ROLE OF CLASS I AND II SOMATIC HLA MUTATIONS

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Curative potential of allogeneic hematopoietic stem cell transplantation (HSCT) in myeloid malignancies is principally related to the graft-versus-leukemia (GvL) effect. Decreased expression of major histocompatibility complexes (MHC) or genomic aberrations in HLA region (6p copy-neutral loss of heterozygosis [LOH] or 6pdel) have been described in haploidentical/mismatched and matched contexts as mechanisms facilitating leukemic relapse. Here, we hypothesize that somatic mutations in class I-II HLA alleles may also contribute to immune escape from GvL decreasing the presentation of immunodominant peptides on leukemic blasts. To that end, we performed a comprehensive genetic characterization of specimens sequentially collected from a cohort of 48 patients with AML and MDS relapsing after HSCT, assessing HLA region along with 173 genes known to have a role in leukemogenesis. Ninety-six paired/serial samples (39 at AML/MDS diagnosis, 48 at relapse after HSCT, and 9 at relapse after chemotherapy) were analyzed. Disruptive HLA mutations were found in 29% of the patients (4% at diagnosis and 25% at post-transplant relapse), in both class I and II loci (median VAF was 33%). In post-transplant group, 75% of those events were found in patients receiving graft from a matched donor, while the remaining 25% was observed after haploidentical transplant. Patients with HLA mutations had more likely a later relapse (median time to relapse: 554 *vs* 150 days after transplant, p=.00042), underscoring a fitness advantage under GVL-related immune pressure (less likely in case of earlier events). Also, HLA mutated subjects were completely refractory to donor lymphocyte infusion-based regimens, (N=6), whereas HLA wild type (wt) patients (61% of the 19) tended to have transient or stable responses, in line with the concept that all the HLA restricted adaptive manipulations may be ineffective in patients characterized by mechanisms of immune escape mediated by HLA loss. When examining the somatic myeloid landscape of those patients, a different pattern of co-mutations was observed compared to HLA wt cases, with enrichment in genetic aberrations in epigenetic regulators (such as TET2, EZH2, EP300, and DNMT3A) in HLA mutated patients.

In conclusion, here we describe the existence of a family of genomic aberrations in HLA region that unveils a new mechanism of HLA loss, possibly contributing to post-transplant immune escape and leukemia relapse, similarly to 6pLOH and MHC downregulation.

C058

EXTRACELLULAR VESICLES AS A CIRCULATING PREDICTIVE BIOMARKER OF ACUTE GVHD IN ALLEGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Acute GVHD (aGVHD) is one of the most important early complication after allogeneic hematopoietic stem cell transplantation (HSCT). Though many factors (e.g. recipient's age, comorbidities, conditioning regimen, HLA compatibility and GVHD prophylaxis) influence the risk of GVHD, the use of validated predictive biomarkers is still uncommon in the real life. Almost all biological fluids, including plasma, are rich in Extracellular Vesicles (EVs). The potential diagnostic power of these biomarkers stands in their surface protein markers profile, as well as into their peculiar microRNAs cargo. Here, we analyzed plasma CD9, CD63 and CD81 positive EVs from 39 patients who underwent allogeneic HSCT for hematological malignancies (AML 41.1%; ALL 17.9%; MDS/MPN 17.9%; other 23.1%) from unrelated (74.3%), related matched (10.3%), haploidentical donor (12.8%), and cord blood (2.6%). EV concentration and size distribution were assessed at different time points (day-1, day+30, day+90 and day>180) by nanosight tracking analysis (NTA). EV membrane phenotype was assessed by cytofluorimetric MACS-Plex assay, which detects an array of 37 membrane proteins, including T, B, myeloid, erythroid and lymphocyte activation markers). Plasma microRNA cargo was assessed by small_RNAseq and droplet digital PCR. A "day -1 signature" of EVs membrane protein markers profile was identified as a predictor of aGVHD at day +90. In particular, high levels of EVs carrying CD3 (p<0.001), CD4 (p<0.001), ROR1 (p=0.002), CD86 (p=0.004), CD133 (p=0.002) and CD69 (p=0.006) were significantly predictive of aGVHD. In conclusion, we here report a peculiar distribution of pre-transplant signature of circulating EVs highly predictive of aGVHD. A validation of these findings is expected to allow early stratification of GVHD risk and to foresee the feasibility of a GVHD risk-adapted therapeutic strategy.

C059

THE IMPACT OF AGE ON HEMATOLOGIC RECOVERY AFTER ALLOGENEIC TRANSPLANTATION

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Introduction: Hematologic recovery is not satisfactory in every patient undergoing an allogeneic stem cell transplantation (HSCT). A significant proportion have been reported to have low platelet counts, despite full donor chimerism. This condition can be related to several factors including: number of CD34+ cells infused , stem cell source, underlying disease, conditioning regimen, GvHD and CMV infection. Recently transplant platforms have changed, including the use of haploidentical transplants and modified GvHD prophylaxis.

Aim of the study: To investigate factors associated with hematological recovery in current transplant years.

Methods: We included 1311 patients with hematological disease undergoing to HSCT from 2000 to 2020 in two transplant center: Genova and Roma, as shown in Table 1, in patients stratified according to age </> 60 years. Platelet counts were taken as a surrogate marker of hematologic recovery.

Results: We first ran a multiple regression analysis on factors influencing platelet counts between 50 and 100 days post-transplant. These were patients age >60 years, GvHD grade II-IV, non sibling donor and a diagnosis of myelofibrosis. Platelet recovery at different time points, up to over 4 years post-transplant, is shown in Figure 1a in patients stratified according to an age cut off of 60 years. Patients younger than 60 years showed significantly improved platelet recovery , at each time point, when compared to patients younger than 60 years; the difference persisted beyond 4 years. There was no difference in platelet recovery in patients aged 18-40 and 41-60. Donor age and year of transplant had no effect on platelet recovery. Figure 1b shown platelet recovery according to risk factors (age, GvHD, myelofibrosis, non sib donor). Transplant related mortality (TRM). We then asked whether low platelet counts predicted TRM. Patients with a platelets count higher than 20 and $50x10^9$ on days 50-100 post-HSCT, showed a reduced transplant related mortality (TRM) as compared to patients with a lower platelet count (13% *vs* 39%: p<0.000001; 11% *vs* 31%, p<0.000001) (Figure 1 c,d).

Conclusions: Platelet recovery post-HSCT seems to be strongly influenced by patient's age, together with GvHD, a diagnosis of myelofibrosis and donor type. Slow recovery in older patients remains statistically significant beyond 4 years after HSCT. Recovery after HSCT has not improved over the past 2 decades. Low platelet counts are a strong risk factor for mortality after allogeneic HSCT. Clinical trials with TPO agonists post HSCT are warranted to assess whether hematologic recovery can be improved, and whether this will translate in reduced mortality.

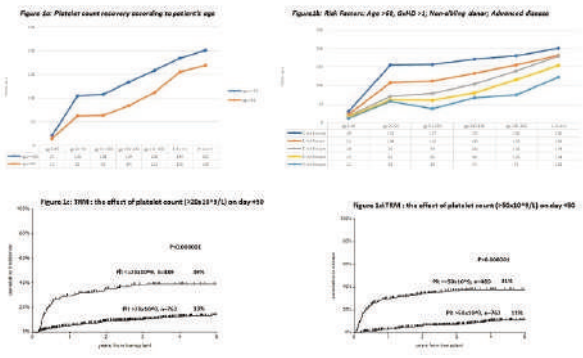

Figure 1.

C060

T-REPLETE HAPLOIDENTICAL STEM CELL TRANSPLANT, WITH MYELOABLATIVE CONDITIONING, FOR MYELOFIBROSIS IN RUXOLITINIB ERA: A SINGLE CENTER EXPERIENCE

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Allogeneic transplant can be curative in myelofibrosis (MF). Haploidentical stem cell transplant (Haplo-SCT) allows optimal timing, nevertheless its efficacy might be hampered by rejection.

Aims: To evaluate the Haplo-SCT in homogeneously treated, MF affected, patients (pts) for engraftment, survival, GVHD and the composite end point of Gr.III-IV aGVHD, severe cGVHD-free/relapse-free survival (refined-GRFS).

Methods: We collected retrospective data of 51 pts, transplanted from 2012 to 2020 at our Unit, affected by PMF (27/51; 53%) or post-TE/PV MF (24/51; 47%). The majority had advanced disease (IPSS-Int2/High: 34/51, 67%. Splenomegaly >22cm: 21/51, 41%). The whole cohort received Haplo-SCT after a myeloablative conditioning consisting of thiotepa (day -6 to -5), fludarabine (day -4 to -2), busulfan (day -4 to -2; one dose omitted in less-fit pts). Overall, 49/ 51 (96%) received unmanipulated bone marrow graft with day +3/+5 post-transplant cyclophosphamide, cyclosporin and mycophenolate from day 0 as GVHD prophylaxis; 2/51 (4%) received peripheral stem cells due to donor needs. 20/51 (39%) received pre-transplant ruxolitinib (RUX).

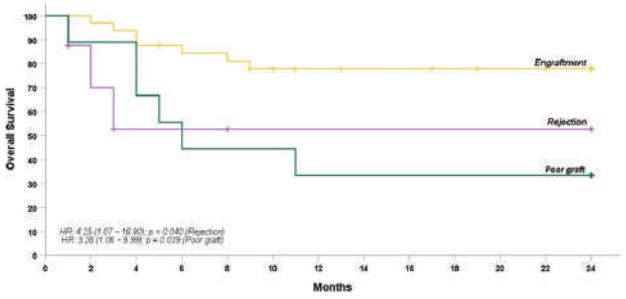

Figure 1.

Results: Median age was 58. Median FU was 11 months (IQR: 4 – 24). Median time to recovery was 24 (15-168) and 33 (12-176) days for neutrophils and platelets. Engraftment (EGF), intended as complete peripheral recovery with full donor chimerism, occurred in 32/49 pts (66%), rejection in 8/49 (16%), PGF in 9/49 (18%); 2 pts, died before day 28, were not evaluable. After 24 months, median OS was not reached (2yr OS: 65%). Lack of EGF was the only factor that significantly impacted OS (multivariate, HR for PGF 3.26; p=0.039; HR for rejection 4.25; p = 0.04). Median PFS was not reached after 24 months (2yr PFS: 52%). Splenectomy negatively impacted PFS (multivariate, HR 5.84; p = 0.023). A trend towards a better PFS was observed in pts exposed to RUX prior to SCT (multivariate, HR 0.19; p=0.13). CI of Gr. II-IV aGVHD was 27% (95% CI: 20% - 33%), Gr.III-IV aGVHD 8% (95%CI: 4% - 12%). 24 months CI of cGVHD was 28% (95%CI: 21%-35%). 24 months refined-GRFS was 51%.

Conclusions: this is the largest experience reported on haplo-SCT in MF. Engraftment is achievable in an acceptable proportion of pts, predicting a favourable outcome. GRFS is a suitable objective in pts undergoing SCT: despite concerns about the alternative source and conditioning intensity, half of the pts are alive, in remission and free from GVHD after procedure.

Hodgkin Lymphoma

C061

BRENTUXIMAB VEDOTIN PLUS CHEMOTHERAPY FOR PATIENTS WITH PREVIOUSLY UNTREATED, STAGE III OR IV CLASSICAL HODGKIN LYMPHOMA: 5-YEAR UPDATE OF THE PHASE 3 ECHELON-1 STUDY (NCT01712490)

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In the ECHELON-1 study, brentuximab vedotin, doxorubicin, vinblastine, and dacarbazine (A+AVD) significantly improved modified progression-free survival (PFS) per independent review in patients (pts) with newly diagnosed Stage III/IV classical Hodgkin lymphoma (cHL) *vs* doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) (Connors, NEJM 2018) and durable PFS per investigator (INV) benefits with A+AVD *vs* ABVD were seen with extended follow-up (Bartlett, Blood 2019; Straus, Blood 2020). We report updated efficacy and safety data after 5 years' follow-up (cutoff date 18 Sep 2020). Pts with newly diagnosed Stage III/IV cHL were randomised 1:1 to up to 6 cycles of A+AVD (n=664) or ABVD (n=670) on days 1 and 15 of a 28-day cycle. Interim PET scan after cycle 2 (PET2) was mandated. Analyses were performed for PFS per INV, peripheral neuropathy (PN) resolution and improvement (improvement by ≥1 grade [G] from worst G by latest assessment) in pts with ongoing PN at end of treatment, rate of secondary malignancies, and pregnancy incidence and outcomes among pts and partners. After 60.9 months median follow-up (95% confidence interval [CI] 55.2–56.7), estimated 5-year PFS per INV was 82.2% (95% CI 79.0–85.0) for A+AVD and 75.3% (95% CI 71.7–78.5) for ABVD, favouring A+AVD *vs* ABVD (hazard ratio [HR] 0.681; 95% CI 0.534–0.867; p=0.002). Estimated 5-year PFS with A+AVD *vs* ABVD in the intention to treat population was 84.9% *vs* 78.9% in PET2– pts (HR 0.663; 95% CI 0.502–0.876; p=0.004) and 60.6% *vs* 45.9% in PET2+ pts (HR 0.702; 95% CI 0.393–1.255; p=0.229). In A+AVD and ABVD arms, 85% and 86% of pts with treatment-emergent PN had complete resolution or improvement of symptoms. In A+AVD and ABVD arms, PN was ongoing in 29% and 21% of pts, respectively, most of which were G1–2. In total, 131 pregnancies were reported; the proportion of ongoing pregnancies or live births in female pts was similar in both arms (A+AVD arm 85%, ABVD arm 74%). With 5 years' follow-up, sustained PFS benefit was observed with A+AVD *vs* ABVD that was independent of disease stage and PET2 status. In addition, treatment adaptation by interim PET2 status is not required for A+AVD and bleomycin exposure is avoided. As most historical cHL relapses occur within the first 5 years (Radford, BMJ 1997), the durable and robust treatment benefit and manageable safety profile of A+AVD in ECHELON-1, suggest that A+AVD is an attractive treatment option for all pts with previously untreated Stage III/IV cHL.

C062

NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA IN THE RITUXIMAB ERA: A STUDY OF FONDAZIONE ITALIANA LINFOMI

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Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare entity whose neoplastic cells retain a B-cell phenotype with expression of CD20. Radiotherapy is recommended for favorable stage IA disease, while for other stages guidelines suggest therapeutic strategies similar to those used for classic HL (cHL). The role of rituximab, although quite widespread, is not completely elucidated. We retrospectively analyzed baseline characteristics of 308 consecutive patients with NLPHL diagnosed in 19 Italian centers from 2000 to 2018. With a median follow up of 8.4 years (IQR: 4.5-12.4) for treated patients, median OS was not reached and estimated 10-yrs OS was 96.4% (93.2% - 98.1%) and 5-yrs PFS was 84.5% (79.7% - 88.3%). Histological transformation rate to diffuse large B-cell lymphoma was 2 x 1000 person-year with a median time to transformation of 25.1 months (IQR: 20.2-31.8). Patients with stage II or more showed superior PFS with immunochemotherapy in comparison to chemotherapy alone (5-yrs PFS was 89.6% *vs* 72.7%, p=0.034). In multivariable analysis no use of rit-

uximab, splenic involvement and bulky disease were associated with an inferior PFS (all p<0.05, as showed in the attached Table 1). Our data suggest an advantage of the use of rituximab combined with chemotherapy±radiotherapy in the treatment of stage II-III-IV NLPHL.

Table 1. Crude (univariable analysis) and adjusted (multivariable model) effect of rituximab and baseline characteristics on progression-free survival in 193 patients with stage II/III/IV nodular lymphocyte-predominant Hodgkin lymphoma who received chemotherapy.

	Univariable analysis		Multivariable model	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Age at diagnosis (years)	1.0 (1.0-1.0)	0.915		-
Stage (III-IV vs II)	2.0 (1.1-3.6)	0.030		-
Hemoglobin (g/dl)	0.9 (0.7-1.1)	0.217		-
Lymphocytes (<8% vs ≥8%)	4.5 (1.1-18.7)	0.041	2.8 (0.6-13.3)	0.184
Spleen involvement (Yes vs No)	3.9 (2.0-7.6)	<0.001	3.2 (1.4-7.6)	0.007
Bulky (Yes vs No)	2.5 (0.9-7.1)	0.085	3.4 (1.1-10.7)	0.034
B symptoms (Yes vs No)	2.2 (1.1-4.6)	0.034	1.9 (0.8-4.7)	0.155
Albumin (<4 vs ≥4 g/dl)	0.9 (0.3-2.3)	0.804		-
Rituximab (Yes vs No)	0.5 (0.3-1.0)	0.037	0.4 (0.2-0.8)	0.015

C063

ABSTRACT WITHDRAWN

C064

BRENTUXIMAB VEDOTIN (BV) CONSOLIDATION AFTER AUTOLOGOUS STEM CELLS TRANSPLANTATION (ASCT) FOR RELAPSED/REFRACTORY (R/R) CLASSICAL HODGKIN LYMPHOMA (CHL): A REAL-LIFE EXPERIENCE BY FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction: Up to 30% of cHL patients (pts) have a R/R disease. Salvage therapy followed by consolidation with ASCT can save only approximately half of R/R pts. In the AETHERA trial, cHL pts at high-risk of progression or relapse who received BV consolidation after ASCT showed a reduced risk of progression compared to a placebo group, with 5-year progression-free survival (PFS) rates of 59% and 41%, respectively. We report here the results of a real-life study on 105 cHL pts treated with BV consolidation after ASCT.

Methods: This retrospective study included R/R cHL pts from 15 Italian centers treated between Apr 2011 and Aug 2020. Eligible pts had received at least 2 cycles of BV after ASCT. The primary aim was PFS and OS assessment and its comparison to data already published.

Results: We included 105 pts, with a median follow-up of 20 months (range 2-108). Pts received a median of 2 lines of treatment before ASCT. The 51% (54 pts) received BV also immediately before ASCT. PET-CT evaluation before and after ASCT reported a Deauville Score (DS) 1-3 in 72 (75%) and 68 (78%) pts, respectively. Considering pre ASCT high-risk features (refractory disease, CR < 12 months, extranodal disease at relapse), 30 (29%) pts presented at least 2 factors. The median number of BV consolidation cycles was 10. A complete schedule of 16 cycles of BV was administered to 57 (54%) subjects (60% of pts who received both consolidation and pre ASCT and 43% of pts treated with BV post-ASCT only). Main causes for treatment interruption were: adverse events (AEs; 15; 33%), PD (13; 28%), consolidation with allo-SCT (8; 17%). Among grade 3-4 AEs leading to treatment interruption, there were 8 peripheral neuropathies, 4 infections, 2 infusion reactions, 1 liver toxicity. The 3-year PFS and OS were 62% (95% CI: 49-72) and 86% (95% CI: 73-93), respectively (Figure 1). The only feature significantly associated with both reduced PFS and OS was a DS 4-5 before ASCT (HR 3.81; 95% CI: 1.80-8.09; p<0.001). Administration of BV pre ASCT was not associated with different risk of progression (HR 0.87, 95% CI 0.44-1.73, p=0.965) or death (HR 0.71, 95% CI 0.20-2.49, p=0.594).

Conclusions: BV consolidation post ASCT is an effective and safe option for R/R cHL pts in line with the AETHERA trial. The use of BV also before ASCT did not negatively impact on its safety and efficacy, and likely allowed to offer to a higher number of pts the option of ASCT.

Figure 1. Kaplan-Meier plots showing OS and PFS.

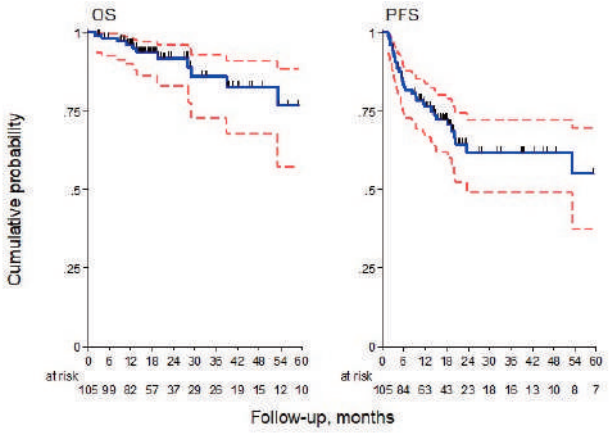

Figure 1.

C065

FERTILITY PRESERVATION IN LYMPHOMA PATIENTS TREATED WITH IMMUNOCHEMOTHERAPY WITH OR WITHOUT RADIOTHERAPY: RESULTS OF A RETROSPECTIVE MULTICENTER STUDY OF THE FONDAZIONE ITALIANA LINFOMI (FERTY CARE)

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In the last decades, a significant improvement in survival of patients (Pts) affected by lymphoma has been observed. Fertility and gonadal function represent an important aspects for long-term lymphoma survivors. We designed a retrospective, multicentric, observational study, with the primary endpoint to describe the different methods of fertility preservation during therapy in the real life. Other end points were: to determine amenorrhea rate and possible risk factors in young female lymphoma patients and to record rate of pregnancy and miscarriage after treatment. All pts, aged from 18 to 40 years old, diagnosed with Hodgkin (HL) or non-Hodgkin's lymphoma (NHL) in the timeframe between Oct 1st/2010 and May 31st/2018, treated with chemoimmunotherapy regimens and/or radiotherapy (RT) were included in the study. A total of 414 women were enrolled in the study. Median age was 28 years old (range 18-40), histology were: HL 308 (74%), PMBCL 56 (13%), DLBCL 43 (10%), FL plus MCL plus T-cell lymphoma 7 (3%). Advanced Ann Arbor stage III-IV was seen in 164 (40%) of pts. First line treatment were: ABVD in 295 (71%), R-CHOP like in 102 (25%), higher intensity regimens (BEACOPP/HD-CHT and ASCT) in 17 (4%) pts. 203 (49%) patients received RT. A relapsed/refractory disease was observed in 48 (11%) cases, and 52% of them received ASCT. Pretreatment data recorded: regular period in 80%, previous pregnancy in 26%, previous miscarriage in 9%. Overall, 76% of the pts received GnRH analogues during chemotherapy; 10% OCs and 14% nothing. Oocytes and ovarian tissue cryopreservation were performed in 55 and 42 pts, respectively (23%). Post treatment data were transient amenorrhea in 216 (75%) and premature ovarian failure (POF) in 33 (9%). After treatment were recorded 43 (10%) pregnancies and 17 (4%) miscarriages. Most pregnancies (88%) were observed in women under 30 years of age and subjected to a single line of therapy. Median age of menopause onset was 34.5 ± 7.8 years old (19.0-47.0). In multivariate analysis median age at diagnosis and number of lines of treatment correlate with higher rate of amenorrhea, risk of POF and menopause. No protective effect of GnRHa administration of amenorrhea, POF and menopause was observed. In conclusion, pregnancy in long term lymphoma survivors should be considered possible and safe. Our study confirm the need to a correct and multidisciplinary approach to fertility preservation in young lymphoma pts.

Infections

C066

INTERIM RESULTS OF PROSPECTIVE OBSERVATIONAL SEIFEM STUDY ON CLINICAL OUTCOME AND INFECTIOUS COMPLICATIONS IN 230 UNFIT AML PATIENTS TREATED IN FIRST-LINE WITH HYPOMETHYLATING AGENTS ALONE OR IN COMBINATION WITH VENETOCLAX

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Introduction: The hypomethylating agents (HMAs) represent an important therapeutic option for older patients (pts) with AML and have become the backbone for combination regimens (eg, with Venetoclax). However, very limited real-life prospective studies are available regarding the clinical outcome of these pts including infectious complications and infectious related mortality (IRM) during treatment.

Patients and Methods: The recruitment of this prospective multicentric study (CE-Id-study:2908) has been completed on December 31, 2020; the study follow-up is still open. We enrolled 230 pts with a median age of 75 years (range 25-94); 157 pts (68%) had >2 relevant comorbidities. Of the 230 cases, 118 (51%) received a first-line therapy with a combination of HMAs+Venetoclax(V) while 112 (49%) were treated with HMAs monotherapy (azacitidine or decitabine). Until now, 1270 cycles of HMAs have been administered (547/1270 with HMAs+V).

Results: The best response achieved, under HMAs treatment, was: CR in 40% of cases (52% in HMAs+V and 28% in HMAs alone, P<0,005), PR in 20% and SD in 14% of cases. The microbiological or radiological proven infectious complications (almost one) occurred in 144/230(63%) pts, mainly pneumonia (in 40% of pts) and/or bacteremia/sepsis (one or

more events in 28% of pts). Febrile neutropenia (one or more episodes) occurred in 38% of pts; 14 cases of Covid-19 (6%) were reported. After a median follow-up of 8 months (1-24) from the start of HMAs therapy, 113 (49%) pts died and 117 (51%) were alive. The 1 yr OS probability was 46% with a median OS of 10 months (9,8 in HMAs+V and 10,5 in HMAs alone). The primary causes of death were: progression of AML (31%), Infection (30%-34/113), Infection+AML (23%), other causes (16%). The directly IRM was 30% and 13/34 pts died of infectious complication while in CR/PR (11/13 in HMAs+V group and only 2/13 in HMAs group). Data on antibiotic prophylaxis, hospitalization, drug-doses modulation, are available and will be analyzed in this study.

Conclusions: These preliminary results confirm, in a real-life setting, a higher CR rate in pts treated with HMAs+V compared to HMAs alone. However, we detected a high rate of infectious complications and a high IRM (30%) with higher infection related deaths in CR/PR pts on HMAs+V group. If confirmed at the end of the study, these data underline the great importance of infection prevention, to reduce infectious deaths, improving OS of this frail AML population.

C067

IMPACT OF LEVOFLOXACIN PROPHYLAXIS WITHDRAWAL ON PRE-ENGRAFTMENT BLOODSTREAM INFECTIONS AFTER AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANT

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Background: Gram-negative bacteria (GNB) blood-stream infections (BSIs) affect mortality in neutropenic hematological patients (pts), particularly if multidrug resistant (MDR). Italy has registered during the past years high prevalence of fluoroquinolone (FQ) resistance and wide spread of carbapenem resistance. In such a context, the benefit of FQ prophylaxis is controversial.

Aims: We aim to evaluate the impact of levofloxacin prophylaxis omission for the prevention of pre-engraftment (PE) BSIs in adult pts affected by hematologic malignancy treated with autologous (ASCT) and allogeneic stem cell transplant (alloHSCT). Primary objective was to compare infection-related mortality (IRM) at day-30 in pts who developed GNB PE-BSIs. Secondary objectives were the incidence, etiology and antimicrobial resistance of PE-BSIs.

Methods: Since February 2019, we modified internal protocol and omitted FQ prophylaxis in pts undergoing transplant. We collected data of neutropenic fever (NF) in four groups of pts: ASCT receiving FQ prophylaxis (group-A1, Jan.2018-Jan.2019), ASCT without FQ prophylaxis (group-A2, Febr.2019-Dec.2020), alloHSCT receiving FQ prophylaxis (group-B1, Jan.2018-Jan.2019), alloHSCT without FQ prophylaxis (group-B2, Febr.2019-Dec.2020).

Results: We detected 25 episodes of NF (38 ASCT) in group-A1, 81 episodes (98 ASCT) in group-A2, 69 episodes (71 alloHSCT) in group-B1, 215 episodes (152 alloHSCT) in group-B2. In group-A1, 2 episodes of PE-BSIs occurred, both sustained by Gram positive bacteria. In group-A2, 22 episodes of PE-BSIs occurred. Pathogens were GNB in 13/22 episodes and MDR-GNB in 1/13 episodes (ESBL-producer Kp). IRM 30 day after ASCT was 0% both in group-A1 and group-A2. In group-B1, 30 PE-BSIs occurred. Pathogens were GNB in 10/30 episodes and MDR in 7/10 episodes (4 KPC-Kp, 2 ESBL-producer Ec, 1 MDR-Pa). In group-B2, 87 PE-BSIs occurred. Pathogens were GNB in 55/87 episodes and MDR in 17/55 episodes (7 ESBL-producer Kp, 7 ESBL-producer Ec, 3 MDR-Pa). Overall regarding alloHSCT in group-B2 we observed higher incidence of PE-BSIs (p 0.04) and GNB PE-BSIs (p 0.02), with a lower incidence of MDR-GNB PE-BSIs (p 0.005). IRM 30 days after alloHSCT was 5.6% in group-B1 and 5.2% in group-B2.

Conclusions: In this study withdrawing FQ prophylaxis has no impact on IRM. We observed an increased rate of PE-BSIs among pts without prophylaxis, whit a decrease in PE-BSIs from MDR-GNB. These data confirm the safety of an approach based on FQ withdrawal.

C068

DETECTION OF SARS-COV-2 INFECTION PREVALENCE IN 860 CANCER PATIENTS WITH A COMBINED SCREENING PROCEDURE INCLUDING TRIAGE, MOLECULAR NASOPHARYNGEAL SWABS AND POINT OF CARE SEROLOGICAL TEST

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Introduction: There are limited data on efficacy of screening procedures to evaluate prevalence of SARS-CoV-2 infection (including asymptomatic cases) in cancer outpatients undergoing antineoplastic therapy.

Patients and Results: From May-1, 2020 to June-15, 2020, during the first wave of SARS-CoV-2 pandemic, 860 consecutive patients, undergoing active anticancer therapy, were evaluated and tested for SARS-CoV-2 with a combined screening procedure including self-report questionnaire, molecular nasopharyngeal swab (NPS) and rapid serological immunoassay (for anti SARS-CoV-2 IgG/IgM). Primary endpoint of the study was to estimate the prevalence of SARS-CoV-2 infection (including asymptomatic cases) in consecutive cancer outpatients by a combined screening modality. A total of 2955 SARS-CoV-2 NPS and 860 serological tests, in 475 patients with hematologic cancers and 386 with solid tumors, were performed. A total of 112/860 (13%) patients self-reported sympthoms potentially COVID-19 related; only 1/860 cases(0.03%) had a positive SARS-CoV-2 NPS and 14 cases(1.62%) had a positive specific serological test (overall prevalence of infection 1.62%). Of the 112 cases who declared symptoms potentially COVID-19-related, only 2.7%(3/112) were found SARS-CoV-2 positive. This suggest that a questionnaire-based triage system, even if accurate and important, has a low positive-predictive value (0,89%; 95% CI: 0,87-0,91%) for the identification of cancer patients with SARS-CoV-2 infection since a differential diagnosis between tumor or treatment-related symptoms and COVID-19-related symptoms is always very difficult. This is the largest study reporting the feasibility of a combined screening procedure to evaluate prevalence of SARS-CoV-2 infection in cancer patients receiving active therapy, during the first epidemic wave, under the restrictive lockdown measures, in one of the areas of active SARS-CoV-2 circulation. Lacking specific recommendations for the detection of asymptomatic SARS-CoV-2 cases, a combined diagnostic screening might be more effective to detect the exact prevalence of SARS-CoV-2 in neoplastic population. The prevalence can obviously change according to the territorial context, the entity of the restrictive measures adopted and the phase of epidemic curve. However, its exact and real-time knowledge could be important to optimally balance risks/benefits of oncologic treatments avoiding (if the prevalence is low) the reduction of dose intensity or the selection of less intensive anticancer therapies.

C069

MDW IS A NOVEL INFLAMMATORY BIOMARKER WITH PROGNOSTIC RELEVANCE IN COVID-19 PATIENTS

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Monocyte Distribution Width (MDW), a new cytometry-based hematologic parameter correlating with cytomorphologic changes occurring during monocyte activation, has recently been described as promising early biomarker of sepsis. Similar to sepsis, in SARS-CoV-2-associated disease (COVID-19), monocyte/macrophage subsets are considered key mediators of the life-threatening hyperinflammatory disorder –commonly defined as 'cytokine storm'– which is part of the complex infection-associated immune dysregulation observed in severe COVID-19 cases (possibly constituting a kind of viral sepsis). Therefore, here we aimed at investigating, for the first time, possible prognostic roles of MDW testing in the monitoring of COVID-19 patients. In this work, we longitudinally measured MDW values (readily available along with automated blood cell count) in a cohort of 87 patients with molecularly-proven COVID-19 diagnosis, consecutively admitted to our intensive/subintensive clinics in early 2020. Statistical analyses were applied to correlate MDW values with common inflammatory markers, disease severity, clinical trajectories and final outcome. We found significant direct correlations between MDW and different inflammatory markers routinely assessed during hospitalization, namely CRP ($p<0.001$), fibrinogen ($p<0.001$) and ferritin ($p<0.01$). Moreover, high MDW values were remarkably associated with fatal outcome (AUC=0.76, sensitivity 0.75, specificity 0.70, MDW threshold 26.4; RR=4.91, OR=7.14) (Figure 1). Furthermore, evaluating MDW dynamics in cases with longer follow-up, we frequently observed progressive MDW increments in patients with worsening inflammatory conditions, while clinical recoveries were consistently associated with MDW decreases. Of note, MDW testing may also help to assess therapeutic response to immunomodulatory treatments, such as tocilizumab. Our pilot study shows that MDW can be useful in the monitoring of hospitalized COVID-19 patients, as it is: (i) easy and rapid to obtain, (ii) directly related to the activation state of a fundamental inflammatory cell subset (*i.e.* monocytes, pivotal both in cytokine storm and in sepsis immunopathogenesis), (iii) strongly correlated with clinical severity of COVID-19-associated inflammatory disorder, and, in turn, (iv) endowed with relevant prognostic significance. Additional studies are needed to define the clinical impact of MDW testing in other settings, including COVID-19 patients with hematologic comorbidities.

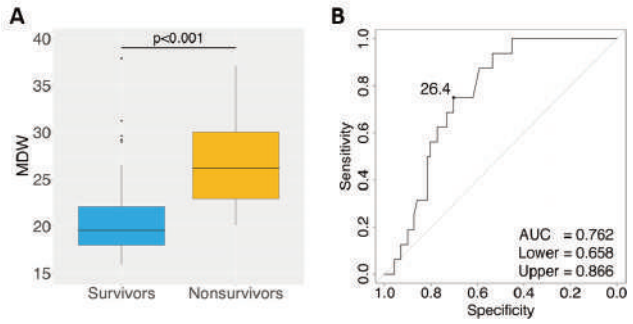

Figure 1.

C070

THE FREQUENCY AND SEVERITY OF COVID-19 IN HEMATOLOGIC PATIENTS VARIES THROUGOUT DIFFERENT PANDEMIC PERIODS: 14-MONTH EXPERIENCE IN A HIGH-IMPACT AREA

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In March 20 the start of COVID-19 pandemic had devastating effects in hematologic patients (hem pts), with a reported related mortality of up to 40%. The aim of our study was to evaluate, in the well-defined epidemiological setting of our patient population, the trend of the pandemic in terms of frequency, severity and prognosis over time after its 1st wave (Mar-Apr20), which was particularly strong in Brescia. From Mar-20 to Apr-21, 259 hem pts with acute leukemia (AL) (21), lymphoma (LY) (90), multiple myeloma (MM) (47), chronic lymphoproliferative disorders (CLD) (38), myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPS) (49), or non-neoplastic disorders (14) acquired COVID-19 and were consecutively recorded. Median age was 71y (20-94), M/F ratio 1,4 Distribution of monthly diagnoses over time was markedly uneven with 108 (42%) in Mar-20, less than 5 from May to October and a mean of 21 from Nov-20 to Mar-21 (2nd wave). Only 49 pts (19%) were managed at home, 82% of them in the 2nd wave. The severity of COVID-19 also varied over time. Of 59 asymptomatic pts, 15 were diagnosed in the 1st wave and 35 in the 2nd, representing 11% and 36% of pts of the two periods, respectively (P=0.0004). Severe/critical pneumonia developed in 78% of pts in the 1st and in 48% in the 2nd wave ($p<0.0001$) (Figure 1). Similarly, COVID-19-related mortality was 36% in the 1st and 21% in the 2nd wave (P=0.0099). However, mortality due to severe pneumonia remained similar (39% *vs* 41%). Overall, age ≥70y (60% *vs* 85%, $p<0.0001$) and male sex (68% *vs* 80%, p=0.045) were associated with reduced survival. Their mortality rates did not differ significantly throughout both pandemic waves. According to hem diagnosis, the mortality decreased from the 1st wave to the 2nd wave in pts with LY (39%>24%), MM (26%>17%), MDS/MPS (39%>8%), and CLD (25%>18%), as opposed to AL (36%>56%). Moreover, pts whose hematologic disease was controlled at COVID diagnosis died more often during the 1st wave (39% *vs* 14%, p=0.009).

In conclusion, the impact of COVID-19 in hem pts varied both in time and severity, being minimal in summer and less frequent and lethal in autumn/winter 2020/21, except for pts with AL. However, the prognosis of hem pts with severe pneumonia did not differ over time, confirming COVID-19 as a critical issue for them. These results may be useful for orienting the management policies of hem pts in different periods of the pandemic.

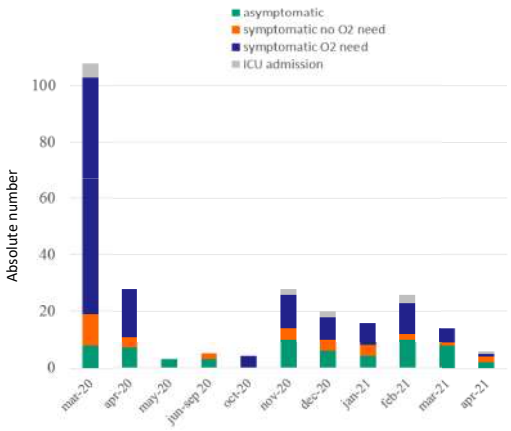

Figure 1.

Acute Leukemia 2

C071

PROGNOSTIC IMPACT OF MINIMAL REDISUAL DISEASE ASSESSMENT IN ELDERLY PATIENTS WITH SECONDARY ACUTE MYELOID LEUKEMIA. A COMPARISON BETWEEN CPX-351 AND INTENSIFIED FLUDARABINE-BASED REGIMENS

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Minimal residual disease (MRD) assessment retains high prognostic value in Acute Myeloid Leukemia patients (AML) undergoing intensive induction therapy. However, most of the data on the prognostic value of MRD come from trials including younger patients treated with conventional 3+7 regimen. AML arising from a previous myelodisplastic syndrome (s-AML) and therapy-related AML (t-AML) are usually under-represented in trial and are unlikely to respond to conventional induction. Few data are available on the kinetics and the prognostic value of MRD in this setting. We evaluated MRD in a cohort of elderly s-AML or t-AML patients receiving induction therapy either with a fludarabine-containing regimen or CPX-351, in order to compare the probability of achieving MRD negativity, to disclose the prognostic value of MRD in this setting and to define the best time-points for MRD assessments. A total of 136 elderly (median age 67, range 60-75) s-AML or t-AML patients were analyzed treated between Jan 2005 and Jan 2020, either with CPX-351 (n=35) or fludarabine-high dose cytarabine-idarubicin (FLAI), with (n=72) or without (n=29) gemtuzumab-ozogamicin (GO). MRD was retrospectively analyzed in patients achieving hematological complete remission (CR) with both multicolour flow cytometry (MFC) and WT1 expression levels. CR was achieved in 83 patients (61%). CR rate was 28/35 in patients treated with CPX-351 (80%), significantly higher when compared to patients receiving FLAI (55/101, 54.5%, p<0.05). The addition of GO to FLAI did not increase CR rate. Among CR patients, a total of 41 (49.4%) and 44 patients (53%) achieved MRD negativity, with MFC or WT1, respectively. MFC MRD negativity probability was higher among patients receiving CPX-351 as induction therapy (MFC MRD negativity rate of 16/28, 57% and 25/55, 45% in CR patients who received CPX-351 or FLAI, respectively, p<0.05). Adding GO to FLAI did not improve MRD negativity probability. MRD showed significant prognostic value in terms of Overall Survival in all treatment group (2-year OS of 74 and 36% in patients with or without residual MFC MRD after induction, respectively, p<0.05). WT1-based MRD lead to similar results. The higher rate of MRD negativity with CPX-351 may be related to a more efficient anti-leukemic activity in this particular setting. The evaluation of MRD with both MFC and WT1-based assessment lead to superimposable conclusions and allowed us to obtain data from virtually all patients.

C072

INTERLEUKIN-2 RECEPTOR ALPHA CHAIN, ALSO CALLED CD25, IS A POTENTIAL TARGET IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is a molecularly heterogeneous disease originating from clonal proliferation of precursor B-lineage cells. In adults, ALL diagnosis is still associated with a dismal prognosis due to the lack of specific targeted therapies. This study was designed to investigate the expression of interleukin-2 receptor alpha chain CD25 in B-ALL and its biological significance, especially following the availability of specific CD25 targeting compounds. The expression of IL2RA (CD25 gene) was detected by flow cytometry (FC), immunohistochemistry and Western blot analysis, in 25 newly diagnosed ALL patients, both Philadelphia positive (12 patients) and Philadelphia negative (13 patients). Similarly, CD25 expression was assessed in four B-ALL commercially available cell lines. Infection with shRNA specifically directed against CD25 was used to evaluate apoptosis induction and cell cycle arrest in primary B-ALL cells established from two patients. Our data suggest that ALL, and in particular Ph-positive ALL, aberrantly expresses the interleukin-2 receptor alpha chain, CD25. Whereas normal B cells display low amounts of CD25, primary ALL cells and ALL cell lines (over)-express CD25. While the high frequency of CD25 on the surface of many different hematological tumor cells has been established and confirmed in our study, there is little investigation focusing on the significance of CD25 expression. Indeed, CD25 may be present on ALL cells and enable oncogenic signaling pathways. In such respect, we observed that CD25 silencing in primary cells promotes cell cycle arrest and apoptosis induction. While these data support the rational to target CD25, ALL cells did not appear to be *in vitro* sensitive to basiliximab, an antibody able to target the Il2RA, but *in vivo* investigations are needed to better assess the effects of this therapeutic approach in ALL context. We concluded that CD25 expression is elevated in patients with B-ALL. Our results also demonstrate that CD25 silencing induces cell cycle arrest and apoptosis. The latter result has important implications from a therapeutic point of view. Targeting CD25 receptor with anti-CD25 antibodies or peptide mimetics could be an effective strategy for targeting leukemic cells. Additionally, high CD25 expression could be exploited for the development of CAR-T therapy

C073

PROSPECTIVE STUDY ON 595 NEUTROPENIC EPISODES IN 230 AML PATIENTS: IMPACT OF DIFFERENT CHEMOTHERAPY REGIMENS ON MUCOSAL DAMAGE DIAGNOSED WITH BEDSIDE ULTRASOUND

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Introduction: Neutropenic enterocolitis (NEC) is a life threatening complication of leukemic and solid tumors patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50-100%. It is characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) is used to evaluate bowel-wall thickening (BWT), and > 4 mm is considered diagnostic of NEC. Early diagnosis and treatment is crucial especially in the era of multidrug resistant (MDR) enteric bacteria. In this work we compared the impact of different CHT regimens on mucosal damage and NEC occurrence.

Methods:The study enrolled from 2007 to april 2021 all AML pts admitted in Hematology Unit, undergoing CHT (N=237). Median age 55 (19-85). Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in CHT-related neutropenic pts.

Results: N=595 chemotherapy-related neutropenic episodes (NE) occurred. N=39 NEC episodes were diagnosed (6.5% incidence rate). N=6

patients died as consequence of NEC (15.4%). CHT regimens number/NEC episodes (15.4%): (i) 3+7 (Idarubicin+ARAC) N=118/17(14.4%), (ii) AML1310 induction N=17/8(47%), (iii) AML 13010 consolidation N=11/0 (0%), (iv) 3+7 (Daunorubicin+ARAC) N=21/2 (9.5%), (v)2+5 (Idarubicin+ARAC) N=62/0 (0%); (vi) 3+3+5 (Idarubicin,VP-16,ARAC) N= 103/7 (6.8%), (vii) Decitabine N= 27/0 (0%); (viii) Clofarabine (20mg and 40mg) N=33/0 (0%), (ix) clofarabine+ARAC N=26/2 (7.7%); (x) FLANG N=54/2 (3.7%), (xi) HD-ARA-C (3gr/mq for 3 consecutive days) N=37/0 (0%); (xii) CPX-351 (induction 1) N=13/0 (0%), induction 2 N=3/0 (0%), consolidation N=5/0 (0%),(xiii) FLAG-Ida N=2/0 (0%), (xiv) ARAC 200mg for 3 days s.c. N=22/0 (0%), (xv) Mirros N=9/0 (0%), (xvi) AIDA (idarubicin) induction N=13/1, cons1 N= 9/0 (0%), cons2 N=8/0 (0%) maintenance N=2/0 (0%). AML 1310 induction had the highest statistical impact on NEC incidence (p=<0.001), it was considered our reference or NEC incidence rate and it was statistically superior to 3+7 Ida, 3+7 Dauno, CPX-351, FLANG, AIDA induction, Clofarabine+ARAC (p=0.04, p=9.5, p=<0.001, p= <0.001, p=0.054, p=0.009, respectively).

Conclusions: We found a statistical different impact on intestinal mucosal damage of chemotherapy regimens. Our finding might help warning the physicians especially in patients colonized with MDR intestinal bacteria. US allowed to detect early signs of NEC and to start prompt treatment.

C074

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND COVID-19 INFECTION. A REPORT FROM THE CAMPUS ALL

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As of February 2020, the Covid-19 pandemic has markedly affected the overall management of patients with hematologic malignancies. In order to define the incidence, features, outcome, impact on management of the Covid-19 infection on adult patients with acute lymphoblastic leukemia (ALL), throughout the Campus ALL network we carried out different surveys in Italy during the year of the pandemic. Out of 756 patients with a diagnosis of ALL followed at 34 Italian hematology centers, 63 (8.3%) developed a SARS-CoV-2 infection, detected by molecular test in all cases but 1. The majority of Covid-19-positive cases was recorded during the period spanning from September 2020 to beginning of April 2021 (57/63, 90.5%). Of the infected patients, 43/63 were men; 21 patients were aged 18-35 years, 17 35-50 years, 15 50-65 and 10 were older than 65. Seventeen (27%) patients had a diagnosis of T-lineage ALL, 26 (41.3%) of B-lineage Ph- ALL and 20 (31.7%) of B-lineage Ph+ ALL; 36 (57.1%) of the infected patients had no concomitant comorbidities, 11 (17.5%) had one comorbidity and 16 (25.4%) more than 1 comorbidity. Source of the infection was mostly nosocomial (26/63, 41.2%) and familial (23/63, 15.9%). It was documented at the onset of ALL in 4 (6.3%), during the induction phase in 10 (15.9%), consolidation in 13 (20.6%), maintenance in 11 (17.5%), after an allogenic transplant in 15 (23.8%), during maintenance or off-treatment in 8 (12.7%), at relapse in 2 (3.2%). The median time to obtain a viral clearance was 34 days (range 7-91). Management of the infection was variable: 29 (46%) patients did not require hospitalization, 28 (44.4%) were hospitalized in a Covid ward and 13 of them required respiratory assistance; 6 (9.5%) were transferred to an ICU unit. In 48 patients (76%) there were no sequelae, in 8 (13%) the infection is still ongoing and 7 (11%) succumbed. Within the 47 patients on ongoing treatment for ALL, therapy was interrupted in 35 (74.4%). In conclusion, the incidence of SARS-CoV-2 infection in ALL patients was similar to that of the general population and was recorded mostly in the last wave of the pandemic. No differences were identified in terms of age, disease subtypes and concomitant comorbidities. The infection was manageable, with 46% of patients not requiring any medical intervention. The death rate was 11% among the infected population. Finally, ALL treatment had to be stopped in most patients. Further details will be provided at the meeting.

C075

PEVONEDISTAT (P), VENETOCLAX (V) AND AZACITIDINE (A) VERSUS V+A IN ADULT PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA WHO ARE UNSUITABLE FOR INTENSIVE CHEMOTHERAPY: A RANDOMIZED, PHASE 2 TRIAL (NCT04266795)

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V is an inhibitor of B-cell lymphoma 2 approved in the USA for patients (pts) with acute myeloid leukemia (AML) in combination with low-dose cytarabine or hypomethylating agents. Treatment with V+A has been shown to improve overall survival (OS) *vs* A alone and is becoming standard of care for pts with newly diagnosed AML unfit for standard intensive chemotherapy. Despite recent advances prognosis remains poor. Novel combination therapies are needed to improve pt outcomes without increasing toxicity. P, an investigational, first-in-class

NEDD8-activating enzyme inhibitor, prevents degradation of select proteins, thereby interfering with protein homeostasis and leading to cancer cell death. In phase 1/2 studies, treatment with P+A has shown promising clinical activity and good tolerability in AML. There is preclinical evidence of synergy with P+V (Cojocari *et al.* Haematologica 2021) likely mediated by P-induced neutralization of pro-survival proteins, including myeloid leukemia cell differentiation protein (MCL-1). Upregulation of MCL-1 may be a primary mode of resistance to V. Thus, P+V may help to prevent or overcome resistance to V and prolong duration of response (DOR). The reported clinical benefit of P+A and V+A in AML and the preclinical evidence of synergy with P+V suggest that combination treatment with all three agents may improve outcomes *vs* V+A in pts with newly diagnosed AML. A phase 1/2 study of P+V+A in secondary AML (NCT03862157) established the recommended phase 2 dose and demonstrated a high response rate among these poor-risk pts. NCT04266795 is a randomized, open-label, controlled, phase 2 study (Figure) with ~85 global study sites. The primary endpoint is event-free survival (EFS: time from randomization to relapse from complete remission [CR] or CR with incomplete blood count recovery [CRi], treatment failure or death from any cause, whichever occurs first). Secondary endpoints include: OS; OS at 6 months, 1 year and 2 years; 30- and 60-day mortality; CR rate; EFS after cycle 6; DOR; time to first response; time to relapse from CR/CRi or death; health-related quality of life; pharmacokinetics; rate of red blood cell and platelet transfusion independence; and hospitalization rate. Exploratory mechanism-of-action studies and molecular characterization of bone marrow aspirates will be performed. Elimination of leukemic stem cells and predictive biomarkers of response will be assessed. Planned enrollment is ~150 pts; recruitment is ongoing.

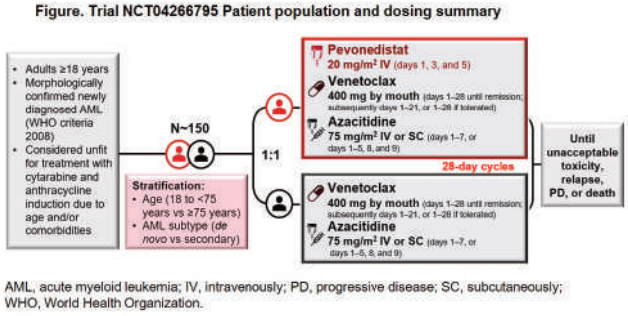

Figure 1.

Chronic Myeloproliferative Diseases 1

C076

THE BET INHIBITOR, CPI-0610, PROMOTES MYELOID DIFFERENTIATION IN MYELOFIBROSIS (MF) PATIENT BONE MARROW AND PERIPHERAL CD34+ HEMATOPOIETIC STEM CELLS

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CPI-0610 is a potent and selective bromodomain and extraterminal domain (BET) inhibitor, with balanced inhibitory activity against BD1 and BD2, under investigation in MF patients (pts) as monotherapy or in combination with ruxolitinib in the MANIFEST trial (NCT02158858). To evaluate the effects of CPI-0610 on bone marrow (BM) biology, correlative analyses were conducted using patient samples from MANIFEST. BM fibrosis (BMF) grading was assessed by local pathologists for BL and post-treatment (most at 24 weeks) biopsies available from 116 evaluable pts. Relative BMF improvement of ≥1 grade was observed in 33% (38/116) of all pts, with 21% (6/29) in arm 1, 41% (16/39) in arm 2 and 33% (16/48) in arm 3. BMF grade worsening was observed in only 6% (7/116) of pts. Additional BM biopsy pairs collected pre-treatment and 24-week post-treatment for exploratory histopathological assessments were available from a total of 37 unselected MANIFEST pts. Immunohistochemistry (IHC) staining of erythroid and megakaryocyte (MK) lineages with CD71 and CD61, respectively, were conducted centrally. Semi-quantitative analysis revealed an overt increase in CD71+ erythroid progenitors in 59% (22/37) of pts. Overall improvement in Mk histotopography, with reduced numbers and tight clusters of CD61+ Mk, was observed in 65% of pts (24/37). CD34+ hematopoietic stem cells were isolated from peripheral blood collected from multiple MF pts at baseline to evaluate the impact of CPI-0610 on MK and erythroid differentiation *in vitro*. When CD34+ cells from MF pts were treated with CPI-0610 in erythroid differentiation conditions in the presence of SCF, IL3 and EPO, a dose-dependent increase in more mature erythroid cell populations was observed. Suppressive effects of ruxolitinib on erythroid differentiation were partially rescued by CPI-0610 in a dose-dependent manner. CPI-0610 treatment of CD34+ cells from MF pts in MK differentiating conditions in the presence of SCF, IL6, IL9 and TPO resulted in a dose-dependent decrease in proliferation of CD34+ cells and an increase in the ratio of late MK (CD34-/CD41a+/CD42b+) and early MK (CD34+/CD41a+/CD42b+). These paired BM biopsy and *in vitro* myeloid maturation results demonstrated an effect of CPI-0610 in promoting erythroid and MK differentiation. These results may partially explain CPI-0610's clinical effects in MF pts, including rising hemoglobin, reduced transfusion dependency and reduction in spleen volume and symptoms.

C077

CPI-0610, A BROMODOMAIN AND EXTRATERMINAL DOMAIN (BET) PROTEIN INHIBITOR, AS MONOTHERAPY IN ADVANCED MYELOFIBROSIS (MF) PATIENTS REFRACTORY/INTOLERANT TO JAK INHIBITOR (JAKI): UPDATE FROM PHASE 2 MANIFEST STUDY

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CPI-0610, a first-in-class, oral, small-molecule inhibitor of BET proteins, potentially promotes disease-modifying activity through altered gene regulation of key oncogenic, fibrotic, and inflammatory factors and may transform the standard of care in MF. Here we present results from MANIFEST Arm 1, a global, open-label Phase 2 study of CPI-0610 monotherapy in advanced MF pts refractory/intolerant to JAKi. Pts are stratified as transfusion-dependent (TD, defined as ≥2U RBCs/mo over 12 wks) and non-transfusion-dependent (non-TD). Eligibility: MF pts intolerant/resistant/refractory/lost response to or ineligible for JAKi; DIPSS ≥Int-2; platelets ≥75x10^9/L; ≥2 symptoms measurable (score ≥1) per MFSAF v4.0; TD per IWG-MRT criteria in TD cohort or spleen volume of ≥450 cc by CT/MRI in non-TD cohort. 1° endpoints-TD cohort: TD to TI (transfusion independence: no transfusion for 12 wks); non-TD cohort: SVR35 (≥35% spleen volume reduction) at wk 24. As of 29 Sep 2020, 27 pts were treated in non-TD cohort (median: 51 wks, range: 2, 147). Mean age 68 y, male: 52%; DIPSS ≥Int-2: 74%; hemoglobin (Hgb) <10g/dL: 63%; primary MF: 70%; 52% with high molecular risk and 63% with *JAK2* mutations. At wk 24, 30% (7/23) pts achieved SVR35 (median % change: -29%, range: -70%, 14%), 48% (10/21) pts achieved TSS50 (median % change: -56%, range: -100%, 25%). 50% (10/20) pts achieved absolute of ≥1.5 g/dL increase in Hgb levels without transfusions with notable Hgb improvement observed in pts who started treatment with baseline Hgb <10g/dL. In TD cohort, 19 pts were treated (median: 32 wks, range: 5, 78). Baseline characteristics: mean age 71 y (SD: 8), 63% male, 94% with DIPSS ≥Int-2, 95% with Hgb <10g/dL, 58% with primary MF, 58% with high-molecular-risk and 68% with *JAK2* mutations. 21% (3/14) of TD pts converted to TI. At wk 24, median spleen volume change is -11% (range: -35%, 90%); 8% (1/13) pts achieved SVR35. 8% (1/13) pts achieved TSS50 (median % change: -22%, range: -70%, 30%) at 24 wks. A total of 46 pts were evaluable for safety. The most common hematological TEAEs of any grade were thrombocytopenia (30%, ≥Gr3: 15%) and anemia (15%, ≥Gr3: 13%). CPI-0610 monotherapy is generally well-tolerated and provides clinical benefits in MF pts refractory/intolerant to rux. SVR35 and symptomatic improvement were observed. Half of non-TD pts demonstrated ≥1.5 g/dL increase in Hgb. Conversion to TI was observed in the TD cohort.

C078

ANALYSIS OF EARLY EVENTS DURING THE FIRST YEAR OF TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC PHASE CML PATIENTS: A "CAMPUS CML" STUDY

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Tyrosine kinase inhibitors (TKIs) revolutionized treatment of chronic myeloid leukemia (CML). However, the first months of therapy are crucial, as optimal response is defined as the achievement of molecular milestones at 3, 6 and 12 months (mo.) and as many toxicities, also causing a TKI switch, are more frequent in the 1st year. To evaluate achievement of early molecular response (MR) and incidence of events leading to a TKI change during the 1st year of therapy, we retrospectively studied 1422 CP-CML patients diagnosed from 2012 and 2019 at 27 Hematology Centres and treated with frontline imatinib (IM) or second-generation (2G) TKIs dasatinib or nilotinib. Optimal MR at 3, 6 and 12 mo. were assessed according to 2020 ELN recommendations. Median age at diagnosis was 60.1 years and 59% patients were males. ELTS risk score was low in 58%, intermediate in 30.6% and high in 11.4% patients. Commonest comorbidities were arterial hypertension (39.5%), previous neoplasm (13.7%), diabetes (11.8%), peripheral vascular diseases (8.2%), COPD (7.8%) and ischemic heart disease (7.0%). Frontline TKI was IM in 795 (55.9%) and 2G-TKIs in 627 (44.1%) cases; IM-treated patients were older (median age 66.9 *vs* 51.1, p<0.001), with higher ELTS score (int/high 65.4% *vs* 55.3%, p<0.001) and more comorbidities (p<0.005 for all diseases). Optimal MR was achieved at 3 mo. by 1020/1233 (82.7%), at 6 mo. by 874/1166 (75.0%) and at 12 mo. by 674/1077 patients (62.6%), respectively. Total number of patients discontinuing TKI in the 1st year was 270/1417 (19.1%), being higher with IM (201/791, 25.4%) than 2G-TKIs (69/626, 11%) (p<0.001). Main causes were primary resistance (8.2%, 11.9% IM *vs* 3.5% 2G-TKIs, p<0.001), extra-hematologic toxicity (6.4%, 8.1% IM *vs* 4.3% 2G-TKIs, p>0.001), hematologic toxicity (1.9%, 2.3% IM *vs* 1.4% 2G-TKIs, p=0.25) and progression (1.1%, 1.3% IM *vs* 0.8% 2G-TKIs, p=0.56). Cumulative incidence of discontinuation at 3, 6 and 12 mo. were 5.4%, 10.5% and 18.9%, respectively; values for IM and 2G-TKIs at the three timepoints were 8.1%, 15.1%, 25.2% and 2.1%, 4.6%, 11% (p<0.001) (Figure 1). This real-world study on over 1400 CML patients shows that almost 20%

discontinue frontline TKI during the 1st year, mostly for primary resistance or toxicity. Discontinuation rates are higher with IM compared to 2G-TKIs, mostly at 3 mo. due to a lower attainment of early MR. The impact of higher risks and heavier burden of comorbidities in IM patients need deeper investigation.

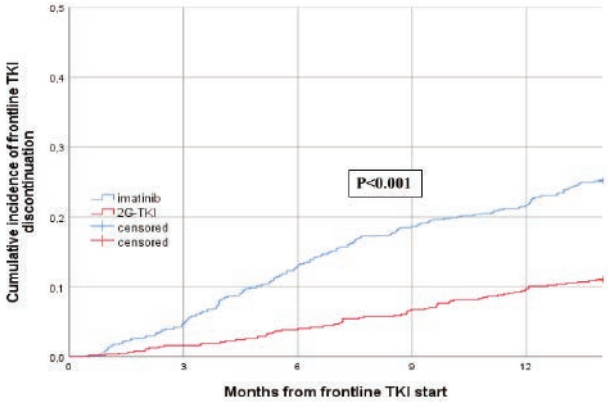

Figure 1. Cumulative incidence of TKI treatment discontinuation according to frontline TKI.

C079

PERIPHERAL BLASTS ARE ASSOCIATED WITH RESPONSE TO RUXOLITINIB AND OUTCOME IN PATIENTS WITH CHRONIC-PHASE MYELOFIBROSIS

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According to the DIPSS and the MYSEC-PM scores, peripheral blasts (PB) are a negative prognostic factor in patients (pts) with primary and secondary myelofibrosis (PMF/SMF). The role of the *JAK1/2* inhibitor, ruxolitinib (RUX), has not been assessed in correlation with PB. After IRB approval, the "RUX-MF" retrospective study collected 742 RUX-treated chronic-phase (CP, defined as PB <10%) pts in 25 Hematology Centers. In 707 pts, PB count was evaluated by morphology at RUX start and correlated with treatment success and outcome. Spleen (SR) and symptoms (SyR) response were assessed using IWG-MRT criteria. Pts were categorized according to PB at RUX start: PB-0 (no PB; n. 444, 62.8%), PB-5 (PB 1-5%; n. 239, 33.8%), and PB-9 (PB 6-9%; n. 24, 3.4%). Pts characteristics at RUX start were: median age 68.1y (24-89); males 57.9%; PMF 53.2%; *JAK2, CALR* and *MPL* mutated: 81%, 12.3% and 2.2% (4.5% triple negative), high DIPSS: 7.2%; PLT<100/WBC >25 x10^9/l: 10.7%/17%; spleen length ≥10 cm: 60.3%, TSS ≥20: 60.4%; ≥1 high-risk mutation (HMR): 74/144 evaluable (51.4%); fibrosis grade ≥2: 73.1%; starting/cumulative RUX dose ≥15 mg BID: 62.5%/50.3%. Higher PB count was associated to lower PLT (p<0.001), higher fibrosis grade (p=0.001) and higher WBC (p=0.04). At 3 and 6 mos, 26.3% and 28.8% of pts achieved a SR, while 65.5% and 75.2% were in SyR, respectively. At 3 mos, both SR (p=0.03) and SyR (p<0.01) were less frequently achieved by PB-5 and PB-9 pts compared to PB-0 pts. This association remained significant for SR at 6 mos (p=0.04) and at any time (p=0.01). After a median RUX exposure of 1.7 y (0.1-7.7), 394 (55.7%) pts stopped RUX, 89 (12.6%) had a leukemic transformation (LT) and 283 (40%) died. In univariate analysis, at 2y PB-9 pts had higher rates of RUX stop (70.8% *vs* 41.9%/33.7% in PB-5/PB-0 pts, log-rank p=0.001) and LT (36.9% *vs* 9.6%/7.1% in PB-5/PB-0 pts, log-rank p=0.003). Median survival times of PB-0, PB-5 and PB-9 patients were 5.9, 5.1 and 2 years, respectively (log-rank p=0.001) (Figure 1). In multivariable Cox analysis, PB confirmed their association with: 1) RUX stop (HR 1.3, p=0.005), with high DIPSS (HR 1.7, p=0.004), TSS>20 (HR 1.4, p=0.01), and PMF (HR 1.4, p=0.004); 2) LT (HR 3.2, p=0.01), with HMR (HR 3.5, p=0.04); 3) survival (HR 1.3, p=0.04) with high DIPSS (HR 2.9, p<0.001). CP-MF pts with PB>5% have a worse response to RUX and a worse outcome. Personalized approaches including newer JAK-inhibitors and combination strategies are needed in these pts.

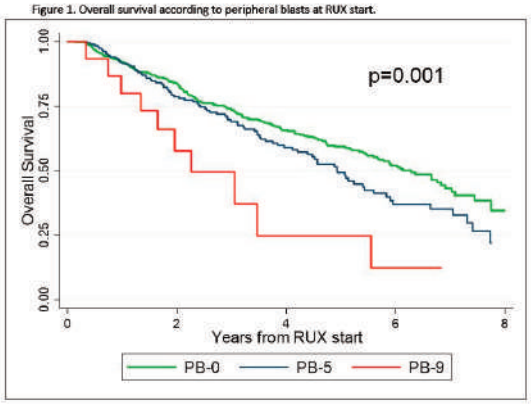

Figure 1.

C080

SCORING PROPOSAL FOR THE UNDERLYING DIAGNOSIS OF SYSTEMIC MASTOCYTOSIS IN PATIENTS WITH UNEXPLAINED SEVERE OSTEOPOROSIS

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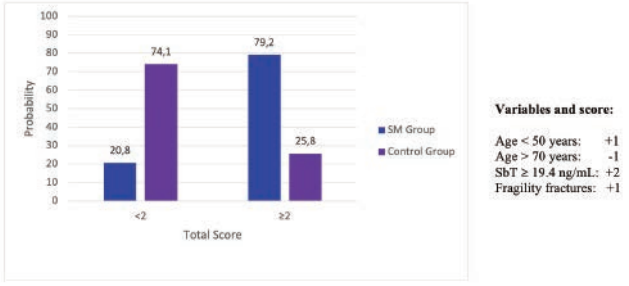
Osteoporosis may represent the only symptom of onset of systemic mastocytosis (SM). As distinct from patients with other mediator-related symptoms, a score to predict the association with an underlying SM in these patients is lacking. Furthermore, normal serum basal tryptase (sbT) levels do not exclude SM diagnosis, whereas high sbT levels might be due to other causes, *e.g.*, familial hypertryptasemia. This study aimed at

analyzing the clinical features of a large series of adult patients referred to our multidisciplinary team for unexplained osteoporosis and suspected SM. Secondly, we aimed at identifying criteria able to predict the diagnosis of SM and provide an indication for bone marrow (BM) studies. One hundred ten patients with unexplained osteoporosis who underwent BM evaluation were retrospectively studied. Diagnosis of SM was based on the 2016 World Health Organization (WHO) criteria. Other causes of secondary osteoporosis had been previously excluded. After BM study, 48 patients (43.6%) were diagnosed with SM, of whom 44 (91.7%) had bone marrow mastocytosis and four (8.3%) had indolent SM, with previously unrecognized skin lesions. Other mediator-related symptoms were reported in 31 patients (64.6%). Sixty-two patients (56.4%) did not fulfill the diagnostic criteria for SM and were used as a control group. SM patients were younger than controls (median age 55 *vs* 63 years, respectively; p=0.005), had higher median sbT level (25.9 *vs* 16 µg/L, p<0.001) and presented more frequently fragility fractures (93.7% *vs* 74.0%, respectively; p=0.009). No significant differences according to gender and mediator-related symptoms were found between the two groups. Based on multivariate analysis, a model to predict the diagnosis of SM before BM study was built, including age <50 years (p<0.001) or not >70 years (p=0.010), sbT level >= 19.4 µg/L (p<0.001) and the presence of fragility fractures (p=0.02) as independent predictive factors. Patients with a score <2 had a lower probability to have mastocytosis (p<0.001; Figure 1). In conclusion, we remark the importance of considering the diagnosis of SM in cases of unexplained osteoporosis. However, our proposed score could avoid unuseful BM studies. In cases with a score <2, searching for the D816V KIT mutation on peripheral blood and testing for familial hypertryptasemia could lower the risk of losing cases of mastocytosis.

Figure 1. Graphical representation of the proposed scoring system.



Score ≥2: Specificity 0.74, Negative Predictive Value 0.82
Sensitivity 0.79, Positive Predictive Value 0.70

Figure 1.

Non Hodgkin Lymphoma 4

C081

EARLY GRANULOCYTE-COLONY-STIMULATING FACTOR ADMINISTRATION DOES NOT IMPACT TOXICITY AND EFFICACY OF ANTI-CD19 CAR-T IN PATIENTS WITH RELAPSED/REFRACTORY B-CELL LYMPHOMA

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Chimeric Antigen Receptor T cells (CAR-T) are an outbreaking treatment option for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL). Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are the most common specific toxicities, while severe cytopenias and infections are often observed as well. Severe cytopenias are known to affect at least 80-90% patients during the first month from CAR-T, with grade 3-4 neutropenia possibly being present in 40% patients 6 months after treatment. Non viral infections have been reported in 25% patients during the first months. Currently, early *in vivo* treatment with G-CSF has been widely avoided both in trials and clinical practice as *in vitro* studies showed that GM-CSF may empower immunological toxic effects. Real life experiences with G-CSF and CAR-T patients, however, are anecdotic and do not support the hypothesis of augmented CRS and ICANS. In this single center study, we analyzed 122 patients affected by DLBCL treated with both commercial CAR-T products (tisa-cel and axi-cel). From March 2020, early G-CSF prophylaxis at day two post-infusion was systematically proposed to 33 consecutive patients. These patients were compared to a control group made of patients who did not receive G-CSF (34 pts) or who received late G-CSF after D5 (55 pts). Efficacy and safety outcomes of G-CSF were considered. Grade 4 neutropenia duration was similar in patients who received early G-CSF compared to control group (4 *vs* 5 days, p=0.18). Nevertheless, significantly decreased incidence of febrile neutropenia was observed in the former group (58% *vs* 81%, p=0.018). Patients experienced similar rate of specific CAR-T toxicities, including any grade and grade 3-4 CRS (p=0.93 and p=0.28, respectively), and any grade and grade 3-4 ICANS (p=0.62 and p=0.88, respectively). We observed no difference in the quality of CAR T-cells expansion, nor in primary disease response rate (best overall response rate 57.6% *vs* 61.8%, p=0.93). In conclusion, early G-CSF administration at day two is safe with no impact on CRS and ICANS and may have a role in reducing febrile neutropenia without affecting anti-lymphoma activity of CAR-T.

C082

FDG-PET IMAGING AND RADIOMICS IN RESPONSE ASSESSMENT OF LYMPHOMA PATIENTS UNDERGOING CAR T-CELL THERAPY

B. Casadei[1,2], A. Paccagnella[3], A. Farolfi[3], L. Argnani[1,2], C. Malizia[3], G. Paolani[4], M. Santoro[4], G. Della Gala[4], S. Strolin[4], L. Strigari[4], S. Guadagnuolo[1,2], F. Bonifazi[2], S. Fanti[3], P.L. Zinzani[1,2]

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Radiomics involves the extraction of quantitative features from medical images, such as positron emission tomography (PET), representing potential surrogate markers of the lymphoma phenotypes. CAR-T cell therapy has revolutionized the treatment of lymphomas but the relapse rate is around 30–60% and almost 20% of patients develop severe Cytokine Release Syndrome (CRS): it is of critical importance the early identification of relapsed/refractory patients and of whom can develop CRS. Primary aim was to early predict the response to CAR-T based on metabolic features extracted from the clinical and baseline PET. Secondary aim was to determine the presence of CRS from the clinical and image-based features. Twenty patients were treated with CAR T-cell and all underwent PET evaluation at baseline (PET_0) and 1 month after (PET_1) CAR T-cell infusion. PET_0 semi-quantitative parameters, namely SUVmax, metabolic tumor volume (MTV), total lesion glycolysis (TLG), were calculated. Pyradiomics library was used for the extraction of 105 radiomics features from each image. A generalized linear model (GLM) was trained to predict the outcome and ROC analysis was used to assess the prediction capability. Patients had a wide range of baseline disease burden, with a median MTV of 129 ml and a median TLG of 971 Bq. Ten (53%) patients achieved a complete (CR) and 9 (47%) a partial response (PR). Of these 9 patients, 6 underwent re-evaluation at 3 months: 1 converted to CR, 4 had a progression to PD and 1 patient maintained the PR. No correlation was found between baseline MTV and TLG and tumor response at PET_1, while they were significantly associated with the severity of CRS ($p<0.5$) (AUC: 0.95 for MTV and AUC: 0.92 for TLG). Two radiomics features, Kurtosis and Median, were statistically significantly correlated with response at PET_1, while the surface area was statistically significantly correlated with moderate/severe CRS. In the GLM, only Median was a prognostic factor of response, with an AUC of 0.81 ($p<0.002$) while surface area was a prognostic factor of moderate/severe CRS with an AUC of 0.81 ($p<0.009$). Baseline FDG-PET radiomics features were able to differentiate between early responder and non-responder patients and between patients with/without moderate/severe CRS. Further correlation between PET_0 radiomics features/clinical baseline characteristics and patients' outcome will be investigated with larger cohort and longer follow-up.

C083

EARLY METABOLIC RESPONSE IN FOLLICULAR LYMPHOMA: A SUBSET ANALYSIS OF THE FOLL12 TRIAL BY THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: A proportion of patients with Follicular Lymphoma (FL) shows an aggressive behavior. Among the prognostic tools, the metabolic response (MR) after immunochemotherapy (ICT) (fPET) has been confirmed with a strong correlation with Progression-free Survival (PFS) and Overall Survival (OS), but only few data are available to define the role of an earlier assessment of MR during the initial ICT. We analysed patients enrolled in the FOLL12 trial, for whom MR was also assessed during the administration of ICT.

Methods: The FOLL12 trial enrolled treatment naïve patients with grade 1-3a, stage II-IV and high tumor burden FL. Complete metabolic response (CMR) was centrally assessed at End of Induction (fPET) using the Deauville scale (DS). In this study we included only patients for whom MR was also assessed during ICT between cycle 4 and 5 (iPET). iPET results were defined based on the local report and were also centrally reviewed applying standard DS. The primary endpoint was PFS.

Results: iPET was performed in 211/807 patients and local report was available in 186 cases, 48% of whom were older than 60 years, 37% had a high-risk FLIPI2, 44% received RB as ICT. Based on local report, iPET was considered positive in 38/186 patients (20%). iPET and fPET were both available for comparison in 174 cases and showed a concordance rate of 82%: 131 out of 140 iPET- confirmed their CMR at fPET (94%). Regarding the 31 iPET+, a fPET- was achieved in 23 cases (68%). In univariable analysis, the 3-year PFS was lower for the iPET+ patients compared to the iPET- (52% *vs* 87%: HR 2.73 (1.51–4.95)) (Figure 1). Considering both iPET and fPET, a positive iPET was associated with an increased risk of progression also if a negative fPET was achieved (HR 2.09 (3.22–19.5)) (Figure 1). iPET was also associated with a different 3-year OS (99% *vs* 89% for iPET- *vs* +; p=0.035). In multivariable analysis the prognostic role of iPET for PFS was confirmed (HR 2.60 (1.41–4.79)) and was independent from FLIPI2 (0-2 *vs* 3-5 HR 1.88 (1.05–3.35)), and for ICT (RB *vs* R-CHOP HR 1.39 (0.77–2.51)). The centralized review of iPET response according to DS is ongoing.

Conclusions: Early MR has a strong prognostic role for PFS in patients with advanced stage FL treated with standard ICT. Considering the higher rates of iPET+ cases compared to fPET, iPET may better contribute to anticipate the identification of FL patients at different risk of progression.

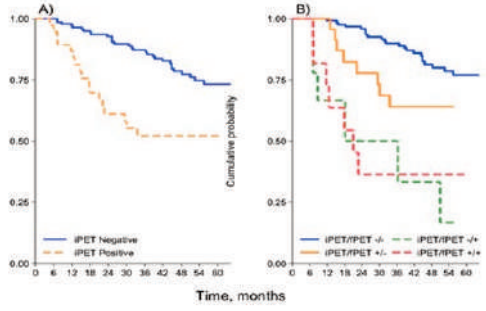

Figure 1. A) Progression free Survival (PFS) by interim metabolic response evaluated with FDG-PETR at cycle 4 of induction therapy (iPET); B) PFS by iPET and metabolic response at end of induction PET (fPET).

Figure 1.

C084

A SIMPLIFIED GERIATRIC ASSESSMENT CAN BE APPLIED TO GUIDE TREATMENT DECISIONS IN LATE-OCTOGENARIAN (LO) PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL). AN ANALYSIS OF 370 PATIENTS OF THE "ELDERLY PROJECT" BY THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction: Elderly patients (pts) with DLBCL are progressively increasing. Treatment with anthracyclines and rituximab is potentially curative but it is not easy to identify pts candidates for this type of treatment, especially in the late octogenarians (>84 years)(LO). Several studies on elderly pts have been conducted, but the median age was usually less than 84 years (y). The Elderly Project (EP) prospectively analyzed elderly pts with DLBCL applying a sGA and creating an elderly prognostic index (EPI) (Merli, JCO 2021).

Aims and Methods: The aim of this study is to evaluate the overall survival (OS) of elderly pts with DLBCL, focusing on LO, in order to define the best therapeutic strategy. The clinical characteristics and outcome of octogenarian pts enrolled in the EP were analysed and stratified by age, sGA and EPI. The type of treatment was defined based on the dose of anthracyclines administered (full dose therapy (FDT): 70-100% anthracyclines, reduced dose therapy (RDT): <70%, palliation (PLT): 0%) and the therapeutic choice was left to the individual clinician.

Table 1.

Factor	Age group		p-value
	80-84 n=241	>84 n=129	Fisher's exact test
CGA			0.100
UNFIT	116 (48)	50 (39)	
FRAIL	125 (52)	79 (61)	
Gender			
M	118 (49)	52 (40)	0.126
F	123 (51)	77 (60)	
IPI			0.820
1	30 (14)	18 (16)	
2	59 (27)	28 (25)	
3-5	131 (59)	65 (59)	
Hb, g/dL			0.738
≥12	120 (52)	67 (54)	
<12	112 (48)	57 (46)	
Stage			0.058
I-II	67 (28)	48 (38)	
III-IV	174 (72)	79 (62)	
ECOG PS			0.392
0-1	180 (75)	91 (71)	
>1	61 (25)	38 (29)	

Results: Among the 1163 pts enrolled in the EP, 241 early octogenarians (EO, 80-84 years) and 129 LO were identified; table 1 shows their clinical characteristics. Overall, 3 y-OS was 51% and median follow up 30 ms (range 1-59 ms). Survival was significantly lower in pts with high *vs* intermediate EPI score (41 *vs* 71% p<0.001), in frail *vs* unfit pts (43 *vs* 60% p 0.001), and in LO *vs* EO pts (37 *vs* 57% p 0.001). FDT did not improve survival compared to RDT (3y OS 62% *vs* 61%), whereas survival of those who received PLT was lower (3y OS 27%). It should be noted that LO pts received more often PLT than EO (50% *vs* 23%), despite having similar clinical and geriatric characteristics, and that in octogenarians the 3-y survival in those who received RDT was independent of age (70% EO *vs* 69% LO). Furthermore, the outcome of pts receiving PLT was improved when RTX was included (OS 9% *vs* 39% at 3y).

Conclusions: The results of this study demonstrate that in LO patients with DLBCL chronological age should not be a precluding factor for the curative intent approach; a sGA should always be integrated in the clinical evaluation in order to identify pts who can tolerate treatment and can also be cured with reduced doses. The addition of RTX can improve the results obtained with PLT in frail pts.

C085

COMPARISON BETWEEN R-COMP AND R-CHOP IN OLDER PATIENTS WITH DIFFUSE LARGE B_CELL LYMPHOMA (DLBCL). A SUBSTUDY OF THE ELDERLY PROJECT BY THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Introduction: Non-pegylated liposomal doxorubicin (NPLD) is considered a good alternative to conventional doxorubicin for the treatment of older patients (pts) with aggressive lymphomas and/or at high risk for cardiological toxicity. The use of R-COMP for the treatment of older pts with DLBCL has been supported by several small retrospective studies. In this report we describe the characteristics and outcomes of pts who were prospectively enrolled in the Elderly Project (EP) and who were treated with R-COMP and compared them with pts treated with conventional R-CHOP.

Methods: This analysis was conducted starting from the dataset of the EP study. The use of NPLD was allowed according to 648/96 law, treatment decision was left to physician discretion and was independent of frailty status. For the purposes of this analysis, we included all pts who were treated with full doses of R-CHOP and R-COMP. The study endpoint were progression free survival (PFS) and overall survival (OS). A propensity score analysis was conducted to account for the main confounding factors.

Results: Overall 691 out of 1163 pts of the EP were treated with R-CHOP (383; 55%) or R-COMP (308; 45%); median age was 71 and 76 years for R-CHOP and R-COMP, respectively (p<0.001) (Table 1). Pts were similarly distributed among different IPI groups for R-COMP or R-CHOP. Based on simplified Geriatric Assessment (sGA) 88%, 11% and <1% of the R-CHOP treated pts and 61%, 32% and 6% of the R-COMP pts were FIT, UNFIT, and FRAIL (p<0.001). Elderly Prognostic Index (EPI) score was low, intermediate, and high in 39%, 54% and 8% of R-CHOP pts and 27%, 49% and 24% of R-COMP pts (p <0.001). PFS at 3-years was 70% for R-CHOP and 64% for R-COMP (p=ns). OS at 3-years was 77% for R-CHOP and 71% for R-COMP (p=ns). The propensity score analysis was conducted in 610 pts and confirmed no significant differences in terms of PFS and OS in the comparison between R-CHOP and R-COMP treated pts (PFS; HR 1.17, 95% CI 0.84-1.63; OS: HR 1.04 95% CI 0.64-1.48). No differences were registered in terms of interruption of treatment due to toxicities (7% for R-CHOP and 11% for R-COMP).

Conclusions: Data from the prospective observational EP study did not show significant differences in terms of efficacy comparing R-COMP to standard R-CHOP. The higher frequency of UNFIT and FRAIL pts among those treated with NPLD suggests R-COMP is a good strategy to offer a curative treatment to these groups of pts.

Table 1. Characteristics of 691 patients treated with R-CHOP or R-COMP.

Characteristics	R-CHOP	R-COMP	Total	NA	p-value
Age, median (range)	71 (65-87)	76 (65-88)	73 (65-88)	-	<0.001
Age >80, n (%)	8 (2)	67 (22)	75 (11)	-	<0.001
Gender M, n (%)	177 (46)	155 (50)	332 (48)	-	0.285
IPI 3-5, n (%)	188 (51)	158 (55)	346 (53)	37	0.344
Hb, median (range)	12.5 (5.8-17.2)	12.4 (7.1-17.5)	12.5 (5.8-17.5)	14	0.228
Hb <12 g/dL, n (%)	131 (34)	129 (43)	260 (38)	14	0.021
B-symp., n (%)	85 (22)	92 (30)	177 (26)	-	0.023
Bulky yes, n (%)	122 (32)	85 (28)	207 (30)	11	0.315
sGA				-	<0.001
FIT	338 (88)	189 (61)	527 (76)		
UNFIT	44 (11)	100 (32)	144 (21)		
FRAIL	1 (<1)	19 (6)	20 (6)		
EPI score				43	<0.001
0-1	141 (39)	76 (27)	217 (33)		
2/5	196 (54)	139 (49)	335 (52)		
6/8	28 (8)	68 (24)	96 (15)		

Myeloma and Monoclonal Gammopathies 2

C086

PROGNOSTIC IMPACT OF GAIN AND AMPLIFICATION OF 1Q IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS RECEIVING CARFILZOMIB-BASED TREATMENT IN THE FORTE TRIAL

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Background: Copy-number alterations of chromosome 1q are frequently found in multiple myeloma (MM) and are associated with poor prognosis. Recently, it has been demonstrated that the number of 1q copies correlates with a high-risk behavior. We aimed to dissect the role of Gain1q (3 copies of 1q) *vs* amplification 1q (Amp1q, ≥4 copies of 1q) in carfilzomib-treated newly diagnosed (ND)MM patients (pts) enrolled in the randomized FORTE trial (NCT02203643).

Methods; Fluorescence *in situ* hybridization (FISH) in CD138+ purified bone marrow plasma cells was centralized and performed at baseline. The cut-off level for Gain1q was 10% of nuclei with ≥3 copies of 1q, while Amp1q was defined as ≥20% of nuclei with ≥4 copies of 1q.

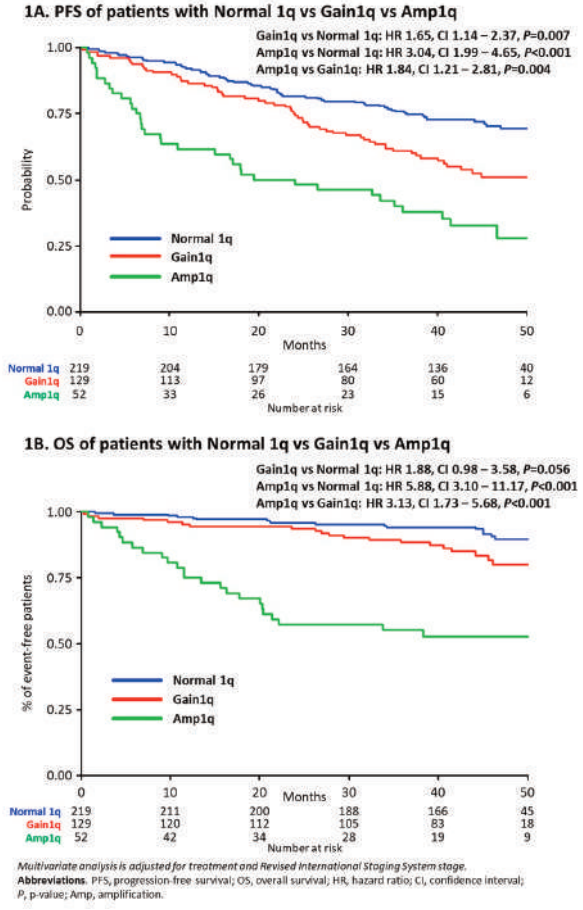

Figure 1.

Results: 474 pts were enrolled. Median follow-up was 45 months (m). Evaluation of 1q by FISH was missing in 70 pts (15%), while in 4 pts (1%) FISH was present but the number of 1q copies was not evaluable. Among evaluable pts, chromosome 1q was normal in 219 (55%) pts, Gain1q was found in 129 (32%) pts, while Amp1q in 52 (13%). Gain1q- and Amp1q-positive pts were well distributed among treatment arms. In the Amp1q group, we observed an enrichment of pts with lactate dehydrogenase (LDH) > upper limit of normal (p=0.002), as compared to Gain1q. In a multivariate analysis, the risk of progression/death was significantly higher with Gain1q *vs* Normal 1q (HR 1.65, 95% CI 1.14-2.37, *p*=0.007) and the highest with Amp1q *vs* both Normal 1q (HR 3.04, 95% CI 1.99-4.65, p<0.001) and Gain1q (HR 1.84, 95% CI 1.21-2.81, *p*=0.004; Figure 1A). Median progression-free-survival (PFS) was not reached in the Normal 1q group, while Gain1q (53 m) and especially Amp1q (21.8 m) groups performed very poorly. The presence of Amp1q *vs* Normal 1q (HR 5.88, 95% CI 3.10-11.17, p<0.001) and Gain1q (HR 3.13, 95% CI 1.73-5.68, p<0.001) predicted a shorter overall survival as well (Figure 1B). Gain1q predicted a shorter PFS compared to Normal 1q in the presence of concomitant standard-risk features (International Staging System [ISS] 1, ISS 2, standard-risk cytogenetics), but not in the presence of high-risk disease (ISS 3, high-risk cytogenetics). On the other hand, the worse prognosis of Amp1q pts was confirmed across all subgroups.

Conclusion. This is a first report on the prognostic role of the number of 1q copies in carfilzomib-treated NDMM pts. Having ≥4 copies of 1q universally predicts a very poor PFS and OS despite the use of a 2nd-generation proteasome inhibitor upfront.

C087

IMPACT OF IMAGING FDG PET/CT MINIMAL RESIDUAL DISEASE ASSESSMENT ON OUTCOMES AND COMPLEMENTARITY WITH MULTIPARAMETER FLOW CYTOMETRY IN NEWLY DIAGNOSED TRANSPLANT ELIGIBLE MULTIPLE MYELOMA (MM) PATIENTS ENROLLED IN THE PHASE II RANDOMIZED FORTE TRIAL

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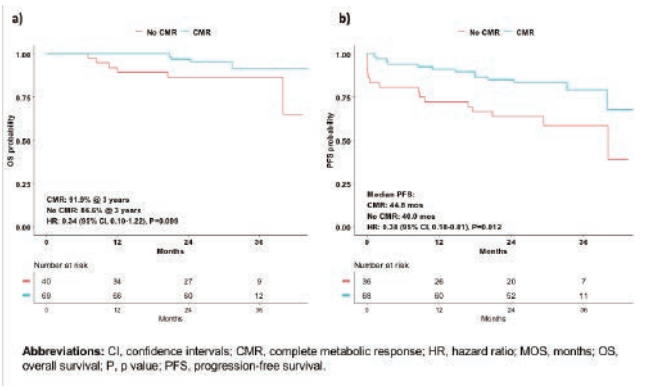
European Myeloma Network

Background: 18F-FDG-PET/CT is the standard technique to define imaging-minimal residual disease (MRD) in multiple myeloma (MM) patients (pts) and proved to be complementary to Multiparameter Flow Cytometry (MFC). The definition of complete metabolic response (CMR) has been recently standardized by the application of Deauville scores (DS). In this analysis, we aimed at confirming the applicability of DS criteria to define PET CMR, the complementarity with MFC and their impact on patient's outcomes in the multicenter phase II randomized FORTE trial for NDTEMM patients.

Methods: 474 newly diagnosed MM pts ≤ 65 years were randomized to receive carfilzomib, lenalidomide, dexamethasone (KRd)_autologous stem cell transplantation (ASCT) *vs* carfilzomib, cyclophosphamide, dexamethasone (KCd)_ASCT *vs* KRd12 and, thereafter, to KR *vs* R maintenance. PET/CT scans were performed locally at baseline (B) and prior to the start of maintenance (PM). DS were applied both in the BM and FLs; CMR was defined as DS < 4 in both localizations (FS and BMS). MFC was performed by 8-color second-generation flow cytometry (sensitivity 10-5) in pts who achieved at least VGPR PM.

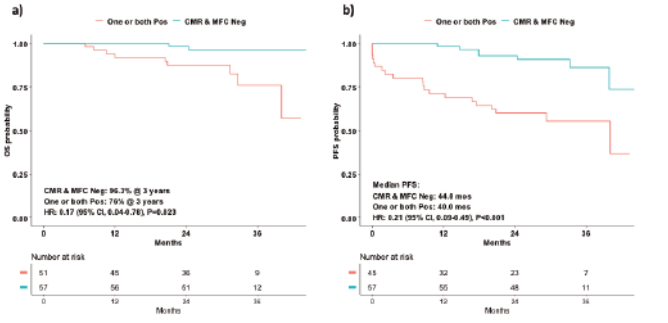
Results: 182/474 pts enrolled in the trial, reflecting baseline clinical features of the entire population, had a B and PM PET/CT evaluation available and were included in this analysis. At B, FS and BMS ≥ 4 were present in 87% and 57% of pts, respectively. At PM, 63% showed CMR. 73% of the pts achieved MFC MRD negativity. In univariate analysis, at Landmark time PM, FS <4 significantly influenced both PFS and OS (HR 0.32, p=0.003; HR 0.28, p=0.038, respectively). Achievement of CMR was a strong predictor for prolonged PFS (HR 0.38, p=0.012), with a trend on OS (HR 0.34, p=0.099) (Figure 1). Patients achieving both CMR and MFC negativity at PM showed significantly extended PFS and OS (HR 0.21, p<0.001; HR 0.17, p= p0.023, respectively) (Figure 2). In Cox multivariable analysis achievement of CMR and MFC MRD negativity were independent predictors of both PFS and OS (HR 0.17, p<0.001 and HR 0.21, p=0.046, respectively for PFS and OS).

Conclusion: In conclusion, the present analysis confirms the applicability and validity of DS criteria to define PET/CT CMR in an independent prospective series of NDTEMM patients. CMR significantly and independently correlated in uni- and multivariable analysis with patient's outcomes in terms of PFS and OS and was complementary to the MFC MRD negativity.



Abbreviations: CI, confidence intervals; CMR, complete metabolic response; HR, hazard ratio; MOS, months; OS, overall survival; P, p value; PFS, progression-free survival.

Figure 1. OS (a) and PFS (b) according to CMR.



Abbreviations: CI, confidence intervals; CMR, complete metabolic response; HR, hazard ratio; MFC, multiparameter Flow cytometry; MOS, months; OS, overall survival; P, p value; PFS, progression-free survival.

Figure 2. OS (a) and PFS (b) according to CMR combined with MFC negativity.

C088

THE ROLE OF UPFRONT AUTOTRANSPLANTATION AND CONSOLIDATION THERAPY IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): FINAL ANALYSIS OF THE RANDOMIZED PHASE 3 EMN02/HO95 STUDY

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The phase 3 EMN02/HO95 study was aimed at comparing intensification therapy with upfront ASCT (either single or double) *vs* bortezomib-melphalan-prednisone (VMP) (R1), and subsequent bortezomib-lenalidomide-dexamethasone (VRD) consolidation therapy *vs* no consolidation (R2), followed by lenalidomide maintenance in NDMM pts aged ≤ 65 years. Results from the final analysis from R1 showed that at a median follow-up of 60.5 months from R1, PFS (the primary study endpoint) was significantly improved with ASCT compared with VMP (median, 57 versus 42 months; HR 0.73, 95% CI 0.62-0.85, adjusted p=0.0001), but not OS (a secondary endpoint). At an extended median follow-up of 70 mos, the final analysis from R2 showed a significant improvement in PFS with VRD consolidation therapy *vs* no consolidation (HR 0.80, 95% CI 0.68-0.96, p=0.016). Estimated rates of OS from R1 (a secondary endpoint) for patients randomized to ASCT or VMP were 69% *vs* 63% (HR 0.81, 95% CI 0.66-0-98, p=0.034). The OS benefit with ASCT was greater for patients with ISS disease stage 2-3 (p=0.047), R-ISS stage 2-3 (p=0.042), and a high-risk cytogenetic profile (p=0.010), and it was the greatest for patients with del(17p) positivity (HR 0.49, 95% CI 0.28-0.86, p=0.013). PFS on next-line therapy (PFS2) from R1 was significantly longer for patients in the ASCT group than for those in the VMP group (HR 0.76, 95% CI 0.64-0.90, p=0.002). Patients randomized to ASCT had a significantly longer time to next treatment (TTnT) in comparison with those who were randomly assigned to VMP (HR 0.71, 95% CI 0.60-0.82, p <0.001). Demographic and clinical characteristics at baseline of patients randomized to upfront ASCT or who received salvage ASCT at the time of relapse following randomization to VMP were comparable. PFS2 and OS in the upfront ASCT group were significantly longer than in the delayed ASCT group (HR 0.52, 95% CI 0.40-0.66, p<0.001; and HR 0.68, 95% CI 0.51-0.93, p= 0.016, respectively). Final results from this study, the largest academic one so far conducted, provided demonstration that upfront ASCT significantly prolonged PFS, OS, PFS2 and TTnT compared with VMP, and that consolidation therapy significantly reduced the risk of progression or death *vs* no consolidation. Patients randomized to upfront ASCT had a significantly longer PFS2 and OS compared with those who received delayed ASCT.

C089

PREDICTIVE ROLE OF DIFFUSION WEIGHTED WHOLE BODY MRI (DW-MRI) IMAGING RESPONSE ACCORDING TO MY-RADS CRITERIA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA AND COMBINED EVALUATION WITH MRD ASSESSMENT BY FLOW CYTOMETRY

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Introduction: Diffusion weighted whole body MRI (DW-MRI) is increasingly used in Multiple Myeloma (MM), but consistent data regarding its prognostic role in the detection of residual disease after treatment are scarce. The Myeloma Response Assessment and Diagnosis System (MY-RADS) imaging recommendations proposed criteria for Response Assessment Category (RAC) with a 5 point scale in order to standardize response assessment after therapy, but this score still needs to be validated. We performed an external validation of RAC criteria in newly diagnosed MM pts in order to evaluate the prognostic role of this technique after autologous transplantation (ASCT) performed as part of first line treatment strategy. Furthermore, we combined the results of MY-RADS with those of minimal residual disease (MRD) assessment by 8 color flow cytometry (FCM, Sn 10^{-5}).

Methods: We retrospectively analyzed the outcome of 64 MM pts diagnosed between Jan 2016 to Jan 2020 who underwent DW-MRI evaluation at day +100 after ASCT (Mel200 conditioning). The predictive role of MRI RAC response on PFS and OS was analyzed.

Figure 1 – post ASCT PFS according to DW-MRI response and MRD

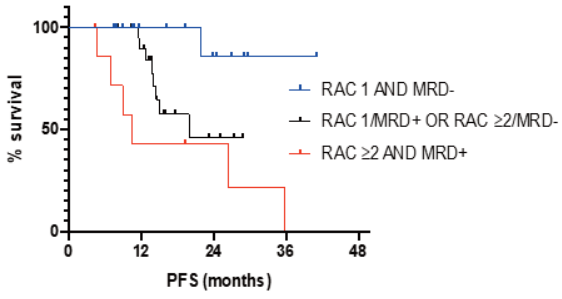

Figure 1.

Results: out of 64 pts, 23 (36%) were ISS stage 3 and 14 (22%) showed high risk cytogenetics. Single ASCT was performed in 41 pts (64%), whereas 23 pts (36%) received double ASCT. Response rates were: VGPR 23%, CR 47% and sCR 14%. MRD after ASCT was available for 46 pts and was positive in 21 (46%). According to MY-RADS,

a complete imaging response after transplant was observed in 37 pts (RAC1: 58%); some residual MM was identified in 27 (42%) [RAC2: 22 (34%), RAC3: 4 (6%), RAC4: 1 (2%)]. After a median follow up of 29 months, a significantly better post ASCT PFS and OS were observed in pts with complete imaging response (RAC1) compared to pts with imaging residual disease (RAC≥2): median PFS not reached (NR) *vs* 26,5 months [p 0,0047, HR 0,28 (95% CI: 0,12-0,68)]; 3-year post ASCT OS 92% *vs* 69% for RAC1 *vs* RAC≥2, respectively [p 0,047, HR 0,24 (95% CI: 0,06-0,99)]. Combining MRD and imaging improved prediction of outcome, with double-negative and double-positive features defining groups with excellent and dismal PFS, respectively: NR *vs* 10,6 months [p 0.001, HR 0,07 – 95% CI: 0,01-0,36)] Figure 1.

Conclusion: The present study supports the applicability of MY-RADS recommendations in MM pts after ASCT; RAC criteria were able to independently stratify pts and to better predict their prognosis. The combined use of DW-MRI with FCM allowed a more precise evaluation of MRD.

C090

A REAL-LIFE STUDY OF DARATUMUMAB-BORTEZOMIB-DEXAMETHASONE (DVD) IN LENALIDOMIDE EXPOSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: A REPORT FROM THE MYELOMA TRIVENETO WORKING GROUP

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Treatment of Lenalidomide refractory (Len-R) Multiple Myeloma (MM) patients still represents an unmet medical need. Up to now, only the OPTIMISMM study evaluating the efficacy of Pomalidomide-Bortezomib-Dexamethasone (PVD) recruited a high percentage of Len-R patients, however this combination was only recently approved. Consequently, in the last years Daratumumab-Bortezomib-Dexamethasone (D-VD) combination was extensively used in this setting, even though only a small fraction of Len-R patients was included in the pivotal trial In this context, the aim of this real-life study was to evaluate the efficacy and the safety of D-VD in Lenalidomide exposed or refractory patients The study cohort included 57 patients (median age 69 years) affected by relapsed/refractory MM. All patients were previously exposed to Lenalidomide, with 77.2% being refractory. Moreover, 89% of cases received ad least a proteasome inhibitor (PI), 17.5% of them being PI refractory. Median line of previous therapy was 2 (1-6), with 22/57 (39%) having received ≥2 lines of therapy. FISH analysis at relapse was available in 30/57 (52.6%) cases and high-risk FISH according to R-ISS was detected in 33.3% of patients. Responses were assessable in 54/57 patients, with overall response rate (ORR) of 79.6% and 43% of cases obtaining at least a Very Good Partial Response (VGPR). D-VD regimen showed a favorable safety profile, with low frequency of grade 3-4 adverse events, except for thrombocytopenia in 21.4% of patients. With a median follow up of 13 months, median progression free survival (PFS) and overall survival (OS) were 17 months and not reached, respectively. Patients achieving at least a VGPR showed improved PFS and OS as compared to patients who did not (p=0.0005 and p=0.0443, respectively). No significant PFS differences were found according to previous lines of therapies (≤2 or >2, 20 *vs* 15 months, p=0.2682) or to high-risk FISH (16 *vs* 20 months, p=0.5432). Len R patients displayed reduced median PFS (16 months) as compared to no Len-R patients (29 months), although not statistically significant (p=0.2876). In conclusion, D-VD represents a reliable therapeutic option in previously Lenalidomide treated patients, with high ORR and VGPR rates and favorable safety profile. Finally, even though a higher validation cohort is required, the benefit of this regimen in Len-R patients in real life is remarkable, placing D-VD as one of the standard of care in this setting.

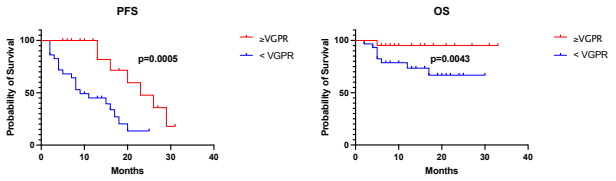

Figure 1.

Chronic Lymphatic Leukemias and other Chronic Lymphoproliferative Syndromes 2

C091

PREVALENCE OF SECOND CANCER DURING LONG TERM FOLLOW-UP IN HAIRY CELL LEUKEMIA PATIENTS TREATED WITH CLADRIBINE: A THIRTY-YEAR EXPERIENCE

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Hairy cell leukemia (HCL) is a lymphoproliferative disease with an excellent prognosis after therapy with purine analogs. Conflicting results have been published concerning second cancer in these patients:we aim to report on the prevalence of second cancer among HCL patients treated with cladribine (2CDA) in first line in the last 30 years. We retrospectively reviewed data of patients treated with 2CDA between March 1991 and May 2019 at 18 Italian Hematological centers. Among 513 evaluable patients (because treated with 2CDA alone), M/F ratio was 4.5 with a median age of 54 years (range 24-88) and ECOG was 0 in 85% of cases. Twenty-seven (5%) patients were diagnosed with a previous cancer: no relapse was reported during follow-up. At a median follow-up of 6.96 years (range 3.81-12.47), 19 second neoplasms were reported in 18 patients (3.5%), lower than what reported in the literature. Six patients had a cancer of urinary tract (4 prostate, 2 kidney, 1 bladder), 5 had a GI cancer (1 esofagus, 1 stomach, 3 colorectal), 4 had an hematological neoplasm (2 multiple myeloma, 1 mantle cell lymphoma and 1 diffuse large B cell lymphoma), 2 had breast cancer and 1 had lung cancer. Precancerous lesions were reported in 1.2% of patients: 4 presented with skin cancer (3 basocellular carcinoma and 1 dysplastic nevus), 1 with pleomorfic parotid adenoma and 1 with colorectal displastic adenoma. Surgical therapy alone was a curative option in 8 patients, while chemotherapy alone was reported in 5 cases. A combination of surgery and chemotherapy +/- hormone therapy was used in 5 cases. Median OS of the population was not reached; 95.7%, 92.8% and 82.3% of patients are expected to be alive at 5, 10 and 15 years, respectively. Among 45 dead patients, 6 cases (1.2%) were due to second cancer. Comparing our data with the Italian registries of cancer for the same period of observation, among the general population the cumulative standard incidence of cancer was 704.4 casesx100.000 inhabitants for males and 484.7 casesx100.000 inhabitants for females. In our population, the cumulative standard incidence of cancer was 543.3 x 100,000 (IC 95%: 283.6-803.1) for males and 492.6 x 100,000 (IC 95%: 0.0-1099.0) for females. No statistically significant differences were identified between the two cohorts. While 2CDA is greatly effective in treating HCL, the occurrence of second cancer is rare. The cumulative incidence of second neoplasms in our population did not significantly differ from that reported among the Italian population.

C092

DIGITAL DROPLET PCR (DDPCR) FOR THE ASSESSMENT OF DISEASE BURDEN IN HAIRY CELL LEUKEMIA

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Background: BRAF-V600E mutation is the pathogenic driver of hairy cell leukemia (HCL), as it is found in the vast majority of cases (> 95%) both at onset and during recurrences. It is absent in other lymphoproliferative diseases thus being highly specific for HCL. The identification of the mutated allele in blood and marrow correlates with the presence of neoplastic cells, therefore it may be considered a marker of active disease. Likewise, the absence of the mutation after treatment may indicate a state of deep response.

Methods: The allelic burden of BRAF-V600E was measured by digital droplet PCR (ddPCR) and expressed as fractional abundance (FA) in 35 HCL patients at different stages of disease (onset, relapse, complete response [CR] after treatment, long-term remission), for an overall number of 55 assays. Peripheral blood (PB) was preferentially used (39/55 assays). Bone marrow (BM) was tested in 16/55 assays. PB was collected in 12 patients at diagnosis, in 7 patients at relapse, in 6 patients at post-treatment assessment of CR and in 14 patients in CR for more than 5 years. BM was analyzed in 4 newly diagnosed cases, 6 relapsed cases and in 6 patients at response.

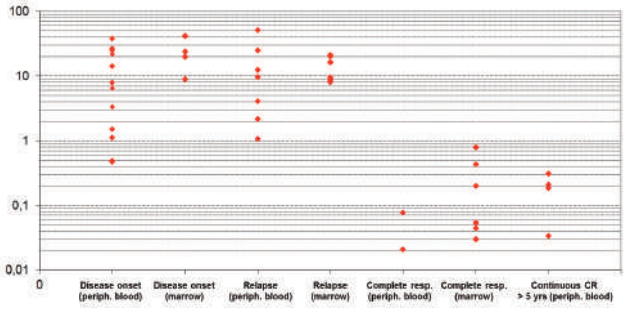

Figure 1.

Results: Mean FA values in PB for patients at diagnosis, relapse and response were 12.26%, 15.07% and 0.02%, respectively. Likewise, mean FA in BM was 23.51%, 13.96% and 0.26%, respectively. Importantly, 4 patients out of 6 evaluated at response were molecularly negative for BRAF-V600E in PB. Mean FA in PB for the 14 patients with long lasting complete response was 0.05%. Ten patients out of 14 achieved a BRAF-V600E negativity in PB. Point values for each of the performed assay are represented in figure (logarithmic scale). Figure shows that BRAF-V600E FA varies considerably when patients with active disease and patients in complete response are considered. In this case series, patients

in CR may still display a positive BRAF-V600E burden, but generally below 1%. Some patients, instead, are indeed molecularly negative.

Conclusions: These preliminary results suggest that ddPCR permits to assess the active tumor burden in HCL at different stages of disease and support the hypothesis that some patients in CR qualify for a complete molecular response. Data are not conclusive on the comparability of PB and BM. An ongoing study is aimed at following patients prospectively by sampling serially PB and BM at each stage (onset, response, relapse, follow-up).

C093

VENETOCLAX AND RITUXIMAB (VENR) FOR THE FRONT-LINE TREATMENT OF YOUNG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND AN UNFAVORABLE BIOLOGIC PROFILE. PRELIMINARY RESULTS OF THE GIMEMA VERITAS STUDY

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The efficacy and safety of the venetoclax and rituximab (VenR) combination has been investigated in a multicenter study of the GIMEMA group (VERITAS study) that included young patients (≤65 years) with previously untreated CLL and an unfavorable biologic profile, an unmutated IGHV and or a TP53 disruption. Treatment consisted of the Ven dose ramp-up (from 20 to 400 mg daily over 5 weeks) followed by Ven 400 mg daily, combined with R for six 28-day courses (375 mg/m2, course 1; 500 mg/m^2, courses 2-6). Patients continued with Ven single agent, 400 mg daily, until month 13. Tumor lysis syndrome (TLS) prophylaxis measures included hydration, allopurinol, or rasburicase. Response assessment included clinical examination, PB and BM evaluation, BM biopsy, and CT scan. MRD was tested centrally in the PB and BM by 8 color flow-cytometry with a sensitivity of at least 10–4. MRD was further evaluated by ASO-PCR with a sensitivity up to 10–5 in patients who showed an undetectable (u) MRD4 by flow-cytometry both in the PB and BM. This study included 75 patients with a median age of 53 years (range 38-65), 56 males, and 37 (84%) patients with Binet stage B/C. Deletion 17p was recorded in 4 (6%) cases and aTP53 mutation in 8 (11%). Seventy-two (96%) patients were IGHV unmutated and 3 (4%) IGHV mutated carried a TP53 mutation. Response at the end of the VenR combination was achieved by 72 (96%) patients and included 41 (55%) CRs and 31 (41%) PRs. Early discontinuation of treatment due to an adverse event (AE) was censored as a treatment failure in 3 (4%) patients. A response with uMRD4 by flow-cytometry was recorded in 61 (81%) cases in the PB, and in both the PB and BM in 41 (57%), while no detectable disease by ASO-PCR, both in the PB and BM, was recorded in 17 (23%). After a median follow-up of 13 months, no patient has progressed with a 12-month progression-free survival of 97.3%. A transient laboratory TLS was observed in 2 patients. Grade ≥3 AEs included neutropenia in 25 (33%) patients, while grade ≥3 infections were recorded in 7 (9%) and included COVID-19 pneumonia in 3. Two patients (2.7%) died due to severe neurologic toxicity related to the concomitant administration of fentanyl in 1 and Covid19 pneumonia in 1. In conclusion, the preliminary results of this study demonstrate the high efficacy in young patients with CLL and an unfavorable biologic profile of the frontline VenR combination, which resulted in a high proportion of CRs and responses with uMRD4.

C094

ABSTRACT WITHDRAWN

C095

OBINUTUZUMAB PLUS CHLORAMBUCIL VERSUS IBRUTINIB IN PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA WITHOUT TP53 DISRUPTIONS. A CAMPUS CLL STUDY

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Introduction: Although, Ibrutinib (IB) and obinutuzumab (G) have significantly improved the treatment landscape of chronic lymphocytic leukemia (CLL), no head-to-head comparison has been reported for IB *vs* G-chlorambucil (G-CHL) in CLL patients.

Aim: The aim of this study was to compare the clinical efficacy of G-CHL and IB in a real-life retrospective study within the Italian CLL Campus network.

Methods: Patients received ibrutinib 420 mg daily until progression or unacceptable toxicity, while G was administrated at 100 mg on day 1, 900 mg on day 2 and 1000 mg on days 8 and 15 of the 1st cycle, then at 1000 mg for cycles 2-6. An IGHV gene sequence homology >98% was considered as unmutated (U-IGHV), as opposed to mutated (M-IGHV). Progression-free survival (PFS), time-to-next treatment (TTNT) and overall survival (OS) were compared with the log-rank test. Minimal residual disease (MRD), assessed by flow cytometry, was considered undetectable when $<10^{-4}$ (uMRD4). A propensity score matching analysis 1:1 was also done, caliper 0.2. The study was approved by the Ethic Committee.

Results: This study included patients without TP53 disruption who received IB (102 patients) or G-CHL at 16 hematologic centers till December 2020. Clinical features of enrolled patients are summarized in Table 1. Patients in treated with G-CHL had a higher CIRS score (p=0.0015), lower creatinine clearance (p=0.0041) and were enriched in M-IGHV cases (p=0.0004). The best overall response rates in the G-CHL and IB arms were 87% *vs* 77%, including 25% *vs* 6% complete remissions (CR, p=0.0029). After a median follow-up of 30 months, the PFS, TTNT and OS was 70% *vs* 93% (p=0.0061), 88% *vs* 97% (p=0.0043) and 91% *vs* 96% (p=0.6642) for the G-CHL and IB arms, respectively. In the G-CHL arm the depth of response in terms of iwCLL responses (No response *vs* partial remission *vs* CR: 30-month PFS, 38%, 68% and 79%; p<0.0001) and responses with uMRD in the PB influenced PFS (data on 87 patients: 30-month PFS, 78% *vs* 53% for uMRD4 *vs* MRD+, p=0.0203). PFS and TTNT were better with IB than G-CHL in U-IGHV (p=0.0190 and 0.0137, Figure 1A and 1C), while they were superimposable for M-IGHV patients (p=0.1900 and 0.1380, Figure 1B and 1D). Similar results were found after patients matching analysis.

Conclusions: Although continuous ibrutinib provides a better disease control in CLL, M-IGHV patients and those achieving an uMRD4 show a marked clinical benefit from a fixed-duration obinutuzumab-based therapy.

Table 1

	G-CHL	IB	p values
VARIABLES			
Age (years)	74.7 ± 6.6	69.2 ± 6.9	0.1064
Male/Female	68 / 35	43 / 38	0.0935
median CIRS	6 (2-18)	4 (0-12)	0.0009
Cl. Creatinine	61±17ml/min	67±16ml/min	0.0011
Stage III-IV	61 (59%)	17 (46%)	0.0743
U / M-IGHV	56 / 47	59 / 21	0.0087
11q deletion	11 (11%)	13	0.5363
RESPONSE			
CR	26 (25%)	5 (6%)	
PR	64 (62%)	64 (80%)	0.0029
SD / PD	13 (13%)	11 (14%)	
MRD STATUS			
U-MRD4	44 (43%)	-	
MRD+	43 (42%)	-	-
not assessed	16 (16%)	80 (100%)	

Figure 1

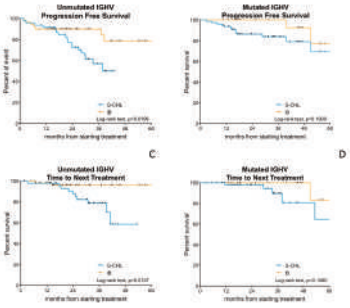

Table 1 and Figure 1.

Chronic Myeloproliferative Diseases 2

C096

CPI-0610, A BROMODOMAIN AND EXTRATERMINAL DOMAIN (BET) PROTEIN INHIBITOR, AS "ADD-ON" TO RUXOLITINIB, IN ADVANCED MYELOFIBROSIS PATIENTS WITH SUBOPTIMAL RESPONSE: UPDATE OF MANIFEST PHASE 2 STUDY

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CPI-0610, a first-in-class, oral, small-molecule inhibitor of bromodomain and extraterminal domain (BET) proteins, potentially promotes disease-modifying activity through altered gene regulation of key oncogenic, fibrotic, and inflammatory factors and may transform the standard of care in myelofibrosis (MF). Here we present results from Arm 2 of the ongoing Phase 2 MANIFEST study, investigating CPI-0610 as "add-on" to rux in advanced MF pts with suboptimal response to rux. Pts are stratified as transfusion dependent (TD, defined as ≥2U RBCs/month over 12 wks), and non-transfusion dependent (non-TD). Eligibility: MF pts having a suboptimal or lost response to rux; DIPSS ≥Int-2; platelets ≥75 x 10^9/L; ≥2 symptoms measurable (score ≥1) per MFSAF v4.0; RBC TD (TD cohort) or spleen volume of ≥450 cc by CT/MRI (non-TD cohort). Pts treated with rux for ≥6 months and on a stable dose for ≥8 wks prior to enrollment. Rux dose escalation is not allowed during the study. Primary endpoints: TD cohort: TD to TI (transfusion independence) [defined as no transfusion for 12 wks per IWG-MRT criteria]; non-TD cohort: SVR35 response (≥35% spleen volume reduction) at wk 24. Secondary endpoints: TSS50 response (≥50% total symptom score reduction) per MFSAF v4.0 at wk 24, safety and PK. As of 29 Sep 2020, 52 pts were treated in the TD cohort (median treatment duration 30 wks, range: 1, 166 wks). 36% (13/36) of TD pts converted to TI 12 weeks. At wk 24, 21% (7/33) pts achieved SVR35 (median % change from baseline: -19%, range: -54%, 48%), and 46% (15/33) pts achieved TSS50 (median % change from baseline: -58%, range: -100%, 24%). In non-TD cohort, 26 pts were treated (median treatment duration 51 wks, range: 2, 111 wks). At wk 24, 29% (6/21) pts achieved SVR35 (median % change from baseline: -17%, range: -90%, 16%), and 38% (8/21) pts achieved TSS50 (median % change from baseline: -45%, range: -100%, 22%). 78 pts were evaluable for safety across the TD and non-TD cohorts. Median exposure was 45 wks. The most common hematological treatment-emergent adverse events (TEAEs) of any grade were thrombocytopenia (45%, ≥Gr3: 23%) and anemia (14%, ≥Gr3: 10%). Early clinical data indicate that CPI-0610 as "add-on" to rux is generally well tolerated. The combination therapy provided clinical benefits in most pts as assessed by SVR, and symptomatic responses. In addition, conversion to TI was also observed in TD patients.

C097

DIFFERENTIAL TREATMENT STRATEGY IN POLYCYTHEMIA VERA PATIENTS WITH STABLE SUBOPTIMAL RESPONSE TO HYDROXYUREA: CLINICAL CORRELATIONS AND IMPACT ON SURVIVAL

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Hydroxyurea (HU) is the most used cytoreductive therapy (tx) for patients (pts) with polycythemia vera (PV). However, many pts may have suboptimal responses (SubOR) to HU. After HU, Ruxolitinib (RUX) may achieve significant responses. The "PV-NET" retrospective study collected 882 WHO2016-defined PV pts in 22 Hematology Centers to investigate if: 1)SubOR influences overall survival (OS); 2)type of SubOR drives 2nd-line tx; 2)RUX switch affects OS. Among the 662 pts who received HU for ≥3 mos, 195 had a complete response (CR), while 467 (70.5%) a SubOR (WBC/PLT count >10/400x10^9/l or need for phlebotomies (PHL) or splenomegaly/symptoms persistence/occurrence at maximum tolerated dose). The index date (ID) was set at 3 mos from HU start (Barosi G et al, BJH 2009). 152 pts (22.9%) had ≥1 HU-related toxicity, comparably in CR and SubOR (p=0.51). Compared to SubOR, CR pts were older (p<0.001), more frequently females (p=0.003), and less frequently had splenomegaly/symptoms (p=0.001) and *JAK2*^{V617F} ≥50% (p=0.004). HU dose ≥1 g/d was more used in CR pts (47.5% *vs* 27.8% in SubOR, p=0.001) but resulted in higher toxicity (33.3% *vs* 17%, p=0.001).

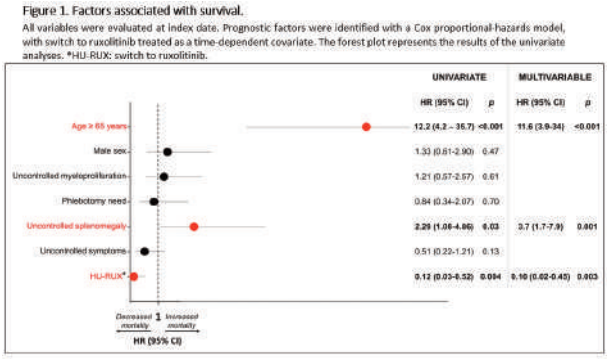

Figure 1.

No association was found between response and HU doses ≥1.5 or ≥ 2g/d. SubOR consisted of: uncontrolled WBC/PLT (n.254, 54.4%), PHL need (n.233, 49.9%), failure to reduce splenomegaly (n. 151, 32.3%) or PV-related symptoms (n.199, 42.6%). Overall, 140 (30%) SubOR pts switched to RUX (HU-RUX) while 327 (70%) continued HU (HU-alone). Uncontrolled myeloproliferation (HR 2.92, p=0.005), splenomegaly (HR 2.59, p=0.003) and symptoms (HR 1.91, p=0.040) significantly predicted an early RUX switch. PHL need (HR 1.89, p=0.004) was also associated with a late (>24 mos) switch. At 12 mos, 22.2% of HU-RUX pts were in CR. OS of SubOR was comparable to CR pts after age adjustment (86.8% *vs* 89.9% at 10 yrs from ID, p=0.33). In SubOR pts, age ≥65 (HR 11.6, p<0.001) and uncontrolled splenomegaly (HR 3.7, p=0.001) predicted a lower OS, while RUX switch correlated with better OS (HR 0.10, p=0.003) (Figure 1). This study highlights the real-world use of low-dose HU, with delayed tx change in SubOR. Notably, baseline absence of splenomegaly/symptoms, *JAK2*^{V617F} burden <50% and use of HU dose ≥1 g/d were associated with CR. Finally, RUX switch achieved significant responses and was associated with longer OS. A more careful management of HU and particularly of SubOR pts may improve outcome in PV.

C098

A MOLECULAR-BASED MODEL TO PREDICT MYELOFIBROSIS PROGRESSION IN PATIENTS WITH 2016-WHO DEFINED ESSENTIAL THROMBOCYTHEMIA

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Background: Essential thrombocythemia (ET) is associated with an increased risk of thrombosis (thr) and progression to myelofibrosis (MF). Thrombosis risk stratification model (IPSET) is based on variables including age >60y, history of thr, *JAK2*V617F genotype and cardiovascular risk factors.

Aim and methods: Aim of this study was to develop a simple, mutation-based, score, to predict MF progression in WHO-2016 defined ET pts. In Florence database (training cohort), 718 pts (65.6% *JAK2*, 12.8% *CALR*1, 7.1% *CALR*2, 3.6% *MPL* and 10.9% triple-negative-TN) were identified. All *JAK2* pts were annotated for variant allele frequency (VAF).

Results: Median age at diagnosis was 57.9y (range 12.9-92.9), women were 64.8%. Median FU was 106.4 months (6.1-421.6) during which 53 pts (7.4%) progressed to MF (23 *JAK2*, 20 *CALR*1, 4 *CALR*2, 5 *MPL*, 1 TN; p<0.0001), and 106 pts (14.8%) died. Palpable splenomegaly was present in 97 (13.5%) patients. 43 (6%) and 235 (33.1 %) patients had constitutional and microcirculatory symptoms, respectively. Univariate analysis for MF-free survival (MFS) identified *CALR1*/*MPL* genotype (p<0.0001, HR 3.8; 95% CI 2.2-6.6), as risk factor for MF progression. *JAK2*V617F VAF as a continue variable was also correlated with a higher risk of MF (p=0.002; HR 1; 1-1.1). A ROC curve was used to determine the best *JAK2* VAF cut-off level predicting MF progression; the curve showed an AUC of 0.76, and the best VAF value was 35%. Accordingly, we divided *JAK2* cohort in those with a VAF ≤35% (77.3%) and >35% (22.7%), the latter displayed a higher risk for MF progression in univariate analysis (Figure 1A; p<0.0001; HR 5.9; 2.4-14.4). Therefore, a two-tiered molecular based model was develop identifying high molecular risk patients (*JAK2*VAF>35%/*CALR*1/*MPL*; 34.7% of total) and low molecular risk patients (*JAK2*VAF≤35%/*CALR*2/TN; 65.3%), (Figure 1B, p<0.0001; HR 6.1; 3.2-11.7) with respective rate of MF evolution of 8% and 1.2% at 10 yrs. The predictive accuracy of the training set was confirmed in external validation cohort of 410 pts from Rome (p<0.01) and 479 from Mayo Clinic, Rochester (p<0.01).

Conclusions: This model, based on simple molecular variables that are routinely required by WHO criteria, identified a high-risk category for MF progression among 2016-WHO defined ET pts.

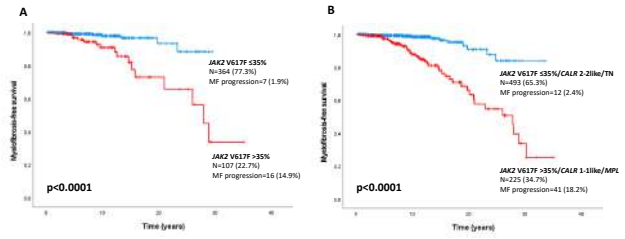

Figure 1.

C099

SINGLE CELL ANALYSIS OF CLONAL ARCHITECTURE IN LEUKEMIC TRANSFORMATION OF MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Evolution to acute myeloid leukemia (sAML) occurs in 15-20% of MPN, it is largely unresponsive to conventional therapy and prognosis is extremely poor. A restricted set of mutations is associated to leukemic evolution (Vannucchi, 2013; Tefferi, 2016), however, the molecular mechanisms underlying progression to AML have not been defined yet. Bulk next generation sequencing (NGS) cannot resolve mutation co-occurrence in the same cell nor elucidate the order of mutations. Aimed at resolving clonal architecture during leukemic progression of MPN, we performed whole exome (WES) and single-cell sequencing (SCS) on paired samples (chronic (CP)/blast phase (BP)).

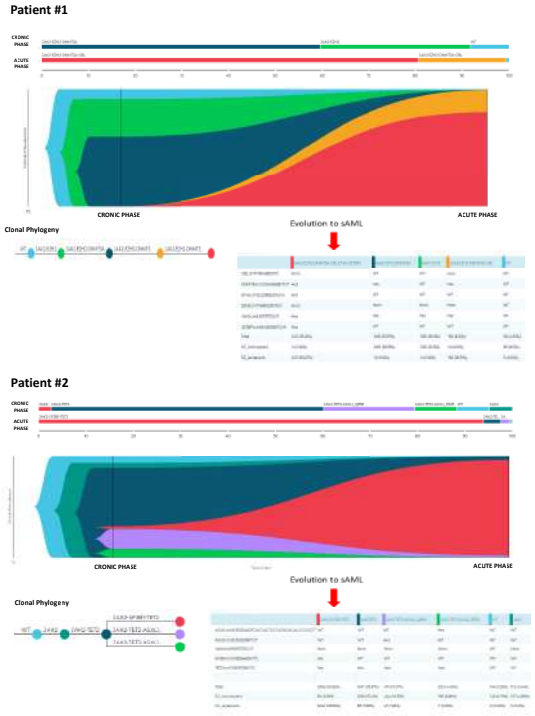

Figure 1.

Methods: We analyzed 12 MPN patients who progressed to sAML. Paired CD34+ cells samples (CP/BP) were subjected to WES and validated by NGS. Concurrently, in five paired samples a targeted SCS for 45 myeloid genes was performed using the Mission-Bio Tapestri platform.

Results: On average 60.000 variants we identified by WES that were unique to BP compared with CP. However, evolution to BP was not associated with recurrent abnormalities. By SCS, of the 5 paired samples, a total of 57375 single cells were sequenced (average 5216, range 2344-8268) with an average of 29628 reads per cell (range 17590-40269) and coverage of 104X (range 56-215). SCS was able to identified 14 low-frequency variants not detected in bulk analysis; however, it failed to discriminate homopolymeric regions including the ASXL1 G646Wfs*12 and 5 variants not covered by the target myeloid-genes panel. We found a significant correlation between variant allele frequency (VAF) from the 2 methods (R =0.84, p<.0001). Driver mutation in CP became undetectable during progression in BP. For all patients we are able to distinguish at least 4 mutated clones and in 3 cases the dynamics of the clones allowed to identify the ones responsible for evolution to sAML. Among these, in all but one, the leukemic clones were already detectable at low frequency (<2%) at CP and became dominant in the BP (FIG.1), but were missed by bulk NGS analysis.

Conclusions: Although bulk NGS is highly informative, only SCS accurately resolves clonal architecture and complexity, identifying rare clones and their dynamics. Moreover, custom panel, rather than commercial ones, might be more effective to avoid misidentification of informative variants. Overall, our findings provide insights into the pathogenesis of AML transformation of MPN.

C100

A DIGITAL DROPLET PCR CUT-OFF TO IDENTIFY POTENTIAL CHRONIC MYELOID LEUKEMIA PATIENTS CANDIDATE TO TREATMENT DISCONTINUATION AND MAINTAINANCE OF A TREATMENT-FREE REMISSION

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Discontinuation has become a new therapeutic endpoint in chronic myeloid leukemia (CML). Unfortunately, about 50% of patients experience loss of major molecular response (MMR). Sustained treatment-free remission (TFR) is based only on the median duration of TKI treatment and sustained stable deep molecular response evaluated by RQ-PCR. We evaluated retrospectively a series of CML patients in deep MR who attempted a TFR with the aim of finding a droplet digital PCR (ddPCR) cut-off predictive of a low rate of molecular recurrence free-survival (MRFS) after discontinuation. Fifty-seven patients, female prevalence (64.9%) and a median age of 47 years (range 21-82) at diagnosis, discontinued treatment in a real-life setting. Sokal score stratification identified 31 patients (54%) as low risk, 22 (38.5%) as intermediate and 4 (7%) as high risk. Thirty-eight patients (67%) carried the b3a2 type of transcript, whereas 19 (33%) the b2a2 type. Seventeen patients received previously interferon-alpha (IFN) (median duration 25 months). Median duration of TKI treatment was 13.8 years. The median duration of deep MR was 2.7 years. Forty-six patients (80.7%) attempted discontinuation after first-line treatment and 11 (17.5%) after subsequent lines (39 patients had received imatinib, 6 patients dasatinib, 12 nilotinib). Fifteen patients (26%) relapsed at a median time of 4.4 months with a MRFS of 68.6% at 30 months. The 57 cases were tested by ddPCR prior to treatment discontinuation: 31 (54.4%) were ddPCR-negative and only 4 of them relapsed (13%), while 26 (45.6%) proved ddPCR-positive and 13 (50%, p=0.002) relapsed. To optimize the use of ddPCR as a possible tool to predict the success of treatment discontinuation we applied a ROC curve analysis to detect a cut-off of molecular residual disease that can better identify candidates to TFR. We obtained an AUC of 68.7% (95%CI 53.1-84.4) and identified a cut-off value of 0.001 copies/µl

(cp/µl) (Figure 1). Sensitivity, specificity, positive and negative predictive value were 73.3%, 64.3%, 42% and 87%, respectively. Before stopping the TKI, 4/15 (27%) relapsed patients had a molecular residual disease ≤0.001 cp/µl *vs* 11/15 (73%) with >0.001 cp/µl ($p<0.001$).

In conclusion, ddPCR can be a more sensitive and accurate method for the detection of molecular residual disease prior to treatment discontinuation. The proposed cut-off value should be validated in a large cohort of CML patients considered eligible to stop TKI treatment.

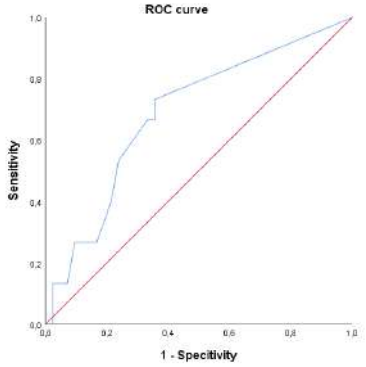

Figure 1.

POSTERS

Lymphomas 1

P01

WIDE LOSS OF B-CELL ANTIGENS AS A NEW ESCAPE MECHANISM AFTER CD19-DIRECTED CAR-T CELLS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Chimeric antigen receptor (CAR) T-cells targeting CD19 represent a promising therapeutic strategy in B-cell malignancies. However, about 60% of Diffuse Large B-cell Lymphoma (DLBCL) patients do not respond or relapse after CAR-T cells, mainly due to high tumor burden and/or low T cell fitness. A significant percentage of B lymphoblastic leukemia (B-ALL) relapses are CD19-negative, indicating a tumor escape mechanism from the immunological pressure driven by CD19-directed CAR-T cells. Molecular mechanisms of CD19 loss have been well studied in B-ALL and include nonsense/frameshift mutations (usually mapping to exons 2 to 5) disrupting the epitope conformation targeted by CAR-T cells, missense mutations retaining CD19 in the endoplasmic reticulum (in-frame insertion in exon 2) and noncoding mutations causing intron retention. Conversely, the mechanism of CD19 loss in DLBCL remains poorly understood, due to the difficulty to study small needle biopsies. In at least one patient, splice sites noncoding mutations were reported.

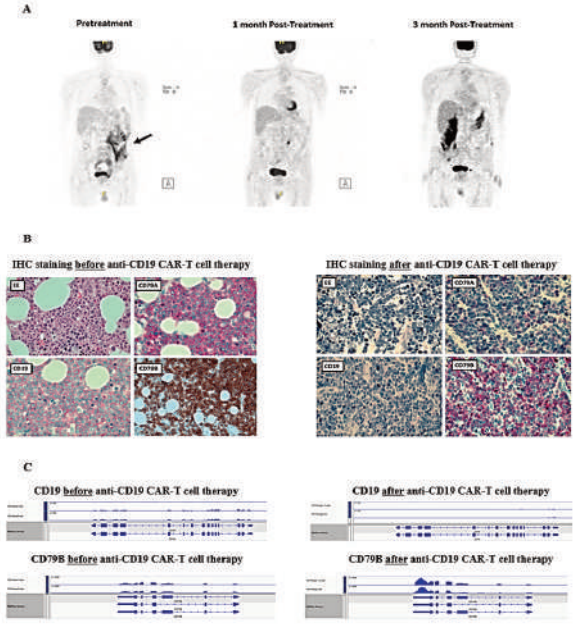

Figure 1. Disease assessment by PET-CT scan before and after CAR-T-cell treatment (A). IHC panels of E.E. and CD19, CD79a and CD79b before CAR-T-cell therapy (left) and at relapse (right) (B). IGV plots of CD19 and CD79b expression levels studied by RNA-sequencing before and after CAR-T-cell therapy. Each line represents one technical replicate.

Figure 1.

Here, we describe for the first time a new mechanism of CD19 escape to CD19-directed CAR-T cells (Tisa-cel) in a 67-year-old male patient with refractory DLBCL transformed from follicular lymphoma. PET-CT scan performed 4 weeks post CAR-T cells revealed >90% reduction in all tumor abdominal masses but, unexpectedly, 3 months later, PET-CT documented a new retroperitoneal mass. Immunohistochemistry of the tumor needle biopsy showed large tumor cells lacking the typical CD19, CD20, CD22 and CD79a B-antigens, that were instead detectable before CAR-T therapy. Notably, CD79b was the only B-cell antigen to remain expressed together with the B-cell transcription factor PAX5. RNA-sequencing of paired samples before CAR-T cell therapy and at relapse, showed the absence of mRNAs translating for all B-cell antigens with the exception of CD79b, indicating a complete loss of expression rather than epitope alternative splicing. Results of whole exome sequencing are ongoing and will be presented. This extraordinary case highlights the importance of investigating the nature of relapse after anti-CD19 CAR-T cells in DLBCL. Interestingly, the wide loss of B-cell antigens with preservation of PAX5 observed in our case is reminiscent of what occurring in Hodgkin lymphoma. Moreover, our results point to the importance of developing novel anti-CD79b CAR-T cells as salvage therapy for DLBCL relapsing as CD19-negative tumors after anti-CD19 CAR-T cells.

P02

ABSTRACT WITHDRAWN

P03

RITUXIMAB, BENDAMUSTINE, AND CYTARABINE (R-BAC) COMPARED WITH RITUXIMAB AND BENDAMUSTINE (BR) IN PREVIOUSLY UNTREATED ELDERLY PATIENTS WITH MANTLE CELL LYMPHOMA (BE-VE-BAC STUDY)

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Both rituximab plus bendamustine (BR), and rituximab, bendamustine, and cytarabine (R-BAC) are considered suitable induction therapies in elderly patients with mantle cell lymphoma (MCL) not candidate to autologous transplant. A direct comparison between the two regimens has never been performed. With this multicenter retrospective observational study, we compared the outcome and the safety features of patients with newly diagnosed MCL, treated with BR or R-BAC between 2009 and 2020 in 8 Departments from north-east of Italy. Primary endpoint was 2-years progression-free survival (PFS). All patients included had a minimum follow-up of 12 months since start of treatment. Inclusion bias were estimated by a propensity score stratified by gender, age, MCL morphology, and MIPI score. 180 patients with MCL with a median age of 72 years (range 53-90) were retrospectively analyzed. According to our propensity score calculation, the probability of receiving R-BAC was higher in younger patients (P<0.0001), but no other significant difference in the distribution of above mentioned prognostic variables was observed between the two groups. We limited our survival analysis to patients with 80 years or less, which allowed us a fair comparison between the two groups (P=NS). This cohort included 155 patients (53 BR, 102 R-BAC) that represented the subjects for the present analysis. Of them, 109 (70%) were males, MIPI was elevated in 63%, 11% had blastoid or pleomorphic morphology, and median follow-up was 46 months (range 12-133). Pa-

tients treated with R-BAC achieved CR in 91% of cases, as compared with 60% for BR (P<0.0001). The 2-years PFS was 87%±3% and 64%±7% for R-BAC and BR, respectively (P=0.001, Figure 1). Median overall survival (OS) was 121 months for R-BAC and 78 months for BR (P=0.08). MIPI score was the only predictive significant variable both in terms of PFS and OS. R-BAC was associated with significantly more pronounced grade 3-4 thrombocytopenia than BR (55% versus 18%). R-BAC doses were frequently reduced (2 days schedule in 38%) as compared to the original scheme. The BE-ve-BAC study indicates that R-BAC, even when administered in the 2-days schedule or with attenuated dose, is associated with significantly prolonged 2-years PFS than BR in elderly patients with previously untreated MCL. As hypotesized hematological toxicity was significantly higher for the latter regimen as compared to BR. Our results will need confirmation in prospective settings.

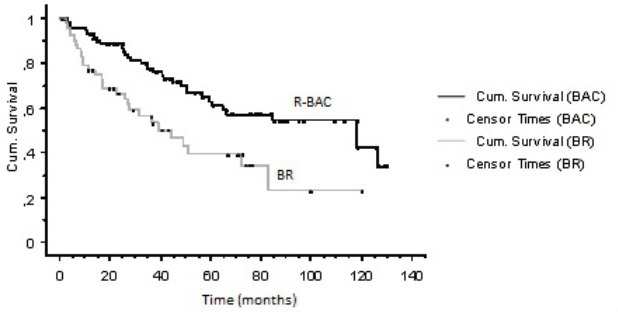

Figure 1.

P04

GUT MICROBIOTA ROLE IN THE RESPONSE TO CHECKPOINT INHIBITOR TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA: AN INTERIM ANALYSIS OF THE MICRO-LINF STUDY

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Single-agent monoclonal antibodies targeting the immune checkpoint PD-1 (programmed death 1) are an efficient and safe therapeutic option in patients with relapsed/refractory B-cell lymphoma. However, many patients progress or lose response to anti-PD1. Recent studies have highlighted the role of the gut microbiota (GM) in influencing the response to chemo-immunotherapeutic agents. Here we hypothesize that the GM dynamics in B-cell lymphoma patients during anti-PD1 therapy correlate with treatment response. From December 2017 to December 2020 we enrolled 17 patients (12 with classical Hodgkin lymphoma [cHL] and 5 with primary mediastinal B-cell lymphoma) treated with anti-PD1 due to relapsed/refractory disease. Feces were collected at baseline, before each therapy cycle, at response assessment (during both therapeutic course and follow-up) and for grade>2 adverse events, and profiled through Illumina sequencing. At each time point, patients compiled a 7-day weighted food intake record that was analyzed by MètaDieta (METEDA). We report the results of the first 6 patients enrolled, all affected by cHL. Median age was 31 years (range 26-71), 5 patients were female. Five patients were refractory to the last therapy, with a median of previous treatments of 3 (range 3-5). All patients discontinued treatment: 3 due to disease progression; 2 achieved complete remission and interrupted to consolidate with autologous stem cell transplantation; the last due to grade 3 adverse event despite partial remission. The median number of anti-PD1 cycles was 15 (range 7-18). The baseline GM separated from that of age/gender-matched healthy controls, being enriched in the pathobiont Collinsella while depleted of health-associated taxa, e.g. Faecalibacterium, Ruminococcus, Coprococcus and Roseburia (p<0.05). The GM dynamics along anti-PD1 treatment were distinct in relation to the therapeutic response, with greater temporal variability of alpha diversity in responders. The latter consumed more fat and fewer carbohydrates. The GM of patients with relapsed/refractory B-cell Hodgkin lymphoma is dysbiotic and shows distinct trajectories during anti-PD1 treatment, closely related to the therapeutic response.

P05

RITUXIMAB PLUS BENDAMUSTINE AND CYTARABINE (R-BAC) IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MANTLE CELL LYMPHOMA: LONG TERM FOLLOW-UP RESULTS OF A PHASE 2 STUDY FROM THE FONDAZIONE ITALIANA LINFOMI

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The activity of the combination of rituximab, bendamustine, and low dose cytarabine (R-BAC) was evaluated in a phase 2 multicentre trial from the Fondazione Italiana Linfomi (FIL RBAC500) in previously untreated patients with mantle cell lymphoma (MCL) who were not eligible to stem cell transplant. Fifty-seven patients (median age 71 years, range 61-79) were recruited and treated with 4 to 6 cycles between 2012 and 2014. Despite some concern in terms of hematological toxicity, the R-BAC regimen was associated with high complete remission (CR) rate (91%), 2-years overall survival (OS) of 86% (74-93), and 2-years progression free survival (PFS) of 81% (68-89). Here, we present long-term survival outcomes. After 7 years of median follow-up (86 months, range 57-107), the median OS and PFS for all patients were not reached (Figure 1A and 1B). The 7-years PFS and OS rates were 56% (95%CI 41-67) and 63% (95%CI 46-72), respectively. Patients who achieved CR (n=53)

had a 7 years PFS of 59% (95% CI 44-71), with the curve that appears to plateau after 6 years. Adverse predictive factors affecting PFS were blastoid morphology (p<0.05), elevated Ki67 > 30% (p<0.05), and failure to achieve CR after 2 cycles (p=0.03). Early-progression of disease (<24 months from start of R-BAC) was associated with impaired overall survival (p<0.05). Eight patients (14%) developed a secondary neoplasia: 1 parotid heteroplasia, 1 parotid nodular hyperplasia, 1 prostate cancer, 1 bladder cancer, 1 larynx, 1 thyroid cancer, 1 lung cancer and 1 secondary acute myeloid leukemia. Among the 25 relapsed patients, 8 did not receive any other treatment. Six had Ibrutinib monotherapy as second line, of whom 4 responded (3 are still in CR), 4 had CHOP or CHOP-like regimens with only partial responses. As per protocol, 23 patients with available marker at diagnosis were followed-up for minimal residual disease (MRD) with ASO-droplet digital polymerase chain reaction (D-PCR). The 4 patients with MRD persistence at the end of induction, either in peripheral blood or bone marrow, had significantly worse 7 years-PFS (p<0.05 for them both). In conclusion, in elderly patients with newly diagnosed MCL, R-BAC showed sustained efficacy over time, which compared favorably with any other reported immuno-chemotherapy regimen (with or without maintenance) in similar populations. With a median OS exceeding 60% after 7-years this regimen has significantly impacted on the life-expectancy of elderly patients with MCL.

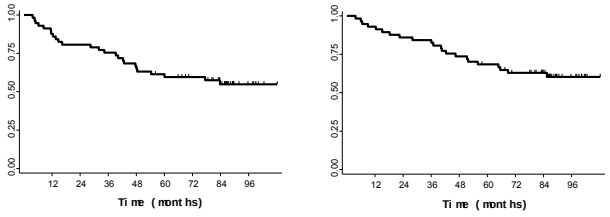

Figure 1.

P06

POLATUZUMAB VEDOTIN PLUS BENDAMUSTINE AND RITUXIMAB (POLA+BR) IN RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (R/R DLBCL): UPDATED RESULTS OF A PHASE IB/II RANDOMIZED STUDY AND PRELIMINARY RESULTS OF A SINGLE ARM EXTENSION

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In the randomized arm of the GO29365 Ph Ib/II study (NCT02257567) Pola+BR improved progression-free survival (PFS) and overall survival (OS) *vs* BR alone in patients (pts) with R/R DLBCL (Sehn *et al*. 2020). A Ph II extension (Ext) cohort of pts receiving Pola+BR was later included. We report updated data from the randomized Ph II arms and Ext cohort. Pts with R/R DLBCL were aged ≥18 years and stem cell transplant-ineligible. Pts with Gr >1 peripheral neuropathy [PN] were excluded. Pola+BR efficacy/safety was assessed with Pola 1.8mg/kg IV and 6 cycles of BR. Primary endpoint was complete response (CR) by PET-CT (modified Lugano) at end of treatment (EOT). Secondary endpoints were objective response rate (ORR), best objective response (BOR), duration of response (DOR), PFS, OS, and safety (end-points assessed by independent review committee; IRC). Median follow-up on Jan 2, 2020, for Pola+BR pts was 42.9 months (mo; randomized N=40), and 9.7 mo (Ext N=106). Baseline characteristics (Table 1) were similar in the study groups. In the randomized Pola+BR arm, 6 pts (15%) had a DOR of >24 mo (range, 26.6–38.6). In the randomized cohorts (Pola+BR *vs* BR), median (m) PFS (95% CI) was 9.2 (6.0–13.0) *vs* 3.7 (2.1–4.5) mo (HR 0.2–0.7); mOS (95% CI) was 12.4 mo (9.0–32.0) *vs* 4.7 (3.7–8.3) (HR 0.4; 0.2–0.7) (Figure). In the Ext cohort (N=106), PET-CR at EOT was 39.6% (n=42; 30.3–49.6; Table 2), consistent with the randomized Pola+BR arm (16/40; 40.0%); BOR rate was 56.6% and best CR was 52.8%. mPFS and mOS (95% CI) were 6.1 mo (5.1–8.0) and 11.0 (8.3–14.2), respectively; however, OS was not mature. In all Pola+BR pts (N=152), mPFS and mOS in primary refractory (n=97) *vs* non-primary refractory (N=55) were 4.8 *vs* 12.6 mo and 7.8 *vs* 32 mo; refractory to last treatment (N=116) *vs* non-refractory (N=36), 5.3 *vs* 14.2 mo and 9.1 mo *vs* not reached; 1 (N=50) *vs* ≥2 (N=102) prior therapies, 10.4 *vs* 6 mo and 14 *vs* 9.5 mo, respectively. There were no new safety signals with Pola+BR. In all pts receiving Pola+BR, 121/151 (80.1%) had Gr 3–4 AEs (mainly cytopenias), 84 (55.6%) had serious AEs and 18 (11.9%) had Gr 5 AEs (mainly infections). 46 (30.5%) pts had PN events of any grade. 4 (2.6%) pts had secondary malignancies. The improvement in PFS and OS seen with Pola+BR *vs* BR persisted with additional follow-up. Efficacy was consistent in the Ext cohort and randomized Pola+BR arm. These data confirm that Pola+BR is effective for pts with R/R DLBCL.

ITT	Randomized Phase II cohort		Extension cohort
	BR (N=40)	Pola+BR (N=40)	Pola+BR (N=106)
Median age, years (range)	71 (30–84)	67 (33–86)	70 (24–94)
Male, n (%)	25 (63)	28 (70)	52 (49)
ECOG PS score, n (%)			
0	17 (43)	12 (30)	30 (28)
1	14 (35)	21 (53)	62 (59)
2	8 (20)	6 (15)	14 (13)
Ann Arbor Stage III/IV, n (%)	36 (90)	34 (85)	84 (79)
Bulky disease (≥7.5 cm), n (%)	15 (38)	10 (25)	28 (26)
IPI score 3–5 at enrollment, n (%)	29 (73)	22 (55)	70 (66)
Median no. of prior lines of therapy (range)	2 (1–5)	2 (1–7)	2 (1–7)
1 line	12 (30)	11 (28)	37 (35)
2 lines	9 (23)	11 (28)	27 (26)
≥3 lines	19 (48)	18 (45)	42 (40)
Refractory, n (%)			
Primary refractory	27 (68)	21 (53)	73 (69)
Refractory to last prior therapy	34 (85)	30 (75)	81 (76)

Definition of refractory: no response or progression within 6 months of last treatment

Table 2. Extension cohort efficacy summary

	Extension cohort; Pola+BR (N=106)	
Response rate*	n (%)	95% CI
CR	42 (39.6)	30.3, 49.6
ORR	45 (42.5)	32.9, 52.4
BOR*	60 (56.6)	46.6, 66.2
Best CR*	56 (52.8)	42.9, 62.6
Time-to-event	Median (months)	95% CI
DOR* (95% CI)	6.2	5.4, 11.6
PFS* (95% CI)	6.1	5.1, 8.0
OS (95% CI)	11.0	8.3, 14.2

*Independent review committee-assessed; †Best response was assessed by either PET-CT or CT results

Figure. Overall survival in (A) randomized and (B) extension cohorts

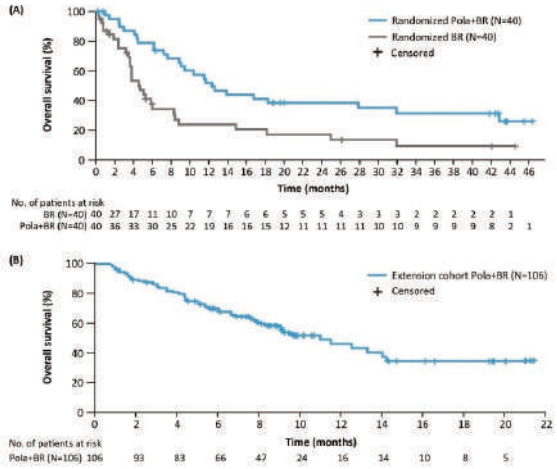

Figure 1. Baseline characteristics.

P07

ABSTRACT WITHDRAWN

P08

OUTCOME OF AUTOLOGOUS TRANSPLANT IN "FIT" ELDERLY PATIENTS WITH RELAPSED/REFRACTORY AGGRESSIVE B LYMPHOMA: RESULTS OF THE PROSPECTIVE RECANZ STUDY BY THE "FONDAZIONE ITALIANA LINFOMI

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Introduction: The majority of patients (pts) with aggressive B lymphoma (aL) are older than 60, and their prognosis is poor when the disease is resistant or relapsed (R/R) after first-line therapy. Standard second-line treatment usually consists of platinum-containing regimens and autologous transplantation (ASCT), but not all elderly pts are eligible for this therapy. Simplified geriatric assessment (sGA) has recently been used to identify elderly lymphoma pts fit for intensive first-line treatment and could also be used in subsequent lines to identify pts FIT to high-dose therapies.

Table 1. Patient Characteristics.

	ABMT as prot	No ABMT	
	N (%)	N (%)	p-value
Gender F	12 (44)	21 (48)	0.808
Age ≥70 y	15 (56)	25 (58)	1.00
DLBCL	24 (89)	42 (98)	0.291
Stage III-IV	18 (75)	34 (83)	0.526
B-symptoms	5 (21)	5 (12)	0.479
ECOG PS 0-1	0	3 (8)	0.281
IPI 3/5	12 (50)	23 (57)	0.611
Time to 1st progression, ≤12months	19 (70)	36 (84)	0.236
Treatment R-DAHP	18 (67)	30 (70)	0.797
Tab.1b Outcome	**Age 65-69**	**Age 70+**	
	N (%)	N (%)	p-value
Response Pre phase, ORR	16 (53)	15 (37)	0.228
ASCT as protocol	12 (40)	15 (37)	1.00
2-yr OS, % (CI95)	69 (45-84)	61 (42-76)	0.672
2-yr PFS, % (95CI)	30 (13-48)	37 (22-53)	0.796

Aims and Methods: This prospective multicenter observational study was designed to evaluate the feasibility and tolerance of ASCT after second line treatment in pts aged 65-75 years (y) with R/R aL and FIT to sGA. Salvage regimens containing platinum (RDHAP, RICE) or gemcitabine were used; stem cell harvest was performed after 1 or 2 cycles and pts with at least partial response (PR) after 3 cycles and FIT to a second sGA underwent ASCT with BEAM or FEAM conditioning.

Results: From May 2014 to August 2019, 75 pts were enrolled from 16 FIL centers and 70 considered eligible. Twenty-seven pts underwent ASCT, a median of 5.6x10^6/kg CD34 were infused. The clinical characteristics of the pts are shown in table 1a, there were no differences between pts reaching ASCT or not. Overall, the most common grade 3-4 non-haematological adverse events were gastrointestinal (11%) and infectious (9%). Forty-three pts did not perform ASCT because: progressive (32) or stable (4) disease, death (1 cardiac event and 1 septic shock), personal or clinician choice (4/1). With a median follow up of 31 months (range 1-62), 2y overall (OS) and event free survival (EFS) by intention to treat were 65% (95%CI:50-76%) and 34% (95%CI:22-46%) respectively. After ASCT OS and EFS were 79% (95%CI:31-86%) and 56% (95%CI:32-75%), respectively, without difference according to age (table 1b): twenty-four (89%) pts achieved CR and 3 progressed and died after 1-8 months.

Conclusions: This study confirms the feasibility and efficacy of ASCT program in R/R elderly pts with aL selected with sGA. Only a minority of pts can benefit from this procedure due to poor response to salvage treatment. These data may constitute a comparative parameter for evaluating the effectiveness of other second-line therapies (CAR-T, bispecific antibodies, biological drugs) in the near future.

P09

BRENTUXIMAB VEDOTIN IN THE TREATMENT OF RELAPSED/REFRACTORY CD30 POSITIVE PERIPHERAL T-CELL LYMPHOMA PATIENTS: A PHASE 2 STUDY OF THE FONDAZIONE ITALIANA LINFOMI

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Options are limited for patients with relapsed or refractory (R/R) peripheral T-cell lymphoma (PTCL) for whom the median overall survival (OS) and progression free survival (PFS) are less than 6 months. Only four agents are FDA-approved for the treatment of R/R PTCL including pralatrexate, romidepsin and belinostat and the objective response rate (ORR) is 25–30% with limited duration of response (DoR) is. For a specific subtype of PTCL, namely R/R systemic anaplastic large-cell lymphoma, single-agent brentuximab vedotin (BV) treatment resulted in an 86% ORR and a 57% complete response (CR) rate. We conducted a phase 2 study to determine the antitumor efficacy of single-agent BV as measured by overall ORR in R/R CD30+ PTCL patients (PTCL not otherwise specified, AITL and transformed mycosis fungoides). The secondary endpoints were: DoR, CR rate, PFS, OS, disease free survival and type, incidence, severity, and relatedness of adverse events. Additional endpoint was the correlation between CD30 expression in patients' biopsy at baseline and response after BV. ClinicalTrials.gov Identifier: NCT02497131. Twenty-five patients were enrolled and 23 received at least one BV infusion (median 5, range 2-16). There were 10 females, 18 patients were in stage IV and 16 subjects were refractory to the last therapy. Median number of therapies received prior to BV was 2 (range 1-6). Final ORR was 28.6%, with 3 CR. CR patients were 2 PTCL not otherwise specified and 1 AITL with response duration of 3.3, 4.5 and 10.7 months, respectively. Best response was achieved at the III cycle. Median PFS was reached at 4.4 months, median OS at 11.4 months and median DoR at 3.4 months, respectively. No correlation between CD30 expression (centrally reviewed) and type of response was observed. Twenty-one hematological toxicities occurred in 12 patients, 14 of them were grade ≥3. Among extra-hematological toxicities (3.5% grade ≥3), 7 were serious adverse events (SAE). To note, 6 out of 7 SAE were lung infection/pneumonia. Five episodes of mild peripheral neuropathy occurred in 4 patients. In terms of response, the ORR and PFS in this trial are comparable to those in similar populations studied with both recently approved agents, such as pralatrexate and romidepsin, and with the other phase 2 study on BV. The ORR and the OS of in the present study places BV among the active agents for PTCL. Safety concerns emerged about infections, claiming for a strict monitoring for these toxicities.

P10

CAR-T CELL THERAPY IN AGGRESSIVE LYMPHOMAS: THE REAL LIFE EXPERIENCE OF "L. E A. SERÀGNOLI" INSTITUTE OF BOLOGNA

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Two anti-CD19 chimeric antigen receptors (CAR) T cell, axicabtagene ciloleucel [axi-cel] and tisagenlecleucel [tisa-cel]) were recently approved for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma (PMBCL) and real-life data are still scarce in literature. Here, we report our real word experience in terms of efficacy, safety and outcome. Sixty-one patients (pts) were screened, 37 of whom were leukapheresed and, to date, 28 pts received CAR-T (4 pts are waiting for infusion while 5 ones obtained a complete response [CR] with the bridging therapy). At time of writing, 24 pts are valuable for analysis. Fourteen pts received axi-cel and 10 ones received tisa-cel, based on slot production availability and histology. Median age at therapy was 57 years (range 20-69), 17 (70.8%) were males, 16 (66.6%) in stage III/IV. Bulky disease was present in 14 pts (58.3%). The median number of previous therapies was 3 (1-7); 6 pts (25%) failed a previous autologous transplantation and 20 (83.3%) ones were refractory to the most recent therapy. Eighteen pts (75%) received a bridging therapy. The median time from apheresis to CAR-T infusion was 46 days (29-91). All pts received lymphodepletion with Flu-Cy. At one months after the infusion, responses were as follows: 9 (37.5%) CR and 9 (37.5%) partial response (PR), with an overall response rate (ORR) of 75%. At three months after infusion 17/24 pts were valuable for response: 10 (58.8%) CR and 3 (21.4%) PR, with an ORR of 76.4%. In particular 3 pts converted from PR at 1 month to CR and 1 pts converted from progressive disease (PD) at 1 month to PR. Two/24 patients were already re-staged after 1 year and are still in CR. Progression free survival was 57.8 % and overall survival was 74.8% at 18 months, respectively. Regarding toxicity, 20 (83.3%) pts developed cytochine release syndrome of any grade (8.3% grade 3 or higher). Immune cell-associated neurotoxicity syndrome (ICANS) of any grade occurred in 33.3% of patients (2.5% grade 3 or higher). One patients died due to ICANS grade 4. One patient experienced a hemofhagocytic syndrome histologically documented and died due to PD. Hematological toxicity was observed in 19 pts (79%) including neutropenia grade 3 or higher in 16 pts (66.6%) without severe infection. Our real-life experience on CAR-T confirms the efficacy reported in clinical studies and the manageability of the related toxicity.

Myeloma and Monoclonal Gammopathies 1

P11

EFFICACY OF CARFILZOMIB-BASED INDUCTION/CONSOLIDATION WITH OR WITHOUT AUTOLOGOUS TRANSPLANT AND LENALIDOMIDE OR CARFILZOMIB-LENALIDOMIDE MAINTENANCE IN HIGH-RISK PATIENTS IN THE FORTE TRIAL

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Background. In the FORTE trial, carfilzomib-lenalidomide-dexamethasone-ASCT (KRd_ASCT) improved progression-free survival (PFS) *vs* KRd without ASCT (KRd12) or carfilzomib-cyclophosphamide-dexamethasone-ASCT (KCd_ASCT). KR maintenance significantly improved PFS *vs* R. The primary aim of this analysis was the impact of treatment on PFS and 1-year sustained MRD negativity (1y-MRD neg) rates according to patient (pt) risk based on cytogenetic data.

Methods. 474 newly diagnosed MM pts were randomized to KRd_ASCT *vs* KCd_ASCT *vs* KRd12 and, thereafter, to KR *vs* R maintenance. Subgroup analyses according to FISH assessed the impact of each single high-risk (HiR) CA [del17p, t(4;14), t(14;16), del1p and 1q gain (3 copies) or amp1q (≥4 copies)] and that of combined HiR CA, defining HiR and double hit (DH) by the presence of ≥1 HiR CA and ≥2 HiR CA, respectively. Standard risk (SR) was defined by the absence of all CA.

Results. SR pts benefited from intensification with KRd_ASCT *vs* KRd12 (HR 0.47, p=0.05) and KCd_ASCT (HR 0.38, p=0.01), with a 4y PFS of 80%, 67% and 57%, respectively. In HiR pts, KRd_ASCT improved PFS *vs* KRd12 (HR 0.6, p=0.04) and KCd_ASCT (HR 0.57, p=0.01). The advantage with KRd_ASCT *vs* KRd12 (HR 0.53, p=0.07) and KCd_ASCT (HR 0.49; p=0.03) was also observed in DH pts. A PFS benefit from KRd_ASCT *vs* KRd12 was observed in pts with del17p (HR 0.61, p=0.3), t(4;14) (HR 0.59, p=0.2) and 1q gain (HR 0.45, p=0.02), while amp1q pts had the worst outcome regardless of treatment (KRd_ASCT *vs* KCd_ASCT, HR 1.16, p=0.73; KRd12 *vs* KCd_ASCT, HR 1.34, p=0.45). KRd_ASCT induced similar 1y-MRD neg rates in SR (50%), HiR (50%) and DH (47%) pts. Lower 1y-MRD neg rates were observed with KRd12 in SR (36%), HiR (39%) and DH (25%) pts. With KCd_ASCT, HiR (48% *vs* 29%, p=0.04) and DH (48% *vs* 17%, p=0.03) pts had significantly lower 1y-MRD neg rates than SR pts. 1y-MRD neg pts showed similar 4y PFS regardless of risk status (SR, 87%, HiR 87%, DH 83%) and treatment arm. KR improved PFS *vs* R in SR (3y PFS 90% *vs* 73%, HR 0.42, p=0.06), HiR (3y PFS 69% *vs* 56%, HR 0.6, p=0.04) and DH pts (3y PFS 67% *vs* 42%, HR 0.53, p=0.1). A benefit from KR *vs* R was observed in pts with del17p (HR 0.59, p=0.37), t(4;14) (HR 0.59, p=0.3), 1q gain (HR 0.54, p=0.07) and del1p (HR 0.23, p=0.08).

Conclusion. KRd_ASCT and KR maintenance were highly effective in SR, HiR and DH pts, with impressive 1y-MRD neg rates, 4y PFS from diagnosis and 3y PFS from maintenance, thus supporting their use in HiR pts.

P12

ICOS AND ICOSL ARE NOVEL BIOMARKERS FOR MULTIPLE MYELOMA

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Introduction and aim. Multiple myeloma (MM) is generally preceded by monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM). The ICOS/ICOSL interaction, an important signaling pathway in the T/B cell crosstalk, also plays a role in the osteoclast function and in neoplastic angiogenesis. The aim of the present study was to assess the biological and potentially prognostic role of ICOS and ICOSL in plasma cell dyscrasia.

Methods: The study enrolled 204 patients with plasma cell dyscrasias followed in a five-year period at our institution. Serum levels of soluble ICOS (sICOS) and sICOSL, were assessed by ELISA. The mice MOPC-21 and human RPMI-8226 myeloma cells and the NOD-SCID-IL2Rg-null mice were used for *in vitro* and *in vivo* studies.

Results. Serum levels of sICOS and sICOSL were analyzed in 36 (17.6%) MGUS, 97 (47.5%) SMM, 71 (34.8%) MM, and 59 healthy controls. Both sICOS and sICOSL were higher in MM than in MGUS and SMM, whose levels were similar to controls (Figure 1A,B). sICOS was higher in Salmon-Durie (SD)-II/III than SD-I (Figure 1C). Levels of sICOS directly correlated with Beta-2-Microglobulin (B2M) levels (p=0.025), M protein (p=0.0003), bone marrow plasma cells (p=0.00035) and, inversely with Hb (p=0.0002). sICOSL correlated with B2M level (p=0.01) and total M protein (p=0.026). Levels of sICOS above the median value significantly associated with shorter overall survival (OS). The optimized sICOS threshold of 40 pg/ml was the best cut-off for OS in our cohort, that, at 36-months, was 67.2% for patients above the cut-off and 89.2% for those below (p=0.00017) (Figure 1D). Multivariate analysis showed that sICOS levels above 40 pg/ml maintained an independent association with an increased risk of death (HR 2.78, 95% CI 1.07-7.20, p=0.035) when adjusted for the SD staging system. Immunophenotyping analysis demonstrated that ICOS and ICOSL can be expressed in myeloma cells. To assess the potential pathogenetic role of ICOS/ICOSL interaction in MM we documented that ICOS-Fc, a recombinant soluble form of ICOS composed by the extracellular portion of ICOS fused to the IgG1Fc portion, inhibited migration of ICOSL+ myeloma cell lines *in vitro* (Figure 1E) and growth of ICOSL+ MOPC-21 myeloma cells *in vivo* (Figure 1F).

Conclusions: The ICOS/ICOSL system may represent a novel prognostic and druggable biomarker that identifies MM patients with a more aggressive disease for whom novel therapeutic strategies are needed.

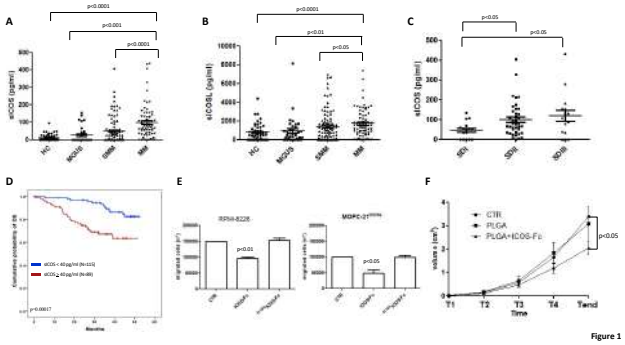

Figure 1

Figure 1.

P13

MELFLUFEN PLUS DEXAMETHASONE (DEX) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) EXPOSED/REFRACTORY TO PRIOR ALKYLATORS – A POOLED ANALYSIS OF THE O-12-M1 AND HORIZON STUDIES

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Background: Melphalan flufenamide (melflufen) is a first-in-class peptide-drug conjugate (PDC) that targets aminopeptidases and rapidly releases alkylating agents inside tumor cells. Melflufen has a mechanism of action distinct from other alkylating agents (Slipicevic et al. AACR 2020. Abs. 1843). In the O-12-M1 (NCT01897714) and HORIZON (OP-106; NCT02963493) studies, melflufen plus dex showed meaningful efficacy and a clinically manageable safety profile in pts with RRMM (Richardson et al. Lancet Haematol. 2020;7:5; Richardson et al. J Clin Oncol. 2020;Dec 9 [Epub]). This pooled analysis examines pts from these studies exposed to prior alkylators.

Methods: Both the O-12-M1 and HORIZON studies included pts with RRMM who received ≥ 2 prior lines of therapy (LoTs) and had a primary endpoint of overall response rate (ORR). Secondary endpoints included progression-free survival (PFS) and safety. Data from the 2 studies were pooled and analyzed according to previous exposure and refractoriness to alkylators before study entry. Refractoriness to prior alkylator therapy was defined as disease that failed to achieve a minimal response or progressed while on therapy, or within 60 d of last therapy.

Table 1. Efficacy by Subgroup.

Patients		n	ORR, % (95% CI)	Median PFS, (95% CI), mo
Total		202	29.7 (23.5, 36.5)	4.4 (3.7-5.1)
Alkylator exposed	**Alkylator refractory**			
0	NA	24	50.0 (29.1, 70.9)	7.1 (3.7-9.0)
≥ 1	0	62	33.9 (22.3, 47.0)	5.3 (4.2-7.9)
1	1	43	23.3 (11.8, 38.6)	4.6 (3.0-6.5)
≥ 2	1	40	35.0 (20.6, 51.7)	3.7 (2.4-4.9)
≥ 2	≥ 2	33	9.1 (1.9, 24.3)	3.1 (1.7-4.0)

CI, confidence interval; mo, months; NA, not applicable; ORR, overall response rate; PFS, progression-free survival.

Results: Of 202 pts (HORIZON: n = 157, cutoff January 14, 2020; O-12-M1: n = 45, cutoff October 29, 2019), 178 (88%) had been exposed to alkylators in ≥ 1 prior LoT (see Table for subgroups). Pts exposed and refractory to alkylators in ≥ 2 LoTs had the highest number of pts refractory to an alkylator in the last LoT (61%), and 82% were refractory to an alkylator within 12 mo of study entry. Meaningful response rates were seen in all subgroups, except for pts who were exposed and refractory to alkylators in ≥ 2 prior LoTs (see Table 1). PFS trended toward being shorter with higher exposure and refractoriness to prior alkylators. Re-

sults should be interpreted with caution due to limited pt numbers. Grade 3/4 adverse events (AEs) were similar between pts exposed to prior alkylators (O-12-M1: 85%; HORIZON: 89%) and the overall population (O-12-M1: 84%; HORIZON: 89%). The most common AEs were hematologic, but were mostly reversible and clinically manageable. Non-hematologic AEs were infrequent and primarily grade 1/2.

Conclusions: Melflufen in combination with dex showed meaningful efficacy and a clinically manageable safety profile in pts with RRMM exposed/refractory to prior alkylators.

P14

FORCED CRISPR/CAS9-MEDIATED EXPRESSION OF NEAT1 CORRELATES WITH THE ACQUISITION OF A CHEMORESISTANT PHENOTYPE IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is a fatal malignant proliferation of antibody-secreting bone marrow plasma cells characterized by a marked genomic instability. The discovery of lncRNAs has added a further layer of complexity to the pathobiology of the disease. *NEAT1* is a highly expressed lncRNA located at 11q13, retained in the nucleus where it forms the core structural component of the paraspeckle sub-organelles. It may act as transcriptional regulator for numerous genes, some of which involved in cancer progression, and it has been reported to play a role in cellular stress response. NEAT1 silencing negatively regulates proliferation and viability of MM cells and negatively affects the expression levels of genes and active fraction of proteins involved in initial and crucial steps of the Homologus Recombination (HR) pathway, highlighting NEAT1 as a pivotal player in the control of DNA integrity. Here, we evaluated whether MM cells could gain survival advantages from the forced expression of NEAT1. We adopted a CRISPR/Cas9 Synergistic Activation Mediator editing system to establish a NEAT1-forced expressing MM cell line. NEAT1-forced expression, validated using qRT-PCR and NEAT1-specif RNA-FISH approaches, did not resulted in significant modulation of MM cells viability or growth rate. Furthermore, NEAT1-forced expression affected the expression levels of two essential paraspeckles proteins, NONO and SFPQ, both reported to be involved in DNA damage response, by acting at post transcriptional level. Accordingly, NEAT1-forced expression directly correlated with an increase in both number and size of paraspeckle sub-organelles. Finally, the forced expression of NEAT1 led to an upregulation of the active form of RPA32 and CHK2 proteins, suggesting a higher efficiency of HR pathway. These results are in line with the lower sensitivity to Bortezomib, Carfilzomib, and Melphalan observed in NEAT1-forced expressing cells, allowing to hypothesize that the NEAT1-mediated upregulation of key responder proteins of the HR pathway could be considered a determinant of chemoresistance in MM cells. Overall, we provided novel important insights into the role of NEAT1 in DNA damage response, contributing to shed light on one of the possible mechanism of action of this lncRNA deregulated in MM. Our result, together with previous data, strongly suggest that NEAT1 should be considered as a new potential powerful therapeutic target for MM treatment.

P15

AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS AGED BETWEEN 65 AND 74 YEARS: NOT TOO OLD TO DO THE BEST

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Background: Autologous stem cell transplant (ASCT) is currently considered the golden standard treatment for newly diagnosed multiple myeloma patients under the age of 65; however, strong evidence of feasibility and safety of ASCT in elderly population is lacking and its role is still controversial.

Aim: To evaluate safety and effectiveness of ASCT in elderly MM patients treated between 2010 and 2019 in our center.

Patients and Methods: 52 newly diagnosed MM patients were included, 30 males and 22 females with a median age of 67 years (range 65-74). ISS was I in 21%, ISS II in 46%, ISS III 32%. Karyotype was evaluated in 80% patients; of them, 7% presented high-risk karyotype. Myeloma frailty score, ECOG, R-MCI and HCT-CI before ASCT were calculated. Bortezomib-based regimens were adopted before ASCT in most patients; conditioning regimen with Melphalan was administered (100 or 140 or 200 mg/mq, according to clinical evaluation). A second ASCT was performed on the basis of clinical response to the first procedure and cryopreserved PBSC availability. Maintenance with Thalidomide or Lenalidomide was administered in a subset of patients.

Statistical analysis: COX regression model, log-rank test and Kaplan-Meier estimator.

Results: Overall response rate (ORR) after induction was 96%, with 36% (19/52) CR, 50% (26/52) VGPR and 9% (5/52) PR. 68% of patients were conditioned with Melphalan 200 mg/mq, 17% with 140 mg/mq and 15% with 100 mg/mq. 85% of patient underwent a single ASCT and 15% double ASCT. Three months after the procedure, 86% patients reached CR or VGPR, obtaining a significant response improvement (p=.006). Median PFS and OS were 35 (95%CI 18-41) and 75 (95%CI 43-77) months, respectively. No patients died because of TRM. In multivariate analysis, disease status after induction was significantly related to better PFS [HR 1.80 (95%CI 1.03-3.14); p=.038] and OS [HR 2.04 (95%CI 1.05-3.96); p=.034]; similarly, disease status after ASCT strongly impacted on PFS [HR 5.22 (95%CI 2.17-12.57); p=.000] and OS [HR 4.40 (95%CI 1.75-11.08); p=.001]. Factors related to patient (age, frailty score, HCT-CI or R-MCI, ECOG), disease (ISS, karyotype) and treatment (ASCT number, maintenance) did not impact on PFS and OS in multivariate analysis.

Conclusion: Our data confirmed that ASCT was a safe procedure in elderly MM patients and its efficacy was strongly associated with the clinical responses achieved after the subsequent steps of the frontline therapy.

P16

ON-DEMAND PLERIXAFOR WITH CYCLOPHOSPHAMIDE AND G-CSF FOR HEMATOPOIETIC STEM-CELL MOBILIZATION IN MULTIPLE MYELOMA PATIENTS: PRELIMINARY RESULTS OF A PROSPECTIVE OBSERVATIONAL STUDY (MOZOBL06877)

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Background: Autologous stem cell transplantation is a standard of care in transplant-eligible newly diagnosed multiple myeloma (NDMM). 5-15% of MM pts mobilized with granulocyte colony-stimulating factor (G-CSF) or G-CSF+cyclophosphamide (G-CSF/CY) fail stem-cell col-

lection (<2×10^6/kg CD34+). Plerixafor (PLX) with G-CSF or G-CSF/CY increases stem-cell yield and lowers the rate of poor mobilizers (PM). We present preliminary results of the prospective, observational MOZOBL06877 study (partially supported by Sanofi investigation funds) to evaluate the performance of stem-cell mobilization with G-CSF/CY plus on-demand PLX in NDMM patients.

Methods: NDMM pts undergoing stem-cell mobilization with CY (2-4 g/m^2) and G-CSF (5-10 mcg/kg/day), with on-demand PLX according to clinical practice (<20 CD34+/ul on 1st count day or <1×10^6 CD34+ cells/kg collected on first apheresis day), could be enrolled. Primary endpoint was the PM rate (patients collecting <2×10^6 CD34+ cells/kg); secondary endpoints were number of patients requiring PLX, stem-cell yields, predictive factors for PLX use and adverse events (AEs).

Results. 192 patients were analysed. 187/192 (97%) patients successfully collected ≥2×10^6/Kg CD34: of these, 153/192 (80%) collected with G-CSF/CY, 29/192 (15%) required the administration of PLX. The PM rate was 5/192 (2.5%): of these, 3/5 did not receive PLX in addition to G-CSF/CY, while 2/5 failed stem-cell collection despite the use of PLX. The median number of CD34 collected was 9.8×10^6/Kg (6.7-14.2), 5.1 (4.3-9.1) with and 10.6 (8.1-14.4) without PLX. The median number of apheresis days was 1 without PLX and 2 with PLX; stem-cell collection efficiency (CD34 number collected/ days of apheresis) was 8.8 without PLX and 3 with PLX. The median number of CD34/ul pre-apheresis was 16 (10-19.5) before and 46 (21-81) after the administration of PLX. Grade 3-4 non hematological AEs occurred in 2% of pts. Factors predicting the use of PLX were ISS 3 (vs. 1, OR 4.43; p=0.008), bone marrow plasma cells at diagnosis >60% (OR 3.85; p=0.006), white blood cell (WBC) count pre-mobilization (OR 6.66; p<0.001) and lenalidomide-based therapy (OR 3.85; p=0.03).

Conclusion. On-demand PLX combined with G-CSF/CY is a safe and effective rescue strategy for stem-cell collection in MM, reducing the PM rate to 2.5%. Extensive bone marrow plasmacytosis, ISS 3 disease at diagnosis, use of lenalidomide during induction and a low WBC count pre-mobilization predicted the use of PLX.

Table 1. Multivariate analysis of factors associated with increased risk of plerixafor need in the study population.

Multivariate analysis		
Variable	**Odds ratio (95% CI)**	**p value**
ISS stage 2 vs 1	0.52 (0.15 - 1.78)	0.3
ISS stage 3 vs 1	4.43 (1.48 - 13.32)	<0.01
Bone marrow plasma cells at diagnosis > 60%	3.85 (1.47 - 10.09)	<0.01
WBC prior mobilization < 4 x 10^9/L	6.66 (2.27-20)	<0.001
Lenalidomide-based induction	3.85 (1.12-14.29)	0.03

Abbreviations: ISS, International Staging System; CI, confidence interval; WBC, white blood cell.

P17

MINIMAL RESIDUAL DISEASE (MRD) BY MULTIPARAMETER FLOW CYTOMETRY (MFC) AND NEXT-GENERATION SEQUENCING (NGS) IN NEWLY DIAGNOSED TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA (NDMM): RESULTS FROM THE FORTE TRIAL

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Background. In the FORTE trial, we evaluated MRD results by MFC and NGS techniques, focusing on outcomes and rates of conversion in different treatment arms.

Methods. NDMM pts ≤65 years (y) were randomized (R1) to KRd induction plus ASCT and KRd consolidation (KRd_ASCT), 12 KRd cycles (KRd12), or KCd induction plus ASCT and KCd consolidation (KCd_ASCT). Pts were then randomized (R2) to KR *vs* R maintenance. MRD was assessed by 8-color 2nd-generation flow cytometry (sensitivity 10^{-5}) in ≥VGPR pts. In ≥CR pts, MRD was also assessed by NGS (Adaptive Biotechnologies, Seattle, US-WA; sensitivity 10^{-5}–10^{-6}). In a logistic regression analysis adjusted for ISS stage (I *vs* II/III) and R1, we evaluated MRD-positive (pos) to -negative (neg) conversions during maintenance and we assessed PFS and OS of MRD-neg *vs* -pos pts in the ITT population. MFC-pos pts also included < VGPR pts; NGS-pos pts included MRD-pos plus < CR pts (excluding CR pts not evaluable by NGS). 1-y sustained MRD-neg by MFC and NGS was evaluated in pts with ≥2 samples available ≥1 y apart.

Results. We previously presented MRD-neg rates by MFC and NGS before maintenance (EHA 2020). At R2, 65% of randomized pts were MRD-neg by MFC (equally distributed in the 2 arms); 39% (48/123) of MRD-pos pts turned neg: 46% (29/63) in KR *vs* 32% (19/60) in R arms (OR 2.27, *P*=0.04). At R2, 72% of CR-evaluable pts were MRD-neg by NGS (equally distributed in the 2 arms); 44% of MRD-pos pts (21/47) turned neg at 10^{-5}: 14/25 (56%) in KR vs 7/23 (30%) in R arms (OR 3.72, *P*=0.04). In the ITT analysis, after a median follow-up of 45 mo from R1, MRD-neg pts before maintenance by both techniques had superimposable prolonged PFS and OS *vs* MRD-pos pts: 3-y PFS was 80% *vs* 52% (HR 0.36, 95% CI 0.26-0.49, *P*<0.001) in MFC-neg *vs* MFC-pos pts and 83% *vs* 55% (HR 0.34, 95% CI 0.22-0.52, *P*<0.001) in NGS-neg *vs* NGS-pos pts (Figure 1); 3-y OS was 96% *vs* 79% (HR 0.24, 95% CI 0.14-0.42, *P*<0.001) in MFC-neg *vs* MFC-pos pts and 97% *vs* 82% (HR 0.30, 95% CI 0.15-0.61, *P*<0.001) in NGS-neg *vs* NGS-pos pts. MRD-neg confirmed a PFS advantage in all subgroups, particularly in the high-risk setting. PFS in 1-y sustained MRD-neg pts was superimposable between MFC and NGS (4-y PFS 88% by MFC and 94% by NGS at 10^{-5}).

Conclusion. We confirmed a high clinical concordance between MFC and NGS. With both techniques, conversions to MRD-neg were high with KR maintenance and the outcomes of MRD-neg pts were similar, as well as those of 1-y sustained MRD-neg pts.

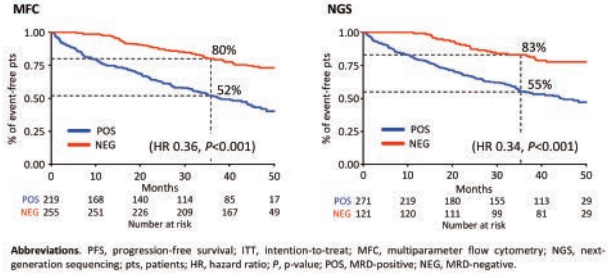

Figure 1. PFS results in ITT pre-maintenance patients.

P18

GAIN1Q IMPACTS ON SURVIVAL OF CYTOGENETIC STANDARD RISK MULTIPLE MYELOMA PATIENTS

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Current multiple myeloma (MM) prognostic stratification is based on cytogenetic aberrations identified by interphase fluorescence *in situ* hybridization (FISH). According to the Revised International Staging System (R-ISS), t(4;14), t(14;16) and deletion of 17p13 are considered high risk (HR) aberrations, while all other abnormalities are considered standard risk (SR). However, some recent studies suggest a worse outcome for t(11;14) cases and growing evidence associates gain 1q21 (+1q) with a poor prognosis. The aim of this study was to evaluate the prognostic

significance of t(11;14) and +1q in a cohort of cytogenetic SR MM patients according to R-ISS. Among 352 newly diagnosed MM patients admitted to our Center, 186 cases of SR MM were identified by FISH. Within this latter group, 71 (38%) cases harbored t(11;14). Comparing t(11;14) *versus* non-t(11;14) SR patients, there were no differences in terms of ISS III stage, lactate dehydrogenase (LDH) levels, serum-free light chain (sFLC) ratio ≥ 100, renal impairment, hypercalcemia, anemia and bone disease at the diagnosis. Considering treatments, no difference was found in type and median number of previous therapies (median = 2 lines). Overall, chromosome 1 alterations, namely +1q and del1p, were present in 36% and 8.6% of cases, respectively, with 7.5% of patients showing > 3 copies of 1q. Of notice, +1q and del1p were significantly higher in the non-t(11;14) compared to the t(11;14) subgroup (42.6% *vs* 25.8%, p=0.0342; 12.1% *vs* 3.0%, p=0.05, respectively).

With a median follow-up of 37 months, median overall survival (OS) of the entire cohort was 102 months. No difference in OS was found between the t(11;14) and non-t(11;14) subgroups (80 *vs* 110 months, p=0.2282). Interestingly, the presence of +1q was associated with a reduced OS in the entire case study (57 *vs* 105 months, p=0.004) and in the t(11;14) and non-t(11;14) subgroups separately (31 *vs* 102 months, p=0.0032 and 62 months *vs* not reached, p=0.0268, respectively). The role of +1q in SR MM was further confirmed by the fact that, excluding +1q, no difference in survival between t(11;14) and non-t(11;14) was demonstrated (102 months *vs* not reached, p=0.65).

In conclusion, the presence of +1q impacts on OS in cytogenetic SR MM and particularly in the t(11;14) subgroup, suggesting that this alteration might be helpful in the risk stratification of non-HR MM. Within SR MM, t(11;14) without +1q does not identify a subset of patients with worse outcome.

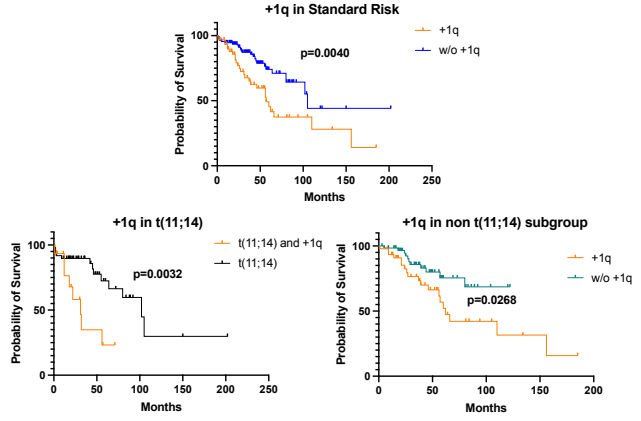

Figure 1.

P19

DIS3 MUTATIONS IN MULTIPLE MYELOMA IMPACT THE TRANSCRIPTIONAL SIGNATURE AND CLINICAL OUTCOME

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Multiple myeloma (MM) is characterized by a profound genomic instability involving ploidy, structural rearrangements, and a wide array of mutations affecting both putative oncogenes and tumor suppressor genes, among which great attention deserves DIS3 that maps at 13q22 and encodes for a highly conserved ribonuclease indispensable for survival in vertebrates. *DIS3* gene mutations (DIS3mts) occur in roughly 10% of MM patients; furthermore, DIS3 expression could be affected by monosomy 13 or del(13q), which occur in approximately 40% of MM cases. Despite several reports on the prevalence of DIS3mts, their contribution to the pathobiology of MM remains largely unknown. We took advantage of the large public Multiple Myeloma Research Foundation (MMRF) CoMMpass dataset to investigate the spectrum of DIS3mts in MM and its impact on the transcriptome and clinical outcome. Among the identified DIS3mts, 80% occurred in the active domains of the protein, 42% of which in three mutational hotspots within the RNase II/R (RNB) domain. DIS3mts showed a clear pattern of co-occurrence with other molecular alterations, mainly del(13q), 1q gain, t(4;14) or MAF translocations. We found that DIS3mts clinical relevance strictly depended on *DIS3* RNA mutational load (>20%) and del(13q) co-occurrence. In particular, bi-allelic DIS3 lesions significantly affected PFS, independently from other predictors of poor clinical outcome, while mono-allelic events mostly impacted OS. DIS3 is a key component of the multi-subunit RNA exosome complex in eukaryotic cells involved in the processing, quality control and degradation of virtually all classes of RNA. Our study further supports and extends the notion that DIS3mts affect the transcriptome, showing a stronger impact on non-coding RNA species, mainly lncRNAs. Indeed, we found that approximately half of the transcripts predicted to be specifically deregulated by the presence of DIS3mts are represented by novel, largely uncharacterized lncRNAs. Among them we identified five distinct transcripts as independent predictors of poorer OS and nine of worse PFS, some of which (*AC015982.2* and *AL445228.3*) predicting both. These findings strongly prompt further studies investigating the relevance of these lncRNAs in MM. Overall, our comprehensive evaluation of the clinically and transcriptional consequences of DIS3mts/deficiency in MM strongly indicates that they may play an important role in the mechanisms of MM transformation and progression.

P20

FRONTLINE THERAPY FOR NON-TRANSPLANT ELIGIBLE MULTIPLE MYELOMA: A CRITICAL APPRAISAL OF PUBLISHED NETWORK META-ANALYSES

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Newly diagnosed multiple myeloma (MM) who are transplant ineligible (NTE NDMM) are usually treated with multiple-drug combinations including proteasome inhibitors, immunomodulatory drugs and alkylating agents. Recently approved combo therapies including anti-CD38 monoclonal agents and/or lenalidomide improve progression-free survival (PFS) as compared with one of the standard treatments. We thus aimed at assessing the relative efficacy of novel daratumumab-based and lenalidomide based triplets/quadruplets as compared with overall standard treatments for NTE NDMM, namely Rd and VMP. Network meta-analyses (NMA) are accepted evidence-based tools for conducting indirect comparisons among treatments, however, the scientific community is still skeptical regarding their robustness. We, therefore conducted an umbrella review: fully published NMAs were retrieved by standard searches (EBMASE, Cochrane Library, MEDLINE/PubMed) and appraised by AMSTAR-2 and ROBIS tools. Three indirect comparisons of PFS were targeted: 1) VRD versus VMP, 2) DaraRd versus VMP, 3) DaraVMP versus Rd. Overall 17 NMA addressing NDMM were published since Jan 2017: 6 fully published ones including both daratumumab- and lenalidomide-based novel treatments were appraised. The overall quality of the NMAs was poor to moderate according to AMSTAR-2 and ROBIS. Each NMA analyzed 6 to 27 trials and 2 ones were company sponsored.

1) VRD was compared to VMP by 3 moderate-quality NMAs, which consistently reported a significant amelioration of PFS or higher SUCRA of VRD, while OS-HR was not conclusive.

2) DaraRd was compared to VMP by 4 NMAs and the pooled PFS-HR ranged from 0.39 to 0.61. A significant amelioration of OS was also

reported by the unique NMA assessing this endpoint.

3) DaraVMP was compared versus Rd by 4 NMAs. Pooled HR ranged from 0.35 to 0.71, which was statistically significant in two ones. DaraVMP achieved the highest SUCRA (0.960) in the latest and largest NMA (Giri et al 2020). Only one NMA compared OS of the two regimens and did not report a significant advantage of DaraVMP.

In conclusion, Dara-VMP, VRD and Dara-Rd show mostly a favorable PFS profile as both directly and indirectly compared with standard frontline treatments for NTE NDMM. NMAs are valuable evidence-based tools, however, their quality needs to be appraised before using their result to support clinical recommendations. Future NMAs are expected to incorporate also safety endpoints in order to allow benefit to risk assessments.

Acute Leukemias and Myelodisplastic Syndromes 1

P21

ROLE OF ALLOGENEIC STEM CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROME: A STUDY FROM THE ITALIAN FISIM REGISTRY

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Background: Allogeneic stem cell transplantation (HSCT) is the only curative treatment for Myelodysplastic Syndromes (MDS), however few MDS patients (pts) are transplant eligible and only a small subgroup of them actually proceed to HSCT. Moreover, what is the best treatment bridge to HSCT remains unknown.

Aims: To assess the actual proportion of MDS pts undergoing HSCT, the impact of pre-HSCT therapies on outcome and the reasons preventing eligible pts to undergo HSCT.

Methods: We included in this study 1293 MDS pts diagnosed between 1994 and 2019 in Piedmont and prospectively enrolled in the FISiM registry. HSCT eligible pts (n=211) were selected with the following consensus criteria (De Witte, Blood 2017): age<70, performance status ECOG≤2, higher r-IPSS risk or lower risk with severe transfusion dependency or life-threatening cytopenia.

Results: Median age of HSCT-eligible pts was 64. According to IPSS-R, 46% of pts were at high/very high risk and 39% at intermediate risk. Sixty-seven pts underwent HSCT, representing 5% of the study population and 32% of HSCT-eligible pts, 42% after intensive chemotherapy (IC), 41% after azacitidine (AZA) and 17% upfront. Response rate to AZA (43%) and IC (50%) were similar, but only 41% of pts treated with AZA eventually underwent HSCT, as compared to 61% in IC group. Median overall survival (OS) after HSCT was 45 months and was not affected by pre-HSCT treatment. HSCT improved OS only in pts at higher IPSS-R risk (median OS 33 *vs* 14 months, p<0.001). The main reasons that prevented eligible pts to receive HSCT were procrastination of HSCT in intermediate risk pts and failure of cytoreductive treatment in the higher risk ones. Some lower risk pts (n=21) were considered for HSCT at progression, when it was no longer an option for lack of eligibility criteria, others (n=16) progressed directly to AML and failed induction treatment. MDS with excess of blasts (n=134) received a cytoreductive treatment as bridge to HSCT that failed in 60% of cases, and only a 16% was rescued by II line therapy and underwent HSCT, whereas 40% progressed to AML.

Conclusions: HSCT should be promptly proposed to high-risk pts to improve survival. Pts at lower IPSS-R risk should be singularly evaluated to balance the risk of transplant-related mortality and progression, to find the best window for transplant. Prospective studies are needed to optimize the best bridge to HSCT, also considering HSCT upfront or in a sequential scheme with IC.

P22

CORRELATIONS OF TYPE OF RESPONSE TO EPO, WHO DIAGNOSIS AND SERUM EPO WITH IMMUNOPHENOTYPE OF ERYTHROID PROGENITORS AND PRECURSORS OF PATIENTS WITH LOW RISK MYELODYSPLASTIC SYNDROMES

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Erythropoietin(EPO) promotes differentiation of committed erythroid cells(COMe) into early erythroid precursors(EEP). Flow cytometry (FCM) analysis can characterize COMe and erythroid precursors(EP comprised of EEP and late erythroid precursors,LEP, that are EPO independent).The RED score assesses erythroid dysplasia using FCM markers. Anemia in lower-risk myelodysplastic syndromes (LR-MDS) is treated with erythropoietic stimulating agents (ESA),but some patients fail to respond(primary refractory, PR) or lose response(secondary refractory, SR). Serum erythropoietin (sEPO) at diagnosis >200mU/mL is the main negative predictor. We aimed at correlating distinctive immunophenotypic erythroid patterns and RED score with type of response to ESA,WHO 2016 category and baseline sEPO in 44 LR-MDS patients. We evaluated and compared with 10 negative controls, 17 PR pts, 17 SR pts and 10 long responders to ESA(LR, meaning a response duration of at least 24 months). 59% of pts had sEPO<200mU/mL (L-EPO), 25% had 200-500mU/mL (M-EPO) and 16% had >500mU/mL(H-EPO). 23%had MDS with single lineage dysplasia(MDS-SLD), 34% multilineage dysplasia(MDS-MLD), 23% ring sideroblasts(MDS-RS) and 20% deletion of 5q(MDS 5q-). FCM analysis was performed on bone marrow aspirates (BMA) for all subgroups at time of diagnosis and, within SR subgroup, at loss of response to ESA(SRII). We used anti-CD34, CD36, CD105, CD117, CD33, CD71 and CD45 antibodies to identify COMe, total BM eryhroid cells (TOTe) and EP. Coefficient of variation of CD71 and CD36 along with hemoglobin level were used to calculate RED score. LR-MDS showed increased TOTe and COMe compared to controls (p=0.013 and p=0.04 respectively), with the exception of MDS 5q- cases where they were reduced (p=0.047). In PR subgroup there was a trend of increased LEP and reduced COMe compared to LR. At SRII COMe were lower than diagnosis(SRI,p=0.039). Finally, RED score was higher in PR compared to LR and SR(p=0.011). MDS 5q- showed lower fraction of TOTe (p=0.047), while MDS-RS cases had increased LEP and RED score (p=0.008 and p=0.012, respectively). H-EPO group showed increased LEP compared to L-EPO cases (p=0.023). These data suggest that increased COMe seems to be positively associated with ESA response, whereas higher LEP and RED score are negatively associated and correlate with MDS-RS and H-EPO. Finally, MDS-5q- cases displayed fewer TOTe and COMe, suggesting a premature erythroid maturation block.

P23

REAL-WORLD TREATMENT PATTERNS AND PATIENT MANAGEMENT IN UNFIT PATIENTS WITH AML RECEIVING FIRST LINE SYSTEMIC TREATMENT OR BEST SUPPORTIVE CARE (CURRENT): ANALYSIS OF THE ITALIAN STUDY POPULATION

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Introduction: CURRENT is a multi-country non-interventional, retrospective study aimed at evaluating the treatment pathways and outcomes, and economic impact of AML patients (pts) unfit for intensive chemotherapy and who received first line systemic treatment (1stL) or Best Supportive Care (BSC) in the real-world setting.

Methods: Here, we analyzed the Italian population of pts enrolled between Jan 2015 and Dec 2018. Pts were followed up until the last recorded visit or death. The main objective was to evaluate the overall survival. Secondary objectives were description of clinical outcomes, patient characteristics, cytogenetic/molecular profiles, treatment patterns and healthcare resource utilization.

Results: 74/81 enrolled pts had complete data for the analysis, 62 treated with 1stL therapy and 12 with BSC; patients and treatment characteristics are shown in Table1. Of the 62 pts in the 1stL arm, 31 (50%) achieved a response (CR+CRi+PR, respectively 15/6/10 pts). CR/CRi had a median duration of 246 days; no CR/CRi/PR was reached in the BSC group. Median time to best response was 134 days in 1stL pts. The median OS from diagnosis was 13.4 months, with the highest among patients receiving HMAs. Median OS was the lowest in BSC pts group (2.7 months). Median PFS ranged from 2.5 months in the BSC arm to 11.8 months in the HMA arm. The most common 1stL treatment was 5-azacytidine (AZA, around 60% of pts). Among those who started 1stL of therapy, only 11.3% received a second or later line of treatment. Independently of the treatment received, most patients needed hospitalization (66.1% and 66.7% of 1stL and BSC pts). Main reason for 1stL patients was infections (45.6%). The use of antibiotics and antivirals (92% and 75% of 1stL and BSC pts), antifungal (58% and 51%) throughout lines of treatment was frequent, for both prophylaxis and curative reasons, while growth factors were less used (6.5% only in 1stL pts). Most patients had blood transfusions (85.5% of 1stL and 100% of BSC pts) during treatment.

Conclusions: Even with some limitations, this study provides a detailed real-world insight in treatment patterns, clinical outcomes, clinicopathologic characteristics, and use of health resources in AML patients unfit to receive intensive chemotherapy in Italy. Caring for these patients requires the use of a variety of resources within the health care system. Outcome for AML patients remains poor, and novel agents or combinations are needed.

Table 1.

Table 1. Patient characteristics and treatment patterns

	Fist-Line Systemic Therapy (n=62)	BSC only
Male	30/62 (48.4%)	10/12 (83.3%)
Median age at diagnosis, years (range)	76.5 (58-88)	77.5 (52-89)
Secondary AML	21/62 (33.9%)	3/12 (25%)
Type of secondary AML:		
- MDS	11 (52.4%)	3 (100%)
- CMML	1 (4.8%)	0
- MPN	2 (9.5%)	0
- t-AML	7 (33.3%)	0
Prior HMA treatment for antecedent disorder:		
Yes	2 (9.5%)	1 (33.3%)
No	17 (81%)	2 (66.7%)
Unknown	2 (9.5%)	0
ECOG performance status		
· 0-1	26/62 (41.9%)	3/12 (25%)
· ≥2	13/62 (20.9%)	3/12 (25%)
· unknown	23 (37.1%)	6 (50%)
Molecular profile		
Any mutation	9 (14.5%)	3 (25%)
· TP53	2 (22.2%)	0
· RUNX1 mutation	1 (11.1%)	0
· ASXL1 mutation	1 (11.1%)	0
· FLT3 mutation	0	1 (33.3%)
· FLT3ITD mutation	3 (33.3%)	1 (33.3%)
· FLT3TKD mutation	1 (11.1%)	0
· CEBPA mutation	1 (11.1%)	0
· NPM1 mutation	2 (22.2%)	2 (66.6%)
No mutation	43 (69.4%)	8 (66.7%)
Unknown molecular profile	10 (16.1%)	1 (8.3%)
Cytogenetic risk		
· Favorable	5 (8.1%)	1 (8.3%)
· Intermediate	27 (43.5%)	6 (50%)
· Poor	24 (38.7%)	3 (25%)
· Unknown	6 (9.7%)	2 (16.7%)
First-line treatment received*:		
Systemic therapy	62 (100%)	-
· HMA (azacitidine)	37 (59.7%)	-
· HMA (decitabine)	25 (40.3%)	-
· LDCA	1 (1.6%)	-
· Venetoclax	1 (1.6%)	-
· Other	1 (1.6%)	-
		-
Treatment combinations:		
· Azacitidine + Decitabine	2 (3.2%)	-
· Azacitidine + Venetoclax	1 (1.6%)	-
BSC only		12 (100%)
Hospitalized Patients		
Yes	41/62 (66.1%)	8/12 (66.7%)
Median Number of Hospitalization (range)	2.0 (1-5)	2.0 (1-2)
***Patients could be treated also with combination of therapies.**		

P24

REFINED EVALUATION OF MINIMAL RESIDUAL DISEASE BY DIGITAL DROPLET PCR IN ADULTS WITH PHILADEPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA: IMPACT OF BLINATUMOMAB ADMINISTRATION IN THE FRONT-LINE SETTING AND OF THE PH-LIKE SIGNATURE

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Despite high complete remission rates (80-90%) with multi-agent chemotherapy, a significant proportion of adults with Philadelphia-negative acute lymphoblastic leukemia (Ph- ALL) relapse and long-term survival rate is slight more than 50%. In the GIMEMA LAL2317, designed for adults with newly diagnosed B-lineage Ph- ALL, two doses of blinatumomab were added in the consolidation phase. Real-time quantitative polymerase chain reaction (RQ-PCR) is the gold-standard tool for minimal residual disease (MRD) monitoring in ALL. However, in samples with a very low MRD burden, digital droplet PCR (ddPCR) proved to be reliable for MRD monitoring with sensitivity at least comparable to RQ-PCR, but with a greater accuracy. We analyzed MRD status by RQ-PCR and ddPCR in a cohort of patients enrolled in the GIMEMA LAL2317 trial and evaluated the efficacy of the first cycle of blinatumomab in eradicating MRD also according to the Ph-like status We performed a sub-analysis on a small cohort of patients that underwent a centralized comprehensive molecular screening at diagnosis including Ig/TR target screening, BCR/ABL1-like predictor assay1, targeted DNA- and RNA- sequencing. MRD status was monitored at specific timepoints (TP), *i.e.* TP2 and TP3, by RQ-PCR and ddPCR2,3. We analyzed 30 Ph- ALL patients: 9/30 (30%) patients were Ph-like according to the BCR/ABL1-like predictor and in 7/9 at least one Ph-like associated genetic lesion was identified. All patients received at least one course of blinatumomab. Before blinatumomab, RQ-PCR showed MRD positivity in 6/9 (66.7%) Ph-like patients and 8/21 (38.1%) non Ph-like patients. DdPCR analysis, evaluable in 29 samples, showed MRD positivity in 5/8 (62.5%) Ph-like cases and 10/21 (47.6%) non Ph-like cases. After blinatumomab, all patients proved MRD negative by RQ-PCR; in contrast, ddPCR proved positive in 3/9 (33%) Ph-like cases (1 being positive not quantifiable) and in 2/21 (9.5%) non Ph-like patients. In this small series, we confirm that Ph-like patients show a slower MRD clearance compared to the non-Ph-like ones, with 66.7% being MRD positive *vs* 38.1%. Importantly, blinatumomab was capable of inducing MRD negative status in all patients analyzed by RQ-PCR in spite of the Ph-like signature. Nevertheless, DdPCR allowed to recover MRD positivity, mostly in Ph-like patients. Survival data are not proved due to the short follow up of the trial. The screening of a larger cohort is warranted.

P25

FLOW-CYTOMETRIC ASSESSMENT OF BONE MARROW MICROENVIRONMENT IN LOW-RISK MYELODYSPLASTIC SYNDROMES

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There is growing interest on the immunologic bone marrow (BM) microenvironment of low risk myelodysplastic syndromes (LR-MDS) for its pathogenic and therapeutic relevance. BM flow cytometry (CFM) of LR-MDS patients have been retrospectively evaluated by 2 CFM experts to evaluate BM microenvironment (BMM) relationship with MDS features, somatic mutations, and response to recombinant EPO (rEPO). Lymphocyte (ly) populations, monocytes, and mastocytes have been evaluated. A total of 136 LR-MDS patients, 60% males (median age 73.6years, 36-90.6), have been studied. As shown in table 1, MDS-RS showed lower B-ly and monocytes compared to MDS-SLD (p=0.008 and p=0.03, respectively) and MDS-MLD (p=0.01 for B-ly). Elderly patients showed higher mastocytes (1.88+1.3% in >65years *vs* 1.2+0.7% in younger patients, p=0.05). Patients with Hb<10g/dL showed lower monocytes (3.4+2.2 *vs* 4.45+3%, p=0.02) compared to not anemic patients; those with PLT<100x10^9/L showed lower T-ly (67.2+15.4 *vs* 72.4+10.3%, p=0.02) and higher B-ly (14.2+11.5 *vs* 10.5+6.5%, p=0.02) compared to those with higher PLT; and subjects with neutrophils <1x10^9/L displayed higher mastocytes (2.5+2 *vs* 1.6+1%, p=0.004) and monocytes (5.5+4.08 *vs* 3.7+2.3%, p=0.004), as compared to those with higher values. Hypocellular patients showed increased total ly (20+9.3 *vs* 13+7.5%, p<0.0001) and NK (21+17 *vs* 16.6+8%), and decreased mastocytes (1.2+1 *vs* 1.8+1.2%, p=0.03) compared to normo-hypercellular ones. The presence of >3 somatic mutations was associated with increased total ly (22.4+14 *vs* 10.8+3.7%, p<0.001). Specifically, mutations in DNA methylation were related to lower T-ly (69+11 *vs* 76+9%, p=0.04) and higher NK (18+8 *vs* 14+7%); mutated chromatin and transcription factors were associated with higher total ly (25+14 *vs* 11+6%, p=0.001 and 22.5+16.5 *vs* 12.3+6.8%, p=0.02, respectively). Mutations in TP53 or PHF6 were associated with increased T-ly (83+0.7 *vs* 73+10%, p<0.001), mastocytes (2.4+1.4 *vs* 1.7+0.7, p<0.01), and monocytes (7.5+5 *vs* 3.8+2.3%, p=0.05), and decreased NK (7+1.4 *vs* 16+8, p<0.001). Patients responsive to rEPO had reduced T-ly (69+14 *vs* 76+10, p=0.05) and increased B-ly (13+11 *vs* 7.5+5%, p=0.01). Different WHO types of LR-MDS show variable levels of BM ly, mastocyte and monocyte percentages. BMM characteristics correlate with cellularity and peculiar molecular alterations. Patients with greater T-infiltrate display higher frequency of disrupting mutations (*i.e.* TP53) and lower response to rEPO.

Table 1.

Table 1: Bone marrow microenvironment cells in low-risk myelodysplastic syndromes (LR-MDS) according to WHO classification. Values are given as mean ± standard deviation.

WHO category	Lymphocytes	T-cells	B-cells	NK-cells	Mastocytes	Monocytes
All patients	14.2±8	71±12	11.5±8	17.2±10	1.8±1.3	4±2.7
MDS-SLD (n=39)	13.9±6	69.9±10	12.8±6	16.9±10	1.7±1	4.4±2.5
MDS-MLD (n=48)	15.3±9	70.4±13	12.7±8	16.7±9	1.8±1.4	3.8±3
MDS-RS (n=37)	12.4±8	71.9±13	8.8±7	19.1±13	1.7±0.7	3.2±2
5q- syndrome (n=5)	17±6	78.6±5	7.7±2	13.8±7	1.5±1	3.8±1.4
MDS/MPN (n=5)	14.3±12	78.2±6	6.2±2	15.6±6	2.3±0.7	9.5±6
MDS-EB1 (n=2)	21±1.4	52±28	34.5±39	13.5±11	4.2±5	2.4±1.3

NK natural killer, MDS-SLD myelodysplastic syndrome with single lineage dysplasia, MDS-MLD MDS with multilineage dysplasia, MDS-RS MDS with ring sideroblasts, MDS/MPN MDS/myeloproliferative neoplasm, MDS-EB1 MDS with excess blasts type 1.

P26

TREATMENT OF NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS: OUTCOME OF A REAL LIFE POPULATION FROM A MULTICENTRIC OBSERVATIONAL STUDY BETWEEN 2016 AND 2018

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Introduction: The outcome of acute myeloid leukemia (AML) in elderly patients is poor due to the heterogeneous biology of the disease and the concurrent comorbidities. Authorized treatments of AML include intensive chemotherapy (IC), hypomethylating agents (HMA) and best supportive care (BSC), but no direct superiority has been demonstrated in the elderly population.

Aim: To describe the approach to the treatment of elderly AML patients and to compare outcome in order to identify clinical and molecular features that could help choosing the right tailored treatment.

Methods: 306 patients aged ≥65 years with newly diagnosed AML between 2016 and 2018 from 11 Italian hematological centers were analyzed to describe clinical and biological characteristics of the disease, patients' comorbidities, kind of treatment, outcome and toxicity (days of hospitalization and severe infectious adverse events, SIAE).

Results: Median age of the study population was 73.6 years (range 65-94); ECOG was ≤2 in 79% of patients; 58% had a hematopoietic cell transplantation-specific comorbidity index (HCT-CI) ≤2 (range 0-11); 36% had unfavorable characteristics (adverse ELN 2017 cytogenetic-molecular markers or secondary AML). Patients were treated with IC (36%), HMA (45%) and BSC (19%); allogenic transplantation was performed in 6%. Complete marrow response (CR) was reached by 64% and 24% for IC and HMA treatment, respectively. In multivariate analysis, median OS was 9.5 months, increasing with lower ECOG at diagnosis (18 months for ECOG 0, p<0.01), achievement of CR (20 months, p<0.01) and allogenic transplantation (4 years-median OS not reached, p<0.05). No differences in OS were found in unfavorable cytogenetic disease despite different treatments (9 months for IC *vs* 10 for HMA, p>0.05). In the other cytogenetic groups, in spite of age, comorbidities or molecular abnormalities, IC showed a better outcome (15 *vs* 11 months, p<0.01, fig.1). Days of hospitalization and SIAE were both higher in the IC treatment (71 *vs* 24 and 1.9 *vs* 1.2 respectively, p<0.01).

Conclusions: IC and HMA confirmed superiority over BSC. Both efficacy and toxicity were higher in the IC group for patients without high risk-disease features. While allogenic transplantation remains the only durable disease-modifying treatment, elderly patients with high risk features remain an unmet clinical need. Further studies are needed to guide the approach to elderly patients in the era of new target therapies.

Figure 1: OS according to the kind of treatment and cytogenetics

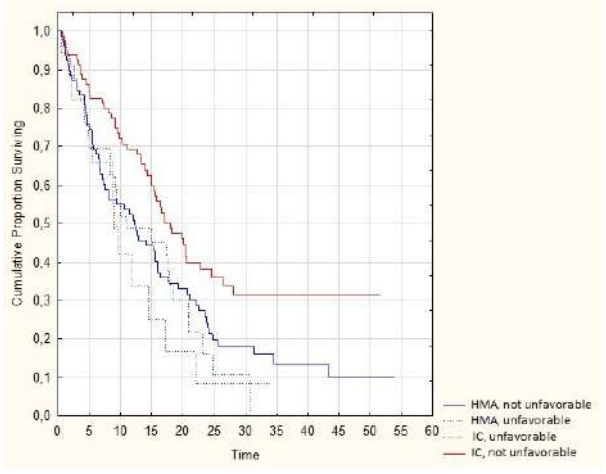

Figure 1.

P27

SEQUENTIAL DEEP AND ULTRA-DEEP SEQUENCING IN LOW-RISK NPM1-MUTATED ACUTE MYELOID LEUKEMIA WITH AN ADVERSE CLINICAL OUTCOME

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NPM1 is the most frequently mutated gene in adult acute myeloid leukemia (AML) defining a distinct entity of the 2016 WHO classification. NPM1 mutated (NPMmut) AML without FLT3-ITD is classified as a low risk AML; however, relapse rate is set around 50%. This heterogeneity of clinical outcome highlights the unmet clinical need of identifying patients at high risk of relapse.

Aim of this study was to assess the presence of co-occurring mutations and their clonal evolution at relapse in de novo low-risk NPM1mut and FLT3-ITDneg AML, in order to gain insights into the molecular pathogenesis of relapse mechanisms, through the application of a deep and ultra-deep targeted NGS. Among 54 patients with low-risk NPM1mut AML diagnosed at our Division, 50% (24/48) relapsed and, among them, 11 subjects with available DNA at multiple time points were included in this study. By NGS a remarkable overlap of mutations between diagnosis and relapse was detected (mutation persistence rate of 75%). At diagnosis the 64% of patients displayed CHIP-related mutations (DNMT3A and/or TET2). Nine complete remission (CR) samples (2 CRMRD- and 7 CR with mMRD) and 6 molecular relapse/progression samples were analyzed by ultra-deep NGS in order to improve the sensitivity of the analysis. Despite the high depth of sequencing coverage, NPM1mut was detected in only 2 out of 13 samples that tested positive at RTqPCR. Sequential analysis of both groups with or without CHIP mutations revealed a uniform evolution pattern, characterized by the disappearance of all co-occurring mutations at CR and the subsequent reappearance at relapse, displaying a high rate of stability and an overlapping allele burden between NPM1 and co-mutations (Figure 1). In the present study, ultra-deep NGS displayed significant inferior sensitivity compared to RTqPCR in detecting mMRD, likely due to discrepancies between genomic sequencing and expression-based assays and to the presence of pseudogenes that could interfere with sequencing. Therefore, we do not consider ultra-deep NGS of a large gene panel a reliable technique to investigate mMRD.

In conclusion, NPM1 mutations, although late driver mutations, occur very early before the expansion of a dominating pre-leukemic hematopoietic clone, suggesting that relapse may rely on the persistence of a chemo-resistant leukemic stem cell clone that harbors both pre-leukemic and NPM1 mutations and persists at very low levels in the absence of an evident clonal hematopoiesis.

Figure 1. Mutational profile at diagnosis/remission/relapse pairs of low risk *NPM1mut* AML patients.
* Patient #3 showed 2 distinct *DNMT3A* mutations at diagnosis: p.R882C (VAF 43%) and p.W409X (VAF 2%), this subclonal mutation grew at remission and relapse.
** Patient #8, showed *DMNT3A* p.G728D at diagnosis (VAF 38%) and relapse (VAF 12%), while *DNMT3A* p.R366C appeared in CR and persisted at relapse (VAF 3% both samples).
*** Molecular relapse. *NPM1mut* persistence detected by RT-PCR in all samples.

Pt.	Diagnosis	Remission	Relapse
#1	NPM1, DNMT3A, NRAS, RAD21	none	NPM1, DNMT3A, NRAS, RAD21, GATA2,U2AF1, WT1
#2	NPM1, GATA2, KRAS, STAG2	none	Not available
#3*	NPM1, DNMT3A, NRAD, TET2, IDH1	DNMT3A	NPM1, DNMT3A, TET2, IDH1
#4	NPM1, GATA2, PTPN11	none	NPM1, GATA2, NRAS
#5	NPM1	none	Not available
#6	NPM1, TET2	none	TET2***
#7	NPM1, DNMT3A, PTPN11	DNMT3A	Not available
#8**	NPM1, DNMT3A	DNMT3A	NPM1, DNMT3A
#9	NPM1, DNMT3A, NRAS, IDH1	Not available	none***
#10	NPM1, FLT3-TKD, SF3B1	SF3B1	NPM1, FLT3-TKD, SF3B1, PHF6
#11	NPM1, DNMT3A	Not available	NPM1, DNMT3A***

Figure 1.

P28

DARATUMUMAB NELLA LEUCEMIA ACUTA LINFOBLASTICA RECIDIVATA O REFRATTARIA. UNO STUDIO DEL CAMPUS ALL

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Acute lymphoblastic leukemia (ALL) blasts express high levels of CD38. The anti-CD38 monoclonal antibody daratumumab (DARA) is being explored in this setting. Pre-clinical studies have documented the activity of DARA in human xenograft models of ALL. The clinical experience is, however, very limited with only few case reports showing some evidence of anti-leukemic activity. In the context of the Campus ALL national framework, we retrospectively collected data on patients (pts) with relapsed/refractory(R/R) or measurable residual disease (MRD)-positive ALL who received DARA. DARA was used off label or was obtained in a compassionate use program between December 2018 and December 2020 in 17 Italian hematological centers. DARA was administered at the approved multiple myeloma schedule. Overall response rate (ORR) was defined as the proportion of pts who obtained a complete (CR) or partial (PR) response or, in MRD-positive pts, a MRD negativity.

Table 1.

Sex	Age	Disease	Previous HCT	Previous lines	Type of relapse	WBC x 10^9/l	BM Blasts (%)	ECOG	Concomitant treatment	Response to DARA	HCT post DARA	DOR, months	N of infusions	Relapse	Alive	Status
M	44	ETP	Yes	1	CR MRD pos	3.3	3	0	no	CR MRD neg	No	23	58	Yes (MRD)	Yes	CR MRD neg
M	26	ETP	No	1	Extramed + MRD pos	4.3	1.4	1	no	CR MRD pos	No	3	11	Yes	No	Relapse
M	40	B-LBL	No	3	Extramed + BM overt	0.3	78	0	CLOFA CTX	CR MRD neg	Yes	3	2	No	No	TRM
M	24	T-ALL	Yes	2	Extramedullary	3	0	1	no	PR	Yes	3	4	No	Yes	CR MRD neg

We obtained information from 20 treated pts (85% males), with a median age of 34 years (range 6-72), 70% of which had a T-lineage ALL. DARA was started at a median time of 13 months from diagnosis and pts had received a median of 3 previous lines of therapy; 80% of pts had a bone marrow (BM) relapse (either isolated or with a concomitant extramedullary localization), with a median BM blast count of 45%. Nine pts had previously received an allogeneic hematopoietic cell transplantation (HCT). DARA was administered with concomitant chemotherapy in 9 cases. At the time of starting DARA, the median ECOG was 2. The ORR was 20%: 2 pts obtained a MRD-negative CR, 1 a MRD-positive CR, 1 a PR, Table 1. 4 pts (2 responders, 2 refractory) proceeded to a HCT after DARA. At the last follow-up, all but 1 pt had stopped DARA and 2 pts are alive and in CR. The median OS was 4 weeks, with a 3-month OS rate of 25%. No unexpected toxicity was observed, with only 1 case of grade 2 infusion reaction. In the largest series so far reported, we confirm the activity of DARA in R/R ALL. Most pts were heavily pretreated, with a poor ECOG and a high disease burden, probably explaining the relatively low ORR. DARA represents a potentially useful therapeutic option, especially for T-ALL for which novel options are fewer than in B-ALL. Pts' selection, as well as an earlier use of the compound (e.g., in MRD-positive cases), is crucial to obtain meaningful results. Clinical trials exploring DARA in combination with chemotherapy in ALL are ongoing.

P29

VENETOCLAX COMBINED WITH HYPOMETHYLATING AGENT HAS SHOWN PROMISING RESULTS IN ACUTE MYELOID LEUKEMIA AND MAY BE A GOOD BRIDGE TO TRANSPLANT: A REAL LIFE EXPERIENCE OF "RETE EMATOLOGICA PUGLIESE"

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Background: VEN-HMA combination represent a significant advance in AML therapy given the very high complete response rates and prolonged response durations both in newly diagnosis (ND) and relapsed/refractory (R/R)-AML setting. Here we report the outcome of patients with ND-AML or R/R-AML treated with VEN-HMA aimed to evaluate efficacy and safety of this combination and its role as a bridge to transplant.

Method: From May 2018 to March 2021, a total of 100 patients (Pts), median age 70 years (range 23-88), 54 with ND-AML and 46 with R/R AML, were included in the analysis. Among R/R AML, 39 (84,7%) relapsed after induction therapy [24 after AML-like therapy (52,1%), 15 after HMA (32,6%)] and 7 (15,3%) after allogenic transplant. After rump-up, all pts received Venetoclax 400 mg/daily orally in 28-day cycles combined with decitabine 20 mg/m^2 days 1-5 of each 28-day cycle 74 patients (74%) or azacitidine 75 mg/m^2 days 1-7 of each 28-day cycle, 26 patients . All pts received a median of 3 cycles (range 1- 20) of venetoclax in combination with HMA. Allogeneic SCT was performed in first or second remission in all eligible patients.

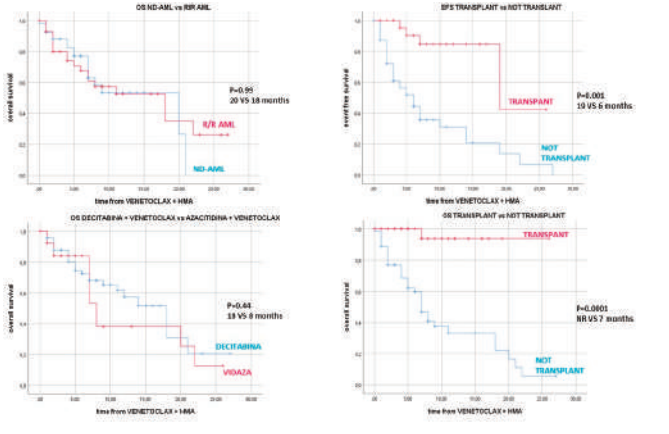

Figure 1.

Results: CR+iCR rate was 68% and 50% for ND- and R/R AML, respectively. In both groups, the median time to response was 2 months (range 1-5). After a median follow-up of 7 mo. (range 2-27), 56 out of 100 patients are alive, including 27 patients (27%) still on therapy and in CR while 44 pts. died, mostly for progressive disease. With a median FU of 11 mo. (range 4-26), 13 R/R and 6 ND-AML underwent to allogenic HSCT, and additional 6 pts are waiting for allo-SCT. Grade 3/4 hematological toxicity was observed in 60% of Pts while 35% experienced non-hematological toxicity (FN 35%, sepsis 16% and clostridium enterocolitis in 2%). There was no statistically significant difference in EFS and OS between ND-AML or R/R AML (p=0.99) as well as between those receiving venetoclax combined with decitabina or azacitidina (p=0.44). In contrast, both median EFS 19 *vs* 6 mo. (p=0.0001) and OS NR *vs* 7 mo. (p=0.0001) was better for transplanted patients compared to did not. The estimate 24 mo. EFS and OS rate was 84% and 93 % respectively in transplant population FIG1.

Conclusion: These real life data demonstrate how the combination Venetoclax and azacitidine or decitabine is safe and effective both ND- and R/R-AML Pts. Most of eligible Pts underwent allo-SCT suggesting as this combination represents a good bridge to transplant.

P30

SYSTEMIC INFLAMMATORY AND AUTOIMMUNE DISEASES ASSOCIATED TO MYELODYSPLASTIC SYNDROMES HAVE NO IMPACT ON OUTCOME

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MDS is associated with features of immunological dysregulation. The coexistence of systemic inflammatory and autoimmune diseases (SIADs) in patients with myelodysplastic syndrome (MDS) or chronic myelomonocytic leukemia (CMML) has been widely recognized, although with distinct results regarding their prevalence and impact on the outcomes. Here, we investigated the prevalence of autoimmune diseases among MDS and CMML patients, comparing characteristics and outcomes in those with and without autoimmune diseases in a monocentric retrospective study in A.O.U. Ospedali Riuniti of Ancona. We analyzed 199 patients with MDS and CMML with median age of 73.5 years (range, 29-94) and 54.8% were male. According to the IPSS-R, 31 (19,1%) patients were classified as very low risk; low risk in 81 (49.7%); intermediate risk in 29 (17,8%); high risk in 12 (7,4%); and very high risk in 10 (6,1%). Clinical autoimmune diseases were identified in 39 of 199 patients (19.6%). The most common autoimmune disease was hypothyroidism (25,6% of patients) followed by rheumatic polymyalgia (15.4% of patients). Autoimmune diseases were more common in female MDS patients, those with RCMD WHO subtype, and those with low or very low risk R-IPSS. Survival analysis showed that median OS was 68.2 months (95%C.I. 37.7-NR) and 92.2 (95% C.I. 62.5-NR) in patients with MDS-SIADs and MDS-noSIADs, respectively (p=0.62). No statistically significant differences were noted grouping patients based on R-IPSS and also there was not any difference in progression free survival comparing patients with MDS-SIADs and MDS-noSIADs. Moreover, in a group of these patients we studied the levels of serum inflammatory cytokines and we found that many of them resulted to be altered: IL6, IL8, IL10, IL18, MCP1, and S100A8/9 were significantly overexpressed respect to normal controls, IL12 was significantly downexpressed. In the MDS patients with SIADs we found the same value of cytokines of patients without SIAD, except for a significant downregulation of IL12 (p=0.0145).

In conclusion, our study shows that SIADs are prevalent in patients with MDS and CMML but they are not associated with different prognosis in MDS patients in terms of OS and PFS, and they may share similar inflammatory mechanisms that underlie MDS pathogenesis.

Chronic Myeloproliferative Diseases 1

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MANIFEST-2, A GLOBAL, PHASE 3, RANDOMIZED, DOUBLE-BLIND, ACTIVE-CONTROL STUDY OF CPI 0610 AND RUXOLITINIB *VS.* PLACEBO AND RUXOLITINIB IN JAK INHIBITOR-TREATMENT-NAÏVE MYELOFIBROSIS PATIENTS

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CPI-0610 is a unique, first-in-class, oral, small-molecule inhibitor of BET (BETi) proteins, designed to promote disease-modifying activity through selective gene regulation of key oncogenic, fibrotic, and inflammatory factors with potential to transform the standard of care in MF. JAK inhibitors (JAKi) are currently approved for treatment of MF, including ruxolitinib (rux) and fedratinib. Approximately one third of JAKi-naïve MF pts treated with rux (35%; 106 of 301) or fedratinib (37%; 35 of 96) achieved a spleen volume reduction ≥35% (SVR35) at 6-12 months. CPI-0610, a potential disease-modifying therapeutic agent with a novel mechanism of action may improve the outcome in MF pts. Clinical activity of CPI-0610 in combination with rux in JAKi-naïve MF pts observed in the phase 2 MANIFEST study was higher (SVR35 at wk 24: 67%) than that observed with rux alone in historical phase 3 trials (Mascarenhas, ASH 2020).

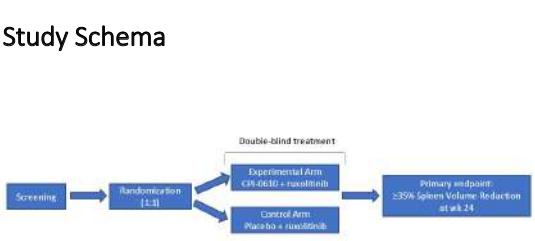

Figure 1.

MANIFEST-2 is designed as a global, phase 3, 1:1 randomized, double-blind, active-control study of CPI 0610 + rux vs. placebo + rux in JAKi treatment-naïve pts with primary MF, post-polycythemia-vera MF, or post essential-thrombocythemia MF. Key eligibility criteria: DIPSS score ≥Int-1; platelet ≥100 x 10^9/L; spleen volume ≥450 cc by CT/MRI; ≥2 symptoms measurable (score ≥3) or a total symptom score (TSS) of ≥10 using the MFSAF v4.0; peripheral blast count <5%, ECOG ≤2. Approximately 310 pts (155 in each arm) will be enrolled in the study. Patient randomization will be stratified by DIPSS risk category (Intermediate-1 vs. Intermediate-2 *vs.* High), platelet count (>200 × 10^9/L vs. 100 – 200 × 10^9/L), and spleen volume (≥1800 cm^3 vs. <1800 cm^3). Double-blind treatment (CPI-0610 or matching placebo) will be administered once daily (QD) for 14 consecutive days followed by a 7 day break, which is considered 1 cycle of treatment (1 cycle = 21 days). Rux will be administered twice daily (BID) for all 21 days within each cycle. Primary endpoint: SVR35 response (≥35% reduction in spleen volume) at wk 24; key secondary endpoint: TSS50 response (≥50% reduction in TSS) at wk 24; other secondary endpoints: safety, PK, PD, changes in

bone marrow fibrosis and myeloid differentiation during treatment, duration of SVR35 response, duration of TSS50 response, PFS, OS, conversion from transfusion dependence to independence, rate of RBC transfusion for the first 24 wks, hemoglobin response, peripheral proinflammatory cytokines.

P32

THE HIGH MOLECULAR RISK (HMR) STATUS DOES NOT ACCURATELY PREDICT POOR OUTCOME IN PATIENTS WITH POST-ESSENTIAL THROMBOCYTHEMIA AND POST-POLYCYTHEMIA VERA MYELOFIBROSIS

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In patients with primary myelofibrosis (PMF) a number of non-driver gene mutations have been associated with impaired outcomes (High Molecular Risk (HMR) category), and are currently incorporated in molecularly annotated prognostic models (MIPSS-70/plus). However, the prognostic value of HMR status in the setting of secondary myelofibrosis (sMF) remains unclear. The aim was to evaluate the prognostic value of HMR status in sMF, 249 consecutive pts with IWG-MRT-defined sMF were included in the study: 133 (53.4%) PPV, 116 (46.6%) PET. Mutational analysis by targeted NGS was performed for all pts. HMR category included *ASXL1*, *EZH2*, *IDH1/2*, *SRSF2*. The nonparametric Wilcoxon rank-sum test, Kaplan-Meier estimate of survival and log-rank test were used as appropriate. For comparison, 661 WHO 2016-defined patients with PMF was included in the analysis.

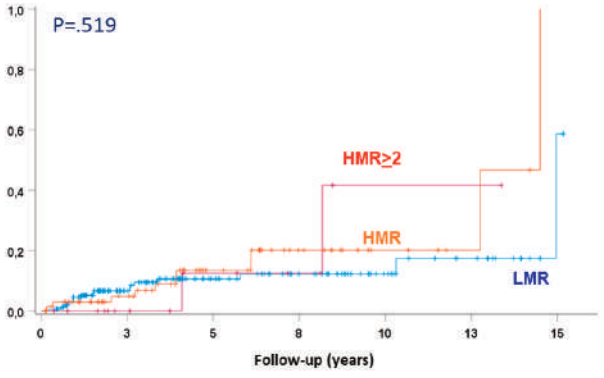

Figure 1.

Results: All PPV pts harbored the *JAK2*V617F mutation compared with 47.6% of PET; 25.0%, 13.8% and 12.1% of PET were *CALR*-type1(T1), type2 (T2) or *MPL* mutated, respectively; while 2.6% were triple negative. In sMF cohort, 86 (34.5%) patients were HMR: 16 (6.4%) had had two or more mutated genes, without significant differences among PPV, PET or PMF. PMF were more frequently *EZH2* (8.5%) and *SRSF2* (10.0%) mutated compared to sMF (respectively 5.6% and 2.4%, p=.04 and P< .0001). With an median follow-up of 4.0 years (yrs) (4.5 yrs PPV and 3.5 yrs PET) 109 deaths were recorded (43.8%), accounting for 42.9% and 44.8% of PPV and PET. 16 PPV (12.0%) and 14 PET (12.1%) pts transformed to acute leukemia. The median survival (OS) in sMF was 8.5 yrs without significant differences between PPV and PET. In PET *CALR* T1 mutation was the most favorable, with median OS of 8.8 yrs, while the OS of pts with *JAK2*V617F and *CALR* T2 mutations was not statistically different, with a OS of 4.7 and 4.1 yrs, respectively. HMR status or ≥ 2 mutations were not predictive of reduced OS (Figure 1), while we found a strong correlation of SRSF2 mutation with shortened survival (HR 3.5, 95%CI 1.40–8.6; P=0.02); median survival was 8.5 yrs in SRSF2 WT compared with 2.1 yrs in *SRSF2* mutated pts. No impact of HMR or >2 mutations on leukemia-free survival was demonstrated. However, we found that the prognostic impact of *SRSF2* mutations on sMF was restricted to PPV pts. We conclude that at variance with PMF, the only HMR-pertinent mutated gene associated with reduced survival in sMF is *SRSF2*. Specific integrated molecular risk scores for sMF are needed.

P33

STIFFER SPLEEN PREDICTS HIGHER BONE MARROW FIBROSIS AND HIGHER JAK2 ALLELE BURDEN IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Introduction and aim: Philadelphia negative (Ph-) myeloproliferative neoplasms (MPNs) are characterized by a variable grade of bone marrow (BM) fibrosis that is higher in primary myelofibrosis (MF), but also present, albeit to a lower extent, in polycythemia vera (PV) and essential thrombocythemia (ET). We aimed at evaluating whether non-invasive spleen stiffness (SS) measurement with a novel spleen-specific and dedicated probe might have a potential role in predicting BM fibrosis and disease severity in Ph- MPNs.

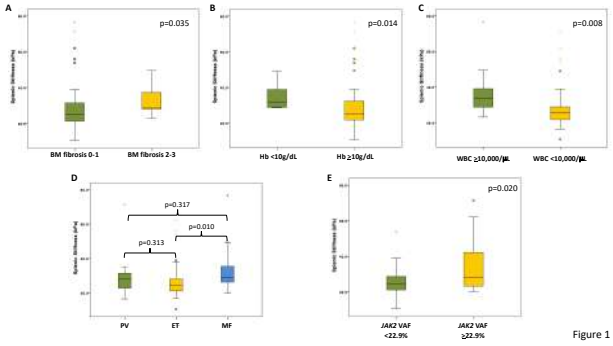

Figure 1

Figure 1.

Methods: In this cross-sectional study, a vibration-controlled transient elastography (VCTE) examination with measurement of SS and liver stiffness (LS) using FibroScan® 630 Expert (Echosens, Paris, France) was applied to consecutive series of Ph- MPNs followed at our center during a 4-week interval. Tumor genomic DNA was analyzed for *JAK2*, *CALR* and *MPL* status by Sanger sequencing. Patients were also analyzed by NGS using the TruSight Myeloid Sequencing Panel (Illumina).

Results: A total 63 patients (9 PV, 32 ET, 22 MF) were included: 52.4% male; median age 72 years (IQR 59-80); 76.2% had JAK2 mutation and 9.5% CALR mutation. The median SS was 26.3 (IQR 22.3-33.6) and the median LS 5.7 kPa (IQR 4.5-7.2). Median SS, but not LS, was significantly higher in patients with grade 2-3 BM fibrosis (28.7 kPa vs. 25.0 kPa, respectively; p=0.035) (Figure 1A), in those with hemoglobin level <10 g/dl (31.5 kPa *vs* 25.4 kPa, respectively; p=0.014) (Figure 1B) and in those with white blood cells (WBC) >10,000/uL (33.5 kPa *vs* 25.5 kPa, respectively; p=0.008) (Figure 1C). The median SS was significantly higher in MF compared to ET, with a median SS of 28.8 kPa (IQR 25.6-

36.3) and 24.3 kPa (IQR 21.1-28.4), respectively (p=0.01) (Figure 1D). SS did not differ between PV *vs* ET (p=0.313) and PV *vs* MF (p=0.317) (Figure 1D). Among the 51 patients who were subjected to NGS analysis, the median variant allele frequency (VAF) of *JAK2* was 22.9%. Notably, patients with a *JAK2* VAF above the median (22.9%) value had a significantly higher SS (p=0.02) (Figure 1E) and, as expected, a higher grade of BM fibrosis (p=0.003). Among the other non-driver genes, *TET2* mutations, occurring in 3 (5.9%) patients, showed a trend toward a higher SS (38.0 kPa vs. 25.2 kPa, respectively; p=0.118).

Conclusions. SS emerges as a non-invasive tool that may reflect the grade of BM fibrosis and the disease burden according to blood count parameters as well as to the *JAK2* VAF value.

P34

AGE-RELATED DNA DAMAGE RESPONSE (DDR) IN HEMATOPOIETIC STEM CELLS FROM CHRONIC MYELOID LEUKAEMIA PATIENTS WHO ATTEMPTED TKI DISCONTINUATION

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Chronic Myeloid Leukemia (CML) is a myeloproliferative disease caused by the 9;22 chromosomal translocation which leads to the formation of the chimeric kinase BCR-ABL. The introduction of imatinib and other tyrosine kinase inhibitors (TKI) allowed excellent control of the disease in the majority of patients. In a number of patients, after a prolonged treatment, Ph+ cells become undetectable or below 10-4, thus allowing a TKI discontinuation attempt. However, about half of CML patients experience a molecular relapse, which in 80% of cases occurs within 6 months from interruption, and need to resume treatment. There is currently no marker to predict the outcome of discontinuation.

Previous studies have shown that age can predict relapse after TKI discontinuation. We hypothesized that this may be linked to the DDR phenotype of leukemic stem cells (LSCs). We aimed to analyze the DDR status of LSCs, measured at diagnosis, in CML patients who attempted TKI discontinuation, and correlated it with treatment-free remission (TFR) duration. CD26 has been proposed as a marker of CML LSCs. We sorted LSCs (CD45+CD34+CD38-CD26+) and normal HSCs (CD45+CD34+CD38-CD26-) from bone marrow samples obtained at diagnosis in 27 CP-CML patients who later discontinued TKI (imatinib, n=15; other TKIs, n=12). The analysis of DDR phenotype was performed by γH2AX immunofluorescence at confocal microscope. Clinical and molecular data were correlated with discontinuation outcome. CD26 staining correctly identified Ph+ cells within CD34+CD38- cells (Figure 1). We found that DDR in the CD34+CD38- population correlates with patients' age (p=0.05) and that CD26+ LSCs present greater DDR compared to normal CD26- stem cells (117±48.9 *vs* 48.8±19.9; p<0.0001). A correlation was also found between DDR at diagnoses and the outcome of TKI discontinuation, which identifies patients in durable TFR as those with less DNA damage at onset (72.2±32.4 *vs* 107.6±18; p=0.034). Another interesting finding of this study was the duration of molecular remission before the discontinuation: in fact, it was clear that the longer the duration of deep molecular remission, the lower the probability of relapse after the TKI discontinuation (p=0.026). We identified DDR and duration of remission as potential biomarkers of relapse in CML patients after TKI discontinuation. Our data also indicate that CML LSCs possess a more pronounced DDR phenotype compared to normal HSCs. These results need to be validated in a larger cohort of patients.

P35

CLINICAL FEATURES AT ONSET AND DURING FOLLOW-UP IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND JAK2-V617F ALLELE BURDEN ≤ 10%

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Essential Thrombocythemia (ET) cases with JAK2-V617F mutation (JAK2+) are generally characterized at diagnosis by a low allele burden (< 50%): however, it is relatively common to diagnose patients with ET JAK2+ and a very low allele burden (≤ 10%). We evaluate the rate and the clinical features of ET JAK2+ patients with very low allele burden (≤ 10%) at diagnosis and we correlate it with major events in the follow-up compared to ET JAK-2+ patients with a relatively higher allele burden. A whole cohort of 222 patients with ET JAK2+ according to WHO 2016 criteria and with an available allele burden measurement behind 2 years from diagnosis in 2 different hematologic Centers was analysed. Allele burden was assessed in granulocyte DNA by quantitative polymerase chain reaction–based allelic discrimination assay. Patients were divided in 2 groups, based on a 10% threshold of JAK2-V617F allele burden. Eighty-four patients (37.8%) were allocated in the very-low allele burden group (≤10AB) and 138 (62.2%) in the higher allele burden group (>10AB). The main clinical features at diagnosis of the whole cohort and according to allele burden are reported in the table: no statistically significant differences were observed between the 2 groups. After a median follow-up observation of 57.2 months (IQR 33.7 – 85.7), 26 thrombotic events (11.7%) occurred in the whole cohort [8/84 (9.5%) in the ≤10AB group *vs* 18/138 (13.0%) in the >10AB group, p=0.429]. Evolution in a myelofibrotic phase was observed in 3 patients (1.4%) of the whole cohort, [1/84 (1.2%) in the ≤10AB group *vs* 2/138 (1.4%) in the >10AB group, p=0.844]: moreover, in the whole cohort 4 patients (1.8%) developed a blastic phase, all in the >10AB group [4/138 (2.9%)] but without a statistically significant difference (p=0.109). At the last follow-up, 5 patients died in the whole cohort, with a 5-year and a 10-year overall survival (OS) of 97.0% (95%CI 94.1 – 99.8) and 93.9% (95%CI 87.3 – 98.6), respectively, without differences between the 2 groups (p=0.398). The presence of a very low allele burden at diagnosis in patients with ET JAK2+ does not seem to correlate with any specific phenotypic feature compared to ET cases with higher allele burden. Furthermore, a very low allele burden is irrelevant in predicting thrombotic episodes, myelofibrotic transformation and OS: some suggestion could be raised as to a lower incidence of blastic evolution in ET patients with very low allele burden, but a larger cohort and further analyses are needed to highlight this issue.

Table 1. Clinical features at diagnosis in the whole cohort and according to allele burden.

	All patients N° 222	Allele burden ≤ 10% N° 84	Allele burden > 10% N° 138	p
Gender, M/F (%)	72/150 (32.4/67.6)	27/57 (32.1 – 67.9)	45/93 (32.6/67.4)	0.943
Median age (years) (IQR)	68.8 (56.8 – 77.1)	67.4 (55.3 – 76.5)	69.6 (58.4 – 78.2)	0.213
Ht, % (IQR)	43.0 (40.9 – 45.5)	42.8 (41.0 – 45.0)	43.0 (40.1 – 46.0)	0.591
Hb, g/dl (IQR)	14.3 (13.6 – 15.1)	14.4 (13.5 – 15.1)	14.3 (13.6 – 15.2)	0.476
WBC, x 10^9/l (IQR)	9.16 (7.40 – 11.19)	8.96 (7.00 – 10.60)	9.52 (7.78 – 11.41)	0.290
PLTS, x 10^9/l (IQR)	702 (573 – 825)	677 (536 – 767)	703 (592 – 875)	0.678
Spleen, n° (%):				
Not palpable	202 (91.0)	79 (93.9)	121 (89.0)	
< 5 cm below costal margin	19 (8.6)	5 (6.1)	14 (10.3)	0.412
≥ 5 cm below costal margin	1 (0.4)	/	1 (0.7)	
Previous thrombotic events, n° (%):				
NO	179 (80.6)	70 (83.3)	109 79.0)	0.427
YES	53 (19.4)	14 (16.7)	29 (21.0)	

P36

IMPACT OF RUXOLITINIB ON THE MANAGEMENT OF POLYCYTHEMIA VERA: A COLLABORATIVE RETROSPECTIVE STUDY BY PH-NEGATIVE MPN LATIAL GROUP

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Ruxolitinib (RUXO) is a JAK1/2 inhibitor that demonstrated its efficacy in patients (pts) affected by Polycythemia Vera (PV) after resistance and/or intolerance to conventional treatments. We report the experience of Latial group with the aim to analyse efficacy and safety of RUXO second line in PV patients. Eighty-three pts started RUXO after a median time of 8.3 years (range 0.7-29.7) from diagnosis of PV due to intolerance (53%) or resistance (47%) to previous therapy. Median age was 70 years (range 31-87). Starting dose of RUXO was 10 mg BID for 78 (94%) pts, 5 mg BID for 5 (6%) pts. The median duration of RUXO exposure was 21.9 months (range 1.2-48.6). Median haematocrit (Ht) level significantly decreased from start of therapy 46%) compared to 3 months (41.2%, p=0.002), 12 (39.9%; p=0.004) and 24-months (39%, p=0.019) during RUXO treatment. Phlebotomy need significantly decreased from baseline (36%) to 3 months of treatment (4%) (p<0.001). WBC count was significantly reduced from baseline to 3-month time point (p=0.003) whereas no significant difference was observed regarding median platelets value or splenomegaly. Constitutional symptoms decreased from 30% at baseline to 3% at 3-month follow-up (p<0.001). No deaths were reported during treatment. Dose reduction occurred in 32 (39%) pts at least once: the majority (66%) occurred within the first year of treatment. Five (5%) pts permanently discontinued RUXO after a median time of 4.7 months (range 0.5-17.3) due to intolerance (80%) or resistance (20%). Nine infectious episodes of grade ≤3 were documented in 8 (10%) pts after a median time of 7.6 months (range 0.8-40.6): 3 bronchitis, 2 cystitis, and 3 viral reactivation (1 HSV1, 2 HZV). Four (5%) pts experienced thrombotic events (2 deep vein thrombosis, 1 splenic vein thrombosis) and an acute myocardial infarction after a median time of 9.1 months (range 4.6-21.1). Mild bleeding events were reported in 6% of the cohort after a median time of 10.5 months (range 5.3-27.5). After a median time of 18.8 months (range 5.2-32.8), 4/83 (5%) pts evolved to secondary myelofibrosis. Five cases of secondary primary malignancy (1 prostate, 1 bladder, 2 non-melanoma skin cancer, 1 laryngeal) occurred after RUXO-start median time of 17.1 months (range 14.9-24.0). None of 83 pts developed a lymphoproliferative disorder. In conclusion, our results confirmed the efficacy and safety profile of RUXO in PV pts outside of clinical trial.

P37

HIGH MOLECULAR RISK MUTATIONS ARE ASSOCIATED WITH CLINICAL RESPONSE AND OUTCOME IN INTERMEDIATE-1 RISK MYELOFIBROSIS PATIENTS TREATED WITH RUXOLITINIB

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Ruxolitinib (RUX) is widely used in patients with myelofibrosis (MF) at intermediate (int)-1 risk. However, information on the impact of high molecular risk (HMR) mutations on response and outcome is scant. After IRB approval, the "RUX-MF" retrospective study collected 742 RUX-treated MF pts in 25 Hematology Centers. Overall, 363 (48.9%) pts received RUX while at int-1 risk according to DIPSS (primary MF, PMF) or to MYSEC-PM (secondary MF, SMF). In 68 int-1 pts, HMR status was evaluated by next generation sequencing (NGS) at RUX start and was correlated with treatment success and outcome. Spleen (SR) and symptoms (SyR) response were evaluated according to IWG-MRT criteria.

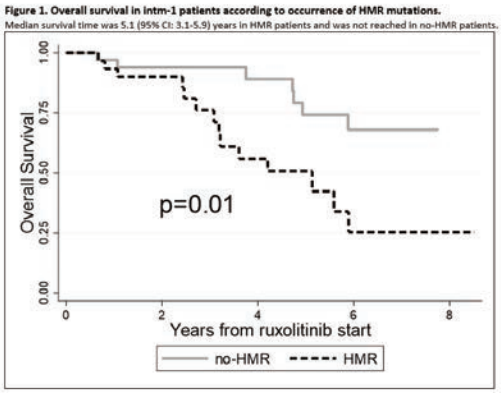

Figure 1.

Outcomes were estimated from RUX start to death/RUX stop/leukemic transformation (LT) or last contact (log-rank p). Characteristics of the 68 int-1 MF pts at RUX start were: median age 66.2y (24-83); males 55.9%; PMF 42.7%; *JAK2*, *CALR* and *MPL* mutated: 75%, 23.5% and 0 (1.5% triple negative); spleen length >10 cm: 39.7%; TSS ≥20: 60.3%; starting/cumulative RUX dose >10mg BID: 73.1%/55.9%. ≥1 HMR was detected in 30 pts (44.1%) (≥2HMR in 7 pts). Specifically, *ASXL-1* was found in 26 pts, *IDH1* in 1, *IDH2* in 2, *SRSF2* in 4 and *EZH2* in 5. *SRSF2* mutations were detected only in PMF (p=0.02); distributions of the other HMR mutations were comparable in PMF and SMF. HMR pts started RUX more frequently with large spleen (p=0.04) compared to no-HMR pts. At 3 and 6 mos, 33.9% and 36.1% of pts achieved a SR, while 67.2% and 72.4% were in SyR, respectively. SR was less frequently achieved by HMR pts at 3 (20% *vs* 45.7%, p=0.03) and 6 mos (22.2% *vs* 47.1%, p=0.04). SyR was not influenced by HMR status. PLT count at 3 and 6 mos was >50 x10^9/l in all cases but two. At 6 months, Hb more frequently decreased <10 g/dL in HMR transfusion independent pts compared to no-HMR (p=0.05). After a median RUX exposure of 2.3 y (0.1-7.7), 35 (51.5%) pts stopped RUX, 4 (5.9%) had a LT and 21 (30.3%) died. In HMR pts, RUX stop (53.3% *vs* 22.4% at

3y, p=0.002) and LT (12.7% *vs* 0 at 3y, p=0.03) were significantly higher. Overall survival was also significantly shorter for HMR pts (log-rank p=0.01) (Figure 1). In int-1 pts, presence of HMR mutations at RUX start is associated with lower responses, increased risk of LT and worse survival. HMR evaluation is crucial for personalized management of these pts.

P38

MYELOPROLIFERATIVE NEOPLASMS AND SPLANCHNIC VEIN THROMBOSIS: CLINICAL AND MOLECULAR FEATURES. A SINGLE-CENTER COHORT STUDY

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Venous thromboses account for approximately 30-40% of vascular complications in MPN, also involving the splanchnic circulation (SVT) with a prevalence of 1-23%. Here, we reported a consecutive single-center series of 54 MPN patients (pts), who developed an SVT at diagnosis or during follow-up between 1979 and 2020.

Table 1. Characteristics of the patients.

	Patients n. 54
Male/female	24/30
Age at MPN diagnosis (years), median (range)	47 (18-78)
MPN subtype, n (%)	
- PV	7 (13)
- ET	9 (16.7)
- pre-PMF	15 (27.7)
- overt PMF	4 (7.4)
- MPN-U	14 (25.9)
- PPV-MF	2 (3.7)
- PET-MF	3 (5.6)
Molecular status, n (%)	
- *JAK2* mutated	45 (83.3)
- *JAK2* allele burden (%), median (range)	27.3 (3.8-97)
- *JAK2* allele burden ≥50%, n (%)	12 (22.2)
- *CALR* mutated	3 (5.6)
- *MPL* mutated	4 (7.4)
- triple-negative	2 (3.7)
NGS, n (%)	
Wild-type	7 (41.2)
HMR	3 (17.6)
DNMT3A	4 (23.5)
TET2	6 (35.3)
TP53	1 (5.9)
Others	2 (11.8)
Cytogenetic abnormalities, n (%)	4 (7.4)
Thrombophilia abnormalities, n (%)	18 (33.3)
Follow-up from MPN diagnosis (years), median (range)	8.3 (0.4-41.1)
Death, n (%)	9 (16.7)
- AML evolution, n (%)	5 (9.3)
- Hemorrhagic complications, n (%)	3 (5.6)
- Infections, n (%)	1 (1.8)
Type of SVT, n (%)	
- PVT	18 (33.3)
- portal and splenic vein thrombosis	11 (20.4)
- portal and mesenteric vein thrombosis	5 (9.3)
- portal, splenic, and mesenteric vein thrombosis	8 (14.8)
- splenic vein thrombosis	4 (7.4)
- mesenteric vein thrombosis	1 (1.8)
- BCS	7 (13)
Age at SVT diagnosis (years), median (range)	46 (20-78)
Follow-up from SVT diagnosis (years), median (range)	7.0 (0.6-42)
Recurrence of SVT, n (%)	10 (18.5)
Esophageal varices, n (%)	25 (46.3)
Bleeding from varices, n (%)	9 (16.7)
Other thrombotic complications after SVT, n (%)	11 (20.4)
- arterial thrombosis	7 (13)
- AMI	4 (7.4)
- ischemic stroke	2 (3.7)
- others	1 (1.8)
- venous thrombosis	4 (7.4)
- CVT	2 (3.7)
- PE	1 (1.8)
- others	1 (1.8)
Bleeding events (excluding variceal ones), n (%)	6 (11.1)

Abbreviations: MPN, myeloproliferative neoplasm; PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; MPN-U, myeloproliferative neoplasm, unclassifiable; PPV-MF, post-PV myelofibrosis; PET-MF, post-ET myelofibrosis; HMR, high molecular risk; AML, acute myeloid leukemia; SVT, splanchnic vein thrombosis; PVT, portal vein thrombosis; BCS, Budd-Chiari syndrome; AMI, acute myocardial infarction; CVT, cerebral vein thrombosis; PE, pulmonary embolism.

We identified 13% of PV, 16.7% of ET, 44.4% of MF, and 25.9% of MPN-U. Most of the cases (83.3%) bear a JAK2V617F mutation, whereas seven (13%) pts were characterized by other molecular markers, *i.e.*, MPL in four and CALR mutations in three cases. The remaining two (3.7%) pts were defined as triple-negative. NGS was performed in 17 (31.5%) cases: the most frequent mutations were found in TET2 (35.3%) and DNMT3A (23.5%) genes, whereas seven (41.2%) pts had no additional mutation. At the time of SVT onset, active antiplatelet therapy was documented in 18.5% of the cases. Among the 16 (29.6%) pts who suffered from SVT during follow-up, cytoreduction was already on-going in 56.3% of the cases, whereas it was then started in all but 16 pts, mainly due to a normal blood cells count. Anticoagulants were started in 43 (79.6%) pts, including ten (18.5%) cases treated with DOACs. After a median follow-up from MPN diagnosis of 8.3 years, nine (16.7%) deaths were recorded: it was due to leukemic transformation in five pts, hemorrhages in three and infections in the remaining patient. 38.9% of the pts suffered from recurrent vascular events, either involving the arterial (13%) or the venous district (25.9%), with 10 (18.5%) pts experiencing a recurrent SVT. In the present study MPN-U seems to represent a distinct clinical entity when compared to other MPN subtypes, as SVT was the initial manifestation in all these cases. Interestingly, during follow-up none of these pts developed clinical features which enabled physicians to re-classify them among one of the classical MPN. Being aware of its limitations, our study confirm that SVT associated with MPN-U represents a more indolent disease as compared with full-diagnosed MPN. Notably, all leukemic evolutions were reported among MF pts after a median follow-up of 15.6 years. Furthermore, our preliminary data support the use of NGS analysis in MPN-related SVT management as it can provide useful diagnostic and prognostic information. However, more than one third of our pts developed recurrent vascular events, confirming the limited efficacy of conventional therapeutic approaches. Updated results will be presented.

P39

PNH CLONES PREVALENCE STUDY IN PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS

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Background: Myeloproliferative neoplasms (MPN) are clonal diseases that confer an increased risk of thrombotic events. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal disease associated with an increased thrombotic risk. The prevalence of PNH clones is little investigated in MPN patients. Early identification of PNH clones may play a role in the etiology of thrombotic event and may provide insights on the pathogenesis and new therapeutic approach.

Objective: The aim of this multicentric study, started in 2017, was to evaluate the prevalence of PNH clones (GPI lacking) in MPN PH negative patients with or without JAK-2, MPL or CARL mutations with hemolytic signs.

Methods: All the participating centers performed the diagnostic test by using a single lyophilized template for granulocytes and monocytes consisting of FLAER-Alexafluor488/CD157-PE/CD64-PC7/CD15-PC5/CD45-PB and a single lyophilized template for erythrocytes consisting of CD235a-FITC/CD59-PE/CD45-APC. Specific calibration beads were provided to standardize the method.

Results: Ninenty-three patients were included in the study, fourty-seven males and fourthy-six females. Median age was 69 years. Anemia, LDH elevation, asthenia and history of thrombosis were considered as major clinical signs and symptoms that may suggest the presence of PNH clone. The prevalence of PNH positive clones was 3.23% (three patients). All three patients had splenomegaly at the time of study enrollment; none of them had thrombosis at the time of PNH suspicion. One

patient affected by CALR positive essential thrombocytopenia, had a small clone (0.52%), clinically irrelevant; one patient affected by JAK-2 V617F- positive myelofibrosis showed a PNH clone of 89.8%, severe anemia and hemoglobinuria and started eculizumab therapy; the third patient affected by CALR positive myelofibrosis showed a PNH clone of 92.6% but without severe anemia and breakthrough hemolysis and eculizumab therapy hasn't started yet.

Conclusion: These data show a prevalence of PNH clones in PH negative MPN patients around 3%. We found association with CALR mutation and JAK-2 V617F mutation and PNH positive clones suggesting that the worsening of malignant process may be bridged by the acquisition of multiple genetic mutations. Furthermore the concurrence of PIG-A mutations with genetic mutations such as JAK2 and CALR may lead to more accurate diagnosis, pathogenic understanding of disease process and development of targeted therapies.

P40

JAK2 NEGATIVE ERYTHROCYTOSIS ASSOCIATED WITH JAK2 GGCC_46/1 HAPLOTYPE, CALR RS1049481_G, AND NORMAL ERYTHROPOIETIN LEVEL: IS THIS A NEW ENTITY?

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The JAK2 haplotype known as "GGCC or 46/1 haplotype" consists of a germline combination of single nucleotide polymorphisms (SNPs) that are inherited together and are frequently associated with the onset of myeloproliferative neoplasms positive for JAK2 mutations. It has been reported a significant association between JAK2 negative erythrocytosis and the simultaneous occurrence of JAK2 haplotype and CALR rs1049481_G allele. In the present study, we investigated the presence of JAK2 haplotype and CALR rs1049481_G allele in a more extensive series of erythrocytosis patients and evaluated a possible correlation with serum erythropoietin (EPO) level. Seventy erythrocytosis patients negative for canonical JAK2 mutations and secondary causes were analyzed. The occurrence of the JAK2 haplotype and of SNP rs1049481 in the CALR gene was investigated by PCR, followed by Sanger sequencing. In silico data from 2504 healthy individuals of the 1000G Project (1000G) were used as a control group. Forty-seven (67.1%) and 23 (32.9%) cases resulted in being positive and negative for the JAK2 haplotype, respectively. The JAK2 haplotype occurred to be associated with erythrocytosis as a statistically significant difference in frequency was detected as respect to 2504 healthy individuals from the 1000G Project ($p<0.0001$). The association was also demonstrated in terms of allelic frequency ($p=0.0010$) and genotype distribution ($p=0.0003$). Regarding CALR rs1049481 SNP, a significant difference in the rs1049481_G allelic rate was confirmed in our cohort compared to 1000G controls ($p=0.0352$). Based on the EPO level, erythrocytosis patients were divided into two groups: normal (58 cases) or subnormal (12 cases). Interestingly, the simultaneous presence of JAK2 haplotype and CALR rs1049481_G was statistically significantly associated with the erythrocytosis group showing normal EPO ($p<0.0001$). This study suggests that the JAK2 haplotype and the presence of the CALR rs1049481_G allele were significantly associated with erythrocytosis cases, negative for JAK2 mutations. Moreover, patients showing two major WHO 2016 diagnostic criteria (erythrocytosis and panmyelosis) without JAK2 mutations and with normal EPO levels can benefit from the search for germline polymorphisms combination in JAK2 and CALR driver genes for a better diagnostic classification. Therefore, the presence of these polymorphisms could represent a novel minor criterion for the diagnosis of "JAK2 negative PV".

Lymphomas 2

P41

THE ROLE OF END OF TREATMENT-PET CT EVALUATED BY DEAUVILLE FIVE-POINT SCALE AS PROGNOSTIC TOOL IN HODGKIN LYMPHOMA

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Introduction: Positron Emission Tomography Computed Tomography (PET CT) is crucial in staging and response assessment in Hodgkin lymphoma (HL). Interim-PET CT (I-PET CT) allows a first patients stratification and customize treatment continuation. Deauville Score (DS) has been developed for I-PET CT interpretation to reduce inter-operator variability [1,2]. End of treatment (EoT) PET CT showed superiority to standard CT in evaluating residual disease. In clinical practice it is used to assess absence or presence of metabolic residual disease, lacking scientific evidence of a correlation between specific DS and prognosis [3]. Our study aimed to analyze EoT PET CT response to demonstrate a possible prognostic correlation between DS and patient prognosis in terms of Relapse Free Survival (RFS) and Overall Survival (OS).

Methods: We conducted a monocentric retrospective study in patients with Classic HL, consecutively treated with ABVD between 2007 and 2018 with at least 1 year of follow-up and with favourable I-PET CT (DS1-3). EoT and I-PET CT images were submitted to blind central revision and DS assessment. Different values of DS at EoT PET CT were compared in terms of RFS. Survival analysis was performed by Kaplan-Meier curves and Log-Rank test. Statistical significance was considered for values of $p<0.05$.

Results: PET CT images of 78 patients were centrally reviewed. All patients are currently alive (OS=100%). After a median follow-up of 60 months (range 17-139) 17 patients (21%) had disease recurrence, with RFS of 60% at 104 months (median not reached). The median time to relapse was 8 months (range 3-39). Patients with EoT DS1 (56 cases) showed a 83% RFS at 100 months (median not reached). Median RFS worsened for higher DS: 77 months for DS2 (12 cases), 2 months for DS3 (2 cases), 26 months for DS 4 (3 cases), and 14 months for DS5 ($p<0.001$) [Fig. 1]. There was a longer time frame to relapse in DS1/2 compared to DS≥3 (median time of 34 and 4 months respectively). Comparing I-PET DS with EoT PET DS, higher RFS was observed in stable or reduced metabolic activity, unlike worsened DS indicates increased risk of relapse ($p<0.001$).

Conclusion: Our study suggests that a systematic evaluation of EoT PET according to DS allows more accurate identification of patients with an unsatisfactory metabolic response and a better prognostic stratification. The joint evaluation of the I-PET and EoT PET show a higher risk of recurrence in case of increased DS.

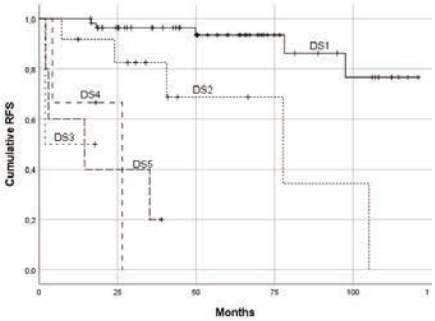

Figure 1.

P42

MIR-22 A SERUM PREDICTOR OF OUTCOME AND TREATMENT RESPONSE IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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The available prognostic tools for patients with DLBCL are not able to identify all the patients refractory to R-CHOP. Liquid biopsies facilitate serial sampling and dynamic patients monitoring. MiRNAs are present in body fluids in a highly stable form, making them interesting candidates as biomarkers. We have previously performed a pilot study on serum miRNAs profile in DLBCL patients, and found that serum miR-22-3p was significantly correlated with PFS. In order to validate the value of circulating miR-22 as reliable non-invasive biomarker and investigate its biological function in DLBCL, we aimed: a) to analyze serum miR-22 in an independent validation cohort of DLBCL patients; b) to compare miR-22 expression in serum and matched tumor samples and c) to assess its functional role in DLBCL pathogenesis and response to R-CHOP. This is a multicentre prospective study on DLBCL patients treated with R-CHOP.

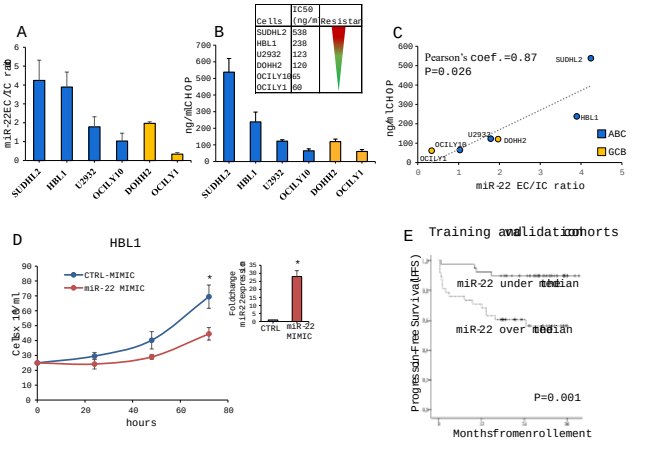

Figure 1.

miR-22 expression profile was evaluated by qRT-PCR in serum samples, in matched tissue samples and in six DLBCL cell lines and related conditioned culture medium. Sensitivity to R-CHOP for each cell line was evaluated determining RCHOP IC50 by cytotoxicity MTT assays after 72h hours of treatment. HBL1 DLBCL cell line was transfected with miR-22 mimic by electroporation, 24h hours post transfection cells were plated and vital cells were counted at 24h, 48h and 72h hours. Our data on 78 DLBCL patients (training + validation cohorts) show that patients with baseline higher serum miR-22 levels had a significant worse clinical outcome in terms of 2-year PFS (p=0.001). Moreover, serum miR-22 was differentially expressed in refractory patients compared to responders (p=0.047). The comparison results of miR-22 expression in serum and paired tumour samples indicate a significant and inverse correlation (Spearman's Rho: -0.469). Assessing miR-22 expression in extracellular (conditioned medium) and intracellular fraction of DLBCL cell lines we observe that the value of extracellular to intracellular ratio of miR-22 levels is directly correlated with the cell resistance to R-CHOP treatment (Spearman's Rho: 0.928). Moreover, a decreased proliferation rate was found upon miR-22 overexpression in HBL1 cells (a cell line with low miR-22 basal expression). Altogether the results of our study suggest that miR-22 may represent a prognostic and predictive biomarker in DLBCL, and may be involved in lymphoma pathogenesis and in mechanisms of response to treatment.

P43

BRENTUXIMAB VEDOTIN (BV) FOLLOWED BY BENDAMUSTINE SUPERCHARGE (BS) FOR REFRACTORY OR RELAPSED (R/R) CLASSICAL-HODGKIN LYMPHOMA (C-HL): 3-YEAR UPDATE OF THE BV+BS-21 STUDY

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Real-world experience in R/R c-HL has shown a gradual transition from traditional cytotoxic agent-based treatment to selectively active agent-based treatment. Clinical trials conducted to define the best partner(s) that can synergize with Bv in R/R HLs present convincing evidence that increasing dosage of bendamustine had good anticancer activity with no dose-limiting toxicity, especially when Bv infusion was followed by increasing doses of bendamustine (enhanced synergistic effect). We report here a prospective series of 34 patients (median age, 44 years; range, 23-59) receiving Bv+Bs-21 (Figure 1) for R/R c-HL during the 2013-2021 period at the Hematology Unit of the Federico II University of Naples, whose clinical presentations were aggressive (>3 lines of previous treatments in 75% of patients, primary refractory disease in 70% of patients, autologous HSCT failure in 35% of patients). Ten patients (29%) experienced grade ≥3 treatment-related adverse events consisting of cytomegalovirus reactivation (median CMV-DNA, 1810 IU/mL; range, 620-170 000 IU/mL) with fever (successfully treated with preemptive therapy with valganciclovir) in 7 cases and neutropenia in 3 cases, all resolved without requiring hospitalization. At post-Bv+Bs-21 re-evaluation, 100% of patients had deep metabolic responses (Deauville scores ≤3) at FDG-PET/CT scans. Thereafter, 5 patients received two additional courses of Bv+Bs-21, 7 patients received allogeneic HSCT, and the remaining 22 patients received autologous HSCT. In this last sub-group, for 12 patients PBSC were previously harvested after two courses of Ifosfamide, Gemcitabine, Vinorelbine and Prednisolone; in the remaining 10 cases PBSC were successfully collected after Bv+Bs-21, with mobilization with G-CSF, vinorelbine-cyclophosphamide and/or plerixafor regimen. The median peak value of CD34+ cells was on day 12 after mobilization treatment (median number CD34+ cells: 3.1 x 106 per kilogram of body weight; range 1.6-4.2 x 106). After HSCT, median day of engraftment of neutrophils and platelets was recorded on day 11 (9-21 days) and day 12 (9-25 days), respectively. At a median follow-up of 42 months (1-94 months) from Bv+Bs-21 regimen termination, the estimated 3-year PFS of the entire population was 94% (95% confidence interval, 84.4%-100%).

In conclusion, our clinical data indicate that bendamustine (an old and low-cost cytotoxic agent) used in a new schedule modality (*i.e.*, administered at increased dose and afterward the first-in-class antibody drug conjugate targeting CD30), has highly synergistic activity in outpatient salvage regimen against R/R HRS cells of patients aged <60 years.

P: peg-filgrastim 6 mg s.c g+6
FDG-PET, at baseline and 5 weeks after treatment
C, serum CMV-DNA on day +1

Bv+Bs-21 schedule: 3-days outpatients i.v. infusions of 1.8 mg/kg of Bv on day 1 of each 3-week cycle combined in sequence to Bs on days 2 and 3 of the treatment cycle at a fixed dose of 120 mg/m² per day, for a total of 4 courses [median-dose intensity during sequential therapy was 100% (range, 88.6% to 102.4%) for Bv and 100% (range, 88.7% to 102.4%) for Bs]

Prophylactic treatment employed in all patients:
-Methylprednisolone 200 mg i.v day +1→g +3
-Difenidramine 50 mg i.v day +1→+3
-Febuxostat 80 mg os days+1→+5
-Trimethropin-sulfamethoxazole 960 (160-800) mg os every 12 h twice a week
-Acyclovir at 800 mg os each day from day 1→ 1 month after treatment
-CMV-DNA monitoring on day +1 of each cycle

Figure 1. Bv+BS -21 schedule and prophylactic treatment.

P44

ARGO, AUTOMATIC RECORD GENERATOR IN ONCOLOGY: MULTICENTRIC VALIDATION OF A NEW TOOL FOR AUTOMATIC CONVERSION OF "REAL-LIFE" HEMOLYMPHOPATHOLOGY REPORTS IN STANDARDIZED ECRF

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Background: The unstructured nature of medical data from Real-World (RW) patients and the scarce accessibility for researchers to integrated systems restrain the use of RW information for clinical and translational research purposes. Natural Language Processing (NLP) might help in transposing unstructured reports in standardized electronic case report forms (eCRFs). We aimed at designing a tool to capture pathological features directly from hemo-lymphopathology reports and automatically record them into eCRFs.

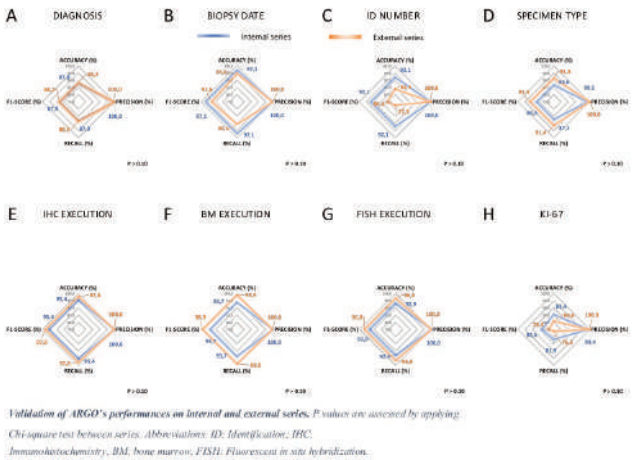

Figure 1.

Method: By Optical Character Recognition and NLP techniques, we built up a tool, named ARGO (Automatic Record Generator for Oncology), and measured its efficiency in recognizing unstructured information from diagnostic paper-based reports of diffuse large B-cell lymphomas (DLBCL), follicular lymphomas (FL), and mantle cell lymphomas (MCL). ARGO was programmed to match data with standard diagnostic criteria, automatically assign diagnosis according to the International Classification of Diseases 10th Revision (ICD10) and populate eCRFs on the REDCap platform. A selection of 239 reports (n. 106 DLBCL, n. 79 FL, and n. 54 MCL) from the Pathology Unit at the IRCCS – Istituto Tumori "Giovanni Paolo II" of Bari and of 93 external reports (n. 49 DLBCL, n. 24 FL, and n. 20 MCL) from other six Italian centers was used to assess ARGO performance in terms of accuracy (A), precision (P), recall (R) and F1-score (F1).

Results: We successfully converted 326 (98.2%) paper-based reports into structured eCRFs incorporating information about diagnosis and tissue of origin of samples (lymph-node, extra-nodal, medullary, and peripheral blood), immunohistochemistry expression of major molecular markers (MYC, BCL2, BCL6, CD10, CD20, Cyclin D1, and the quantitative assessment of Ki-67/MIB1 proliferation index) and DLBCL cell-of-origin subtype [Hans et al., Blood, 2007]. Overall, ARGO showed high performance (nearly 90% of A, P, R and F1 from 7/8 data fields analyzed from internal and external series of reports) in capturing identification report number, biopsy date, specimen type, diagnosis, and additional molecular features (Figure 1A-H). Conclusions. We developed and validated an easy-to-use tool that converts RW paper-based diagnostic reports of major lymphoma subtypes into structured eCRFs. ARGO is cheap, feasible, and easily transferable into the daily practice to generate REDCap-based eCRFs for clinical and translational research purposes.

P45

PATTERN OF CARE IN INDOLENT NON-FOLLICULAR LYMPHOMA: A REPORT FROM NF10 PROJECT, AN INTERNATIONAL, PROSPECTIVE, OBSERVATIONAL STUDY OF FONDAZIONE ITALIANA LINFOMI

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Background: Indolent non follicular B-Cell Lymphomas (INFL) include small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphomas (LPL) and marginal zone lymphomas of splenic (SMZL), nodal (NMZL) and extranodal (ENMZL) subtypes. In 2010 the NF10 study was started by the Fondazione Italiana Linfomi as a prospective registry specifically devised for investigating the prognosis of this group of lymphomas.

Methods: The prospective enrollment of consecutive adult patients with newly diagnosed INFL with no exclusion criteria was activated in 47 centers in Europe and South America.

Results: From July 2010 to January 2020, 1535 cases were registered

and 1328 validated. One hundred thirty-six (10%) cases were SLL, 316 (24%) LPL, 95 (7%) CD5-low grade and 781 (59%) MZL, including 259 (19%) SMZL, 84 (6%) NMZL, 334 (25%) ENMZL or 104 (8%) disseminated subtypes. Median age was 67 years (range 22-93), 54% of patients were males; Ann Arbor stage was III-IV in 82%; 11% had B symptoms, 6% had ECOG performance status > 1, lactate dehydrogenase and β2-microglobulin were elevated in 24% and 54% of cases, respectively. Six percent of cases were HCV positive (HCV+ rate was 7.8% among MZL cases). Regarding HBV infection, 14% of patients were HBcAb-positive and 2% of patients were HBsAg-positive. Immediate systemic therapy was planned in 50% of patients. SMZL, SLL and CD5-low grade were the subtypes with the lower rates of immediate therapy (48%, 47% and 14% respectively) whereas ENMZL were addressed to systemic therapy in 63% of cases. When systemic therapy was prescribed rituximab (R) was used in 85%. In 76% of patients R was combined to cytotoxic therapy. Regarding immunochemotherapy regimens, R was combined with bendamustine in 49%, alkylating agents in 34%, CHOP-like in 13%, and fludarabine in 3%. With 43 months of median follow up, 5-year progression free survival and overall survival (OS) were 61% (95CI: 58-65) and 87% (95CI: 84-89) respectively; the initial choice of deferring immediate therapy did not impact on OS.

Conclusions: We provide a complete report on the approach to patients with INFL showing that immediate therapy is required in half of the cases with a heterogeneous approach among INFL subtypes. The NF10 study confirms that a web-based world-wide cooperation allows the collection of a relevant and complete data set, providing a platform for future prognostic and pathobiological studies.

P46

IMPACT OF PRE TRANSPLANT SALVAGE THERAPIES ON OUTCOME OF HODGKIN LYMPHOMA PATIENTS PERFORMING ALLOGENEIC TRANSPLANT

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Allogeneic transplant is an effective salvage therapy in Hodgkin lymphoma (HL) patients relapsed or refractory to previous treatments. In recent years immunotherapies (conjugated antibody and check point inhibitors) showed interesting results and were used as bridge therapies to allotransplant. The aim of this retrospective study in Lazio Region was to evaluate the impact of this new therapies on allogeneic hematopoietic stem cell transplantation (HSCT) outcome in comparison with the standard chemotherapies used in the past. We selected all consecutive patients with diagnosis of HL transplanted in four Hematology Transplant Unit and we collected data obtained from patients' records concerning all the treatments before HSCT. Forty-six patients were observed, 40 patients performed salvage chemotherapy and autologous stem cell transplant as consolidation therapy 6 were treated with standard chemotherapy without transplant because of progressive disease. Sixteen patients relapsed after transplant and 30 showed a progressive disease. Therapies used as a bridge to HSCT were: Brentuximab Vedotin (BV) in 22 patients, Nivolumab alone in 2, BV and Nivolumab in 11 and chemotherapy in 11 patients. Response to these salvage therapies before HSCT were: 19 complete remission, 6 partial remission, 12 stable disease and 9 progressive disease. The transplant source was bone marrow in 19, peripheral stem cells in 25 and cord blood in 2 patients. Fifteen patients experienced relapse after HSCT and 20 patients died: 7 for progressive disease, 8 due to infections and 5 due to acute or chronic GVHD all in complete remission. After a median observation period from HSCT of 32 months (range 0.3-144 months) the overall survival is 49% the event free survival is 41% and the progression free survival is 54%. No differences in overall survival were observed according to bridging therapy. Analysing salvage treatments we observed no relapses in patients treated with Nivolumab, 41% of relapse in patients treated with BV and 55% in patients treated with chemotherapy. The causes of deaths were progressive disease or infections (79%) in patients treated with BV or chemotherapy and GVH or infections (100%) in patients treated with Nivolumab. In conclusion it seems that whatever the treatment used before HSCT results depend on the response obtained, check point inhibitors cancel relapse incidence but could increase the risk of GVH.

P47

CIRCULATING TUMOR DNA (CT-DNA) FOR MINIMAL RESIDUAL DISEASE MONITORING THROUGH IMMUNOGLOBULIN GENE REARRANGEMENTS IN PATIENTS WITH LYMPHOID NEOPLASMS

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The ease of acquiring cell-free DNA (cfDNA) from peripheral blood (PB) makes it an interesting tool for minimal residual disease (MRD) assessment in patients with hematological malignancies. Several pre-analytical factors must be solved, since the quantity and quality of extracted cfDNA can significantly affect the sensitivity of MRD analysis. The PreAmp system successfully increases the amount of the tumor fraction of PB-derived cfDNA (ctDNA) at diagnosis, with a complete correspondence between genomic DNA (gDNA) and ctDNA post-amplification sequence analysis (Della Starza *et al.*, SIES 2020). Here, we studied the feasibility of MRD evaluation on ctDNA in acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) samples by immunoglobulin gene rearrangements (IGH) quantification. We screened by PCR the diagnostic gDNA of 7 CLLs (PB), 4 ALLs (bone marrow) and 7 DLBCLs (lymph node) to identify the disease-specific IGH rearrangement. The SsoAdvancedTM PreAmp Supermix (BioRad) was applied to all 18 diagnostic ctDNA and 27 follow-up (FU) ctDNA samples (6 CLLs, 7 ALLs, 14 DLBCLs). All post-amplification products were sequenced for comparison with gDNA and analyzed by ddPCR for quantification. The sequence of all amplified products resulted superimposable to that of gDNA. The amplification system allowed to detect ctDNA by ddPCR (otherwise not detectable) that reached the gDNA levels of quantification. Of 6 CLL FU samples, MRD on gDNA was positive in 1 case at 4E-03. MRD on ctDNA of the same sample was quantified at 1E-02 and negative in the other 5. In ALL FU samples, both gDNA and ctDNA were negative. At diagnosis, ctDNA was positive in 5/7 DLBCL patients and gDNA in 4/7. In 14 PB FU samples, either at interim and end of induction timepoints, gDNA was always negative and ctDNA positive in 4/7. Interestingly, a persistence of positive ctDNA with a gDNA negativity has been observed in 2 of 3 DLBCL relapsed patients. The PreAmp system increases the ctDNA amount, making the ddPCR MRD monitoring possible without an analytical bias. The limitations of the IGH monitoring in DLBCL resides in the low sensitivity of patient-specific primers. Thus, we are moving towards NGS approaches to identify the target and to monitor MRD, in order to validate the predictive value of MRD monitoring in DLBCL in a non-invasive manner on a larger cohort of patients.

P48

THE PROGNOSTIC SIGNIFICANCE OF MYC AND BCL2 PROTEIN DOUBLE EXPRESSION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA, NOS, IN THE ABSENCE OF MYC REARRANGEMENT

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The revised 2016 WHO Classification distinguishes diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS) from high grade B-cell Lymphomas (HG with *MYC* and *BLC2* or *BCL6* gene rearrangement, or NOS) and other entities. DLBCLs NOS with MYC and BCL2 protein double-expression by immunohistochemistry, also known as double expressor lymphomas (DEL), have been associated with a worse outcome in all categories. However, unlike *MYC* rearranged cases, DEL have been managed with standard R-CHOP when not associated with HG features or gene rearrangements. In this retrospective observational study we enrolled all consecutive patients with a diagnosis of DLBCL, NOS, between 2013 and 2019 treated and followed-up at University of Verona. Exclusion criteria were age >80, different treatment than R-CHOP, transformation from indolent lymphoma, HG histology, and presence of *MYC* rearrangement. Expression of MYC and BCL2 proteins was defined with standard cut-offs (MYC ≥ 40% and BCL2 ≥ 50%). One hundred and ninety seven patients were included in the study. Median age was 63 (22-80), 117 were males, and median follow up was 24 months (4-94). Progression free survival (PFS) at 2 years was 68% ± 4% and overall survival was 77% ± 3%. Forty-nine patients (25%) were DEL, and 86 (44%) had international prognostic index (IPI) 3 to 5. Multivariate analysis showed that the IPI of 3-5 (HR 2.56; 4.34-1.53), and DEL (HR 2.77; 4.76-1.66) were the only independent significant variables associated with adverse PFS. Considering these two factors, a three-risk group model was built, recognizing a low-risk group (43% of all patients analyzed, no risk factors), an intermediate-risk group (45%, one risk factor), and a high-risk group (12%, both risk factors) with highly significantly different 2-year rates of PFS of 85%, 62%, and 33%, respectively as illustrated in Figure 1. Our analysis showed that DEL, together with IPI, can easily recognize patients with DLBCL, NOS, in the absence of *MYC* rearrangements, who do not benefit of R-CHOP. This score should be tested in prospective settings, in order to early identify patients that may be candidate to alternative therapies.

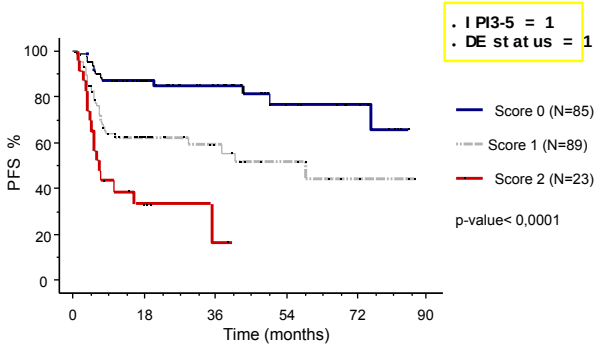

Figure 1.

P49

A NON-INTERVENTIONAL STUDY OF OBINUTUZUMAB IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED FOLLICULAR LYMPHOMA (URBAN): IMPACT OF COVID-19 PANDEMIC ON ENROLLMENT AND SAFETY

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Background: The introduction of new agents has improved the prognosis of follicular lymphoma (FL). Unfortunately, COVID-19 pandemic may have affected disease management.

Objective. The URBAN study is the only study that aims to assess effectiveness and safety of Obinutuzumab-based treatment in patients with untreated advanced FL with FLIPI≥2 in a real-world setting. The objective of this interim analysis is to evaluate patient characteristics, potential changes in the management due to COVID-19 pandemic, preliminary efficacy at the end of induction (EOI) and safety data 1 year after the first patient enrolled.

Methods: Participants should have received at least 2 cycles of Obinutuzumab-chemotherapy induction before the accrual (retrospective part of the study). The study, as per clinical practice, includes 6-8 induction courses, 2-year maintenance, and 1-year follow-up period after the last dose of Obinutuzumab. Two time periods have been analyzed: pre-COVID-19 phase (until 24-Feb-2020) and COVID-19 phase (25-Feb-2020 to 09-Oct-2020; clinical cut-off date).

Results. 266 patients (median age 61 years, 56% females) were enrolled: 186 patients began the treatment pre-COVID-19 (pre-C group) and 80 patients during COVID-19 (C group). Among the observed trends, not statistically significant, more patients in the C group *vs* pre-C group initiated treatment with B-symptoms (26.2% *vs* 19.4%) and more advanced (III-IV) disease stage (95% *vs* 90.9%). Fewer patients in the C group than in the pre-C one (41.2% *vs* 48.9%) received bendamustine, resulting in reversed rates for CHOP (55% *vs* 44.1%). As to safety, in the retrospective part of the study neutropenia occurred in 22.9% of patients, infusion-related reactions in 12.8%, thrombocytopenia in 9.4%, and infections in 7.5%. In the prospective part they were 17.7%, 0.8%, 2.3%, and 8.6%, respectively. One patient had a grade 3 COVID-19 infection. No data about potential COVID-19 impact on maintenance strategies, yet. Preliminary efficacy data, available for 164 patients at EOI, show that 134 patients (81.7%) achieved a PET-CT CR, and 25 (15.2%) a PR.

Conclusions: The interim analysis of the URBAN study shows that Obinutuzumab-based treatment is associated with a good safety and efficacy profile. Furthermore, the observed trends might suggest that COVID-19 might have influenced the clinical approach to the treatment of FL patients: the adoption of a watch-and-wait strategy could be wider and the use of bendamustine reduced.

P50

COMPARISON OF FIRST-LINE TREATMENT WITH BENDAMUSTINE PLUS RITUXIMAB VERSUS R-CHOP FOR PATIENTS WITH FOLLICULAR LYMPHOMA GRADE 3A: RESULTS OF AN ITALIAN MULTICENTER, RETROSPECTIVE STUDY

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Background: In the setting of follicular lymphoma (FL), frontline therapeutic regimen with R-CHOP represented for many years standard of care for patients with symptomatic advanced FL, but more recently also bendamustine plus rituximab has become an option of treatment. In clinical practice, the choice of the therapeutic regimen for grade 3A FL is still an open question.

Aims: We designed a retrospective, multicenter, observational study, to compare outcomes and toxicities of patients diagnosed grade 3A FL treated either with R-CHOP or RB first-line therapy. *Methods*. We retrospectively assessed 145 patients affected by FL grade 3A treated with a first line therapy in 15 Italian FIL (Fondazione Italiana Linfomi) centers between January 1, 2014, through 30 May, 2018.

Results: Seventy patients were treated with RB and 75 with R-CHOP. In the RB group the median age at time of diagnosis was 67 (range 36-85 years) compared with 59 years (range 29-77 years) in the R-CHOP group, with a statistically significant difference between the two groups ($p<0.001$). Patients in RB group achieved a similar percentage of overall response rate (96% *vs* 100%) and a better complete remission (87% *vs* 80%, $p=0.035$) compared with patients in R-CHOP group. Rates of toxic effects did not differ between the treatment groups. Late toxicity in terms of secondary malignancies occurred in 7 patients (10%) in RB group and 5 in R-CHOP group (7%). A total of 64 patients (85%) received maintenance treatment with rituximab after R-CHOP and 59 patients (84%) after RB. The progression free survival (PFS) difference between individual treated with R-CHOP and RB did not show differences, with a 4-year PFS 76.6% in RB group and 77.7% in R-CHOP group ($p=0.745$). Overall survival OS was significantly longer with R-CHOP than with RB (HR=0.16; 95% IC, 0.04-0.74; $p=0.007$), but mainly influenced by different median age in the two groups; in a final analysis adjusted by age and gender, no statistical difference was observed for OS. *Conclusion*. With the limitations of the study design, our results suggest that RB and R-CHOP as first-line treatment in FL3A seem to be valid treatment options, with similar outcomes and toxicities. PFS and OS showed no statistical difference in a final analysis adjusted by age and gender. We consider possible selection biases in the choice of chemotherapy therapy related to clinical variables of patient and disease that are challenging to handle in a retrospective analysis.

Myeloma and Monoclonal Gammopathies 2

P51

NINE HUNDRED DARATUMUMAB INFUSIONS IN NINETY MINUTES FOR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A SINGLE-CENTRE OBSERVATIONAL STUDY

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Background: Daratumumab was the first fully human anti-CD38 monoclonal antibody (mAb) tested in clinical trials, demonstrating efficacy as a single agent and in combination with proteasome inhibitors (PIs, DaraVD) or immunomodulatory drugs (IMiDs, DaraRD) in patients with relapsed/refractory multiple myeloma (RRMM). Daratumumab displays an excellent safety profile, with moderate-grade infusion-related reactions (IRRs) occurring mostly during the first infusion. Although evaluated in a few patients, some retrospective single-centre experiences of rapid infusion of 90 minutes (min) of daratumumab starting from the third infusion are reported in the literature, confirming that the procedure is safe even for patients undergoing fractional doses.

Aim: Moving from the observation of a low rate of adverse reactions even in patients with advanced disease, we adopted 90 min of rapid infusion protocol since February 2019, in order to confirm safety and describe the potential advantages.

Methods: Single-center study of 900 daratumumab fast infusions, between February 2019 and December 2020, administered in 72 patients. The only inclusion criterion was the previous delivery of four doses of daratumumab, according to standard practice. Previous IRR was not an exclusion criterion. All patients were treated at our institution (Haematology Unit, Careggi Hospital of Florence, Italy).

Results: No adverse events were observed during rapid infusion, neither 30 min after completion. We confirmed safety of rapid infusion on 38 (53%) patients defined "at risk" by the presence of cardiovascular diseases (arterial hypertension, arrhythmia or valvulopathy) or pulmonary comorbidities (COPD, asthma and allergic rhinitis). Ninety-minutes daratumumab administration was also well tolerated in 8 patients with cardiac or renal amyloidotic involvement. Reducing the duration of the daratumumab infusion not only improved the patient's quality of life by reducing hospitalization times, but also had an impact on cost savings for the healthcare system: our 2 years of experience of 90 min infusion of Daratumumab resulted in a potential cost saving of € 13.707, € 8.177 in 2020 alone.

Conclusion: Daratumumab infusion time of 90 minutes is well tolerated, thus allowing a considerable saving of time for RRMM patients and potentially ameliorating their adherence to treatment.

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IDENTIFYING TRANSPLANT-INELIGIBLE PATIENTS (NTE) WITH MULTIPLE MYELOMA (MM) WHO MAY NOT BENEFIT FROM NEW STANDARD THERAPIES: A RETROSPECTIVE ANALYSIS OF A SINGLE CENTRE

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Background: Daratumumab incorporated into frontline regimens has significantly prolonged PFS in NTE patients with multiple myeloma but it has to be administered indefinitely until progression and it is a high-cost treatment so it would be extremely relevant to identify specific pop-

ulations that may not benefit from daratumumab within the marked heterogeneity of elderly patients. Understanding factors affecting early mortality (EM: death within 6 months) could help with this aim.

Methods: We analyzed NTE MM patients recorded in our database from 2010 to 2020, calculating retrospectively simplified frailty scores, based on age, ECOG PS and CCI (Facon *et al*., 2000) to evaluate its applicability in a real-world population. Secondly, logistic analysis and Cox regression analysis were performed to search factors affecting EM with the aim to improve discriminating power of Facon frailty score.

Results: Among 189 patients, 44 (23%) were older than 80 years. CCI>1 was detected in 40% of patients, PS ≥2 in 33%, R-ISS stage 2-3 in 81% and renal failure in 23% of patients. All patients received IMiDs- and PIs-based regimes and EM occurred in 23 (12.2%). According to Facon frailty scale, 132 patients (70%) were classified as frail and 57 (30%) non-frail with EM of 12% and 0, respectively (p=0.02), comparable with published data. In order to improve the predictive value of this score, we looked for all potential variables affecting EM by binary logistic analysis that selected CCI>1, PS ≥2 and albumin level ≤ 3g/dL but not age. Therefore, replacing age (included in the Facon simplified score) with albumin (albumin level ≤3g/dL at the same weight as age> 80), we built a new score able to stratify patients in frail (score 3-5, n= 55, 29.5%) and non-frail (score 0-2, n=155, 70.5%). Univariate Cox analysis found CCI>1, PS ≥2, albumin level ≤3 g/d, Facon frailty score and our new frailty score as factors significantly affecting EM but stepwise Cox regression analysis selected only our new score system with EM of 23% in frail and 6% in non-frail patients (p=0.002).

Conclusion: our analysis suggests that simplified frailty score overestimates the number of frail patients in the real life setting, not allowing to identify true frail individuals. Using other parameters instead of chronological age such as albumin level, Facon score could be improved in the ability to detect patients with the highest risk of EM and could help in developing personalized treatments in NTE MM patients.

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ABSTRACT WITHDRAWN

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DARATUMUMAB COMBINED WITH DEXAMETHASONE AND LENALIDOMIDE OR BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS: REPORT FROM THE MULTIPLE MYELOMA GIMEMA LAZIO GROUP

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The multiple myeloma (MM) treatment has changed over the last years due to the introduction of novel drugs such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs). Despite improvements in the MM outcome, MM remains an incurable disease. Daratumumab is a human IgGK monoclonal antibody targeting CD38 with tumor activity associated with immunomodulatory mechanism. In combination with standard of care regimens, including bortezomib and dexamethasone (Vd) or lenalidomide and dexamethasone (Rd), daratumumab prolonged progression-free survival (PFS) in patients (pts) with RRMM as well as in new diagnosis MM. This led its approval for MM patients requiring treatment. We report the real life experience of the Multiple Myeloma GIMEMA Lazio Group in 171 heavily treated pts who received daratumumab in combination with Vd or Rd. Ninety-one pts (53%) were male and 80 pts (47%) were female. Median age was 64 years (range 37-83), median level of hemoglobin was 10.9 g/dL (6.9-16.7); median level of creatinine was 0.9 mg/dL (range 0.4-7.4). According to ISS, 71 pts (48%) were ISS I, 41 pts (28%) were ISS II, 36 pts (24%) were ISS III. One hundred eight pts (71%) have bone lesions. One hundred twenty pts (70%) received a single previous line of therapy; 32 pts (19%) received 2 previous lines of therapy and 19 pts (11%) received ≥ 3 lines of previous therapy. One hundred thirty-three (78%) pts received DRd and 38 pts (22%) DVd. One hundred sixty three pts were evaluable for hematological response. The overall response rate was 80%: 85 pts (52%) obtained a PR; 34 (21%) pts a VGPR; 15 (9%) pts a CR and 3 (1.8%) pts a sCR. After a median follow-up of 13.5 months (range 0-30), the overall survival (OS) and PFS at 12 months were 84% and 77%, respectively. No statistical difference was found in OS stratified according to treatment; pts treated with DRd have a better PFS compared to pts treated with DVd (p=0.007). According to the number of line of therapy, pts treated with daratumumab at II line have a better PFS compared to pts treated at ≥ 3 lines of therapy (p=0.003). The most common grade 3/4 hematologic treatment-emergent adverse events (TAEs) were neutropenia, thrombocytopenia and anemia. The most common non-hematologic TAEs of grade 3/2 were peripheral sensory neuropathy (19%) and infections (23%), specifically pneumonia (10%). No grade 3/4 infusion-related reactions were observed. Our data support that DRd or DVd therapy is effective and safety in RRMM pts.

P55

CLINICAL FEATURES OF LONG SURVIVING MYELOMA PATIENTS

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In January 2020 an invitation to collect data of patients living longer than ten years after initial treatment for multiple myeloma was extended to Italian haematological centres. After ethical approval, 9 centres sent data of 151 patients. A preliminary analysis shows slight excess of young (median 60 yrs, r.:33-82) women (53%); 85% of the whole population is alive. Previous MGUS was documentable in 56% of cases; Ig type were: Gkappa 47%, Glambda 25%, Akappa 10%, A lambda 9%, BJ 9%. Increased (> 2 mg/dl) serum creatinine was detected in 43% of cases, DS stage III A/B 43%. After first line, 46% of patients were in CR, all treated by transplant procedures, 20% are in CCR, 20% were in VGPR, 25% in PR, 9% in PD. 53% of CR patients relapsed after an average time of 66 months (r.: 8-): 30% of them attained a second CR, the others did not. Thirteen patients were refractory to the initial therapy: 5 of them have never reached CR after other lines of therapy and 4 are alive. Approximately 75% of patients received second line treatment, 45% third line, 35% fourth line, 20% fifth line, 10% sixth line. We do not report persons attaining 20 years of survival, for which is difficult to accept even the possibility of operational cure. We cannot consider CR after induction as necessary and sufficient condition for long survival, as about half of the patients never attained CR. However, all patients living longer than 15 years (14 very long survivors, 12 of which are alive) were in CR

after induction and had received intensive high dose therapy (6 tandem auto-, 5 single auto-, 3 allo-BMT). The impact of treatments for relapsing disease seems significant, as one third of the patients survived more than 10 years thanks to four lines of therapy.

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NETWORK META-ANALYSIS OF RANDOMIZED TRIALS IN MULTIPLE MYELOMA: EFFICACY AND SAFETY IN FRONTLINE THERAPY FOR PATIENTS NOT ELIGIBLE FOR TRANSPLANT

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The treatment scenario for newly diagnosed multiple myeloma not eligible for transplant (NEMM) currently include the combination of bortezomib (V), melphalan (M) and prednisone (P)(VMP) +/- daratumumab (D)(D-VMP) or lenalidomide (R) and dexamethasone (d) (Rd) +/- V(VRD) or D(D-Rd). However, the lack of direct head-to-head comparisons between approved regimens strongly complicate the decision-making process. Here we performed a network meta-analysis (NMA) of phase 2/3 trials in this setting to determine the potential best regimen(s) according to efficacy and safety. A total of 27 studies including 16,456 patients and 21 therapeutic regimens (thalidomide (T)/d (Td), MP, Vd, Rd, cyclophosphamide (C)/R/P (CPR), MPT, MPT+T maintenance, MPR, MPR+R maintenance, VMP, Rd for 18 months (Rd_18), MP+carfilzomib (KMP), VRd, VTd, CTd, D-VMP, VMPT+VT maintenance (VMPT), D-Rd, VMP+siltuximab (VMPS), ixazomib+Rd (IRD), pembrolizumab+Rd (PRD)) were identified in the timeframe 1999-2021. 4 efficacy (progression free survival, overall survival, complete response rate and overall response rate) and one safety (rate of the most frequent grade 3-4 adverse event) indicators, were extracted from each study and used within the NMA to build a ranking chart. With a mean surface under the cumulative ranking curve (SUCRA) of 87.58, D-VMP reached the highest position in the chart. Unfortunately, the integration of all SUCRA scores by calculating a mean value could be misleading. To overcome this limitation, we undertook a principal component analysis approach, that automatically and unbiasedly grouped the 19 regimens into 3 different subgroups according to their efficacy/safety profile. On these bases we identified: 1) D-VMP, D-Rd, VMPT and VMPS as the preferred regimens to be used as first line approach; 2) Nine (VMP, IRd, Rd, Rd(18), Vd, KMP, VRd, VTd, CTd) less effective more "safe" regimens; among them, VRd and IRd represent the best compromise between safety and efficacy; 3) Nine regimens with a low probability of being beneficial in frontline. Interestingly, we observed that 3 out of 4 regimens within the best group include melphalan. Overall, we demonstrated that 1) first line treatment for NEMM should include a regimen between D-VMP (preferred), D-Rd, VMPT or, for frail patients, VRd or IRd; these results support the possibility of using highly effective Rd-based regimens (KRd) at first relapse; 2) melphalan still deserves a role within the overall treatment strategy of NEMM.

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CLINICAL VALUE OF COMBINED SEROLOGICAL ANF WHOLE-BODY DIFFUSION WEIGHTED MRI (WB DW-MRI) MONITORING DURING CONTINUOUS TREATMENT FOR MULTIPLE MYELOMA

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Background: Continuous treatment with novel agents is a standard of care for Multiple Myeloma (MM). Whole Body Diffusion Weighted Magnetic Resonance (WB DW-MRI) has great sensitivity for MM focal lesions. IMWG radiological algorithm recommend radiological re-evaluation under treatment in case of suspected serological relapse. We explore the role of combined serological and WB-DW-MRI monitoring during continuous therapy.

Methods: We analyzed 47 MM patients (pts) who underwent sequential serological and assessment before therapy (T0) and at six month intervals during continuous treatment (T1 and T2). WB DW-MRI response was rated according to My-RADS 5 points scale. Serological response was evaluated using IMWG criteria. Weighted Cohen's kappa was used to test concordance between hematological and radiological response.

Results: 11 pts (23%) received transplant plus lenalidomide (R) maintenance, 15 pts (32%) were treated with continuous Rd, 21 pts (45%) were addressed to R-based triplet in first relapse. Median time between T0-T1 was 7 months; T2 was available both for serological and radiological response in 27 pts (67%) (median time between T0-T2 13 months). Figure 1 a and b showed RAC distribution according to serological response at time points T1 and T2. Serological response at T1 was as follow: ≥VGPR 58%, PR 27%, MR+PD 15%. Radiological response at T1 was as follow: complete imaging response (RAC 1) 30% (12 pts), partial response (RAC 2+3+4) 48% (19 pts), radiological progression (RAC 5) 22% (9 pts). Serological response at T2 was as follow: ≥VGPR 74%, PR11%, MR+PD 15%. Radiological response at T2 was as follow: complete response (RAC 1) 52% (14 pts), partial response (RAC 2+3+4) 22% (6 pts), radiological progression (RAC 5) 26% (7 pts). We found a fair concordance between radiological and hematological response at T1 (agreement: 68%, kappa coefficient 0.22, p=0.021) as well as at T2 (72%, kappa coefficient 0.32, p=0.015). Radiological progression (RAC5) with sustained hematological response (≥VGPR) was observed in 3 pts at T1 and in 3 pts at T2 (cfr figure 1). In these cases of suspected radiological progression, after confirming focal lesions increase, change of treatment was made according to local guideline.

Conclusions: Our retrospective data suggested the potential role of WB DW-MRI longitudinal monitoring at specific time points for early detection of focal progression under continuous treatment, regardless of persistence of serological good quality remission.

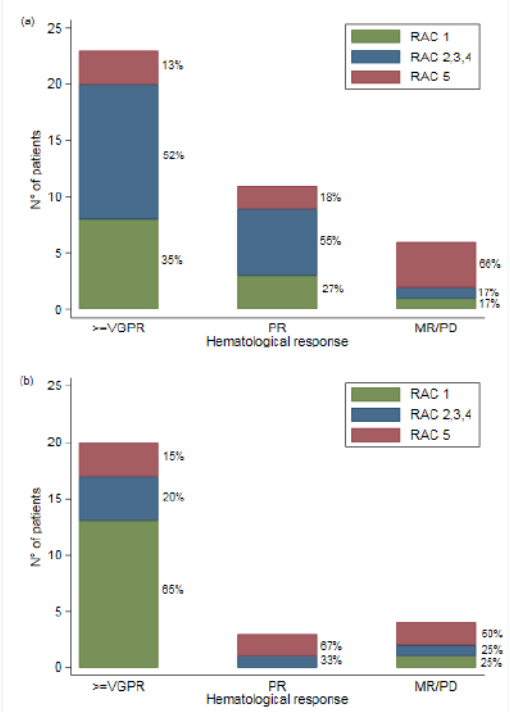

Figure 1.

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ROLE OF SERUM FREE LIGHT CHAIN ASSAY FOR DEFINING RESPONSE AND PROGRESSIVE DISEASE IN IMMUNOGLOBULIN SECRETORY MULTIPLE MIELOMA

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The IMWG guidelines recommend using electrophoresis and immunofixation to define response and progression (PD) in immunoglobulin (Ig) secretory multiple myeloma (Ig-MM). Empirical modified criteria including the serum free light chain (sFLC) assay have been proposed, however the impact on clinical outcomes evaluation has to be explored and validation is needed. We analyzed the inclusion of sFLC assays (Freelite) in the definition of response and PD for Ig-MM. Response and PD were categorized as by IMWG criteria. Progression-free survival (PFS) and overall survival (OS) were conventionally defined. Increasing sFLC levels (defined as for oligosecretory MM) at the time of conventional PD as well as an sFLC escape (sFLCe, *i.e.* increase in sFLC without any IMWG criteria of PD) were noted as sFLC PD. Progression/sFLCe-free survival (ePFS) was the time from the start of treatment to the date of first PD or sFLCe, or death. Second time to progression/sFLCe (2nd TTPe) and OS after progression/sFLCe (OS after Pe) were the time from first PD or sFLCe to second PD or sFLCe, and to the date of death, respectively. 339 Ig-MM patients (pts) treated with a first line novel agent-based therapy and who had sFLC measurements serially available (*i.e.* every 3 months) were retrospectively analyzed. The median follow-up was 54 (IQR 25-84) months. At baseline, 231 (68%) pts showed an sFLC measurable disease. 148 (44%) pts achieved a complete response and 198 (60%) a normal sFLC ratio (sFLCR). sFLCR normalization was an independent prognostic factor for extended PFS (HR=0.46, p=0.001) and OS (HR=0.47, p=0.006) by multivariable analysis. 175 (52%) pts experienced PD by either increased monoclonal Ig (M-Ig) (n=137) or development of organ damage without changes in M-Ig (n=38). Overall, 77 (23%) pts showed a sFLC PD, including 31 (9%) who experienced sFLCe. Median values for PFS and ePFS were both equal to 36 (95% CI=32-42, and 32-40, respectively) months. The median 2nd TTPe was 14 months for PD with no M-Ig and/or sFLC changes, 20 for sFLC PD, and 26 for M-Ig PD without increased sFLC (p=n.s.). sFLC PD adversely affected the OS after Pe compared to M-Ig PD without increased sFLC (median 30 *vs* 48 months, HR=0.52, p=0.012). These data strongly support including the sFLC assay for definition of response and PD in Ig-MM. sFLCR normalization has a prognostic role regardless of conventional response. PFS and ePFS are similar. Increasing sFLC levels negatively impacts the OS after Pe.

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IXAZOMIB-BASED INDUCTION FOLLOWED BY SINGLE-AGENT IXAZOMIB MAINTENANCE IN TRANSPLANT INELIGIBLE, NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: UPDATED RESULTS OF THE EMN10-UNITO TRIAL

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Introduction: Ixazomib represents an appealing option for the management of elderly patients (pts) with multiple myeloma (MM), due to its oral administration and the lack of peripheral neuropathy. Here we present updated results of the phase II EMN10-Unito study investigating Ixazomib in combination with dexamethasone (Id), Cyclophosphamide-dexamethasone (ICd), Thalidomide-dexamethasone (ITd) or Bendamustine-dexamethasone (IBd) as induction therapy followed by single-agent Ixazomib maintenance in transplant-ineligible newly diagnosed (ND) MM pts.

Methods: Treatment consisted of 9 28-day induction cycles of Ixazomib 4 mg on days 1,8,15 and dexamethasone 40 mg on days 1,8,15,22 or Id plus either Cyclophosphamide 300 mg/m^2 orally on days 1,8,15 or Thalidomide 100 mg/day or Bendamustine 75 mg/m^2 iv on days 1,8; followed by Ixazomib maintenance (4 mg on days 1,8,15) for up to 2 years. The primary endpoint was the selection of the most effective induction regimen considering a 2-year progression-free survival (PFS) ≥65% as satisfactory.

Results. 175 pts (Id 42, ICd 61, ITd 61 and IBd 11) with a median age of 74 years were enrolled. Two of the four investigational arms were prematurely closed due to low-enrollment (IBd arm, 11 patients enrolled) and high risk of inefficacy (Id, 42 patients enrolled). The median PFS was 10 months with Id, 19 with ICd, 12 with ITd, and 14 with IBd, with a 2-year PFS probability of 32%, 41%, 25% and 40%, respectively. After the induction phase, ICd and ITd resulted in higher ≥ partial response (PR; 75%-84% vs. 57%; p<0.05) and VGPR (46%-48% *vs* 24%; p<0.05) rates as compared to Id. Grade 3-4 non-hematological adverse events (AEs) were more frequent in the ITd arm (48%) as compared to the Id (17%), ICd (19%) and IBd (36%) arms. Overall, 58% of patients started ixazomib maintenance. 19% of pts improved the response obtained during induction by at least one IMWG category. The median PFS from start of maintenance was 14.9 months. Grade 3-4 AEs occurred in 14% of patients. Grade 1-2 peripheral neuropathy (PN) was reported in 16% of patients without grade 3-4 events.

Conclusions: Safety and efficacy data suggest that ICd was the most promising induction strategy. Continuous treatment with single-agent Ixazomib confirmed its efficacy and tolerability in elderly NDMM pts.

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EFFICACY AND SAFETY OF IXAZOMIB INDUCTION AND MAINTENANCE IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS ACCORDING TO THE IMWG FRAILTY SCORE: A POST-HOC ANALYSIS OF THE EMN10-UNITO TRIAL

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Introduction: The IMWG frailty score stratifies NDMM patients ≥65 years in 3 categories (fit, intermediate-fit and frail), according to age, comorbidities and functional abilities, with different prognosis. The phase II EMN10 study investigates 4 Ixazomib-based induction regimens followed by Ixazomib maintenance in elderly NDMM patients. We conducted a post-hoc analysis of safety and efficacy of these combinations according to IMWG frailty score.

Methods: NDMM, transplant-ineligible pts were enrolled. Treatment consisted of 9 induction cycles of Ixazomib combined with dexamethasone (Id), Cyclophosphamide-dexamethasone (ICd), Thalidomide-dexamethasone (ITd) or Bendamustine-dexamethasone (IBd), followed by ixazomib maintenance. Pts were classified fit, intermediate-fit or frail according to IMWG frailty score.

Results. 171 pts started treatment, 75(44%) were fit, 53(31%) intermediate-fit and 43(25%) frail. Median follow-up was 27 months. ORR and VGPR rates after induction were similar in fit (71%;42%), intermediate-fit (74%;38%) and frail pts (76%;40%). No significant differences in PFS were observed among fit (14.1 months; HR: 0.75, p=0.27), inter-

mediate-fit (14.8, HR: 0.68, p=0.12) as compared to frail pts (12.2 months). OS was longer in fit pts (NR; HR: 0.36, p=0.02) and intermediate-fit (NR; HR: 0.58, p=0.15) as compared to frail ones (36.7 months). Grade(G) 3-4 non-hematological AEs during induction were higher in frail pts (37%) *vs* fit (24%) and intermediate-fit (26%) ones. Treatment discontinuation was higher in frail (21%) *vs* fit (11%) and intermediate-fit pts (9%). When comparing PFS with three- *vs* two-drug induction, both fit (HR: 0.75) and intermediate-fit (HR: 0.69) pts benefited from the use a triplet; no difference was observed in frail pts (HR: 1.01). Overall, 102 pts (60%) started maintenance: 46(45%) were fit, 35(34%) intermediate-fit and 21(21%) frail. No difference in PFS from start of maintenance was observed in intermediate-fit (HR: 0.92) and frail (HR: 1.14) pts *vs* fit ones. Maintenance discontinuation due to AEs was similar in the three groups (fit: 11%; intermediate-fit: 15%; frail: 10%).

Conclusions: Ixazomib-based regimens showed similar efficacy irrespectively of frailty status, although toxicity was higher in frail pts. Frail pts did not seem to benefit from a triplet over a doublet. Ixazomib maintenance was effective and well tolerated in all frailty subgroups, representing an appealing option in elderly MM.

Acute Leukemias and Myelodisplastic Syndromes 2

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GENETIC LANDSCAPE AND CLONAL EVOLUTION PATTERNS OF CEBPA-DOUBLE-MUTATED ACUTE MYELOID LEUKEMIA BASED ON NEXT-GENERATION SEQUENCING: A SINGLE CENTER RETROSPECTIVE ANALYSIS

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Introduction: Although CCAAT/enhancer binding protein alpha double mutated (CEBPA DM) acute myeloid leukemia (AML) is considered a low-risk disease according to 2017 ELN recommendations, relapse remains a major cause of treatment failure and death. To assess the broader prognostic impact of the genetic landscape, we sequenced a panel of 40 myeloid disorders-related genes in a single center patient cohort (n=25).

Methods: 16 CEBPA DM AML diagnosis samples, along with 9 CEBPA single mutated (SM) samples, were sequenced by targeted next-generation sequencing (Ion Torrent) using Oncomine Myeloid Research Assay. 4 CEBPA DM and 2 CEBPA SM AML relapse samples were analyzed as well. All patients received intensive chemotherapy according to 2017 ELN recommendations.

Results: With a median follow-up of 3.2 years (range 0.4-12), 5y OS was 61% and 14% for CEBPA DM and CEBPA SM patients respectively. CEBPA DM patients had a significantly lower risk of death as compared to SM patients (OR 1.65; 95%CI 0.02-0.9, p=0.049). Overall, the most frequently mutated genes were FLT3 (45.8%), NPM1 (33.3%), DNMT3A (33.3%), WT1 (29.2%), GATA2 (29.2%), STAG2 (16.7%) and TET2 (16.7%). CEBPA DM and SM patients had a different mutational pattern, with GATA2, FLT3, DNMT3A and TET being the most frequently mutated genes in CEBPA DM *vs* NPM1, FLT3, DNMT3A and WT1 in CEBPA SM patients. NPM1 (77,8%% *vs* 6,7%; p<0.01) and ASXL1 mutations (44,4% *vs* 0%; P=0.02) were more frequent in CEBPA SM patients, confirming their mutually exclusivity with CEBPA biallelic lesions. Overall, mutations in WT1 and FLT3 were associated with increased relapse rate (p=0.02 and p=0.01 respectively), while patients with GATA2 mutations had a strong trend towards better 5y OS (83% *vs* 32%, p=0.053). Patients with less than 5 concurrent mutations had a lower OR of death (OR 0.21, 95% CI: 0.06-0.7, p=0.015). Each single unitary gain in the number of mutated genes increased the hazard ratio (HR) of death by 27.7% (95% CI: -1.4%-+65%, p=0.064). Matched diagnosis and relapse samples analysis suggested different features of clonal evolution: while WT1, DNMT3A, NPM1, and IDH1 consistently persisted at relapse, CEBPA and GATA2 mutations were unstable during disease course. ZRSR2 and PRPF8 mutations were found in relapse samples only.

Summary: Our study offers insights into the genetic landscape of CEBPA mutated AML highlighting the potential contribution for the risk stratification and individualized treatment strategies.

P62

COMPREHENSIVE GERIATRIC ASSESSMENT, ALLOGENEIC HSCT AND SURVIVAL IN AML PATIENTS 65-75 YEARS OLD

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Background: Acute myeloid leukemia (AML) in patients over the age of 65 carries a dismal prognosis, due to poor control of the disease with chemotherapy alone. Allogeneic hematopoietic stem cell transplantation (HSCT) can provide significant anti-leukemic effect in eligible patients.

Methods: 44 consecutive de novo or secondary AML patients, aged 65-75 years, were diagnosed in our Unit between September 2018 and August 2020. Patients were risk-stratified according to ELN 2017 criteria and classified as FIT, UNFIT and FRAIL according to a comprehensive geriatric assessment (CGA), which included ECOG,CIRS,ADL and IADL scores. Only fit patients with intermediate-adverse ELN 2017 risk score were eligible for HSCT.

Results: Median age was 70 years (range 65-75). 9 patients presented with favorable, 16 with intermediate and 15 with adverse risk. 19 patients were classified as FIT, 12 as UNFIT and 13 as FRAIL. All FRAIL patients were treated with best supportive care. UNFIT patients were treated with HMAs (n=8), Flt-3 inhibitors (n=1), CPX-351 (n=1), FLA (n=1), one refused therapy. In the FIT group, induction was standard chemotherapy (n=9), CPX-351 (n=5), HMAs (n=1), LDAC (n=1), allo-HSCT upfront (n=3). Median CIRS score was 1 (1-4). 12/19 had ECOG score 0, 18/19 had IADL over 5/8. Complete remission (CR) was achieved in 15/44 patients (39%) and in 15/19 FIT patients (79%). At last follow-up,14/44 patients were alive (median 135 days, range 1-813), with an actuarial 2 year survival of 21%. Survival was 0% for FRAIL and UNFIT patients,52% for FIT patients (Figure 1). 13/19 FIT patients were allografted. Reasons for not grafting were early relapse (n=2), refusal (n=2), waiting list (n=2).Donor type was HLA haploidentical (n=6), MUD (n=4), MSD (n=2), cord blood (n=1).Conditioning was non myeloblative (n=2), reduced intensity (n=9) or myeloablative(n=2). GvHD prophylaxis consisted of CsA, MMF and post transplant cyclophosphamide in 9/13. Acute GvHD grade II-IV developed in 5 patients (38%) and chronic GvHD in 3 (23%). Actuarial 2 year survival is 60%, and median survival from diagnosis is 365 days (60-813); cause of death was transplant related (n=2, 15%) and relapse (n=2, 15%).

Conclusions: CGA has a strong influence on treatment strategies in elderly AML. An allo-HSCT was performed in 68% of FIT patients with promising results bed availability was the most important factor delaying transplantation. New therapies are required for UNFIT and FRAIL patients.

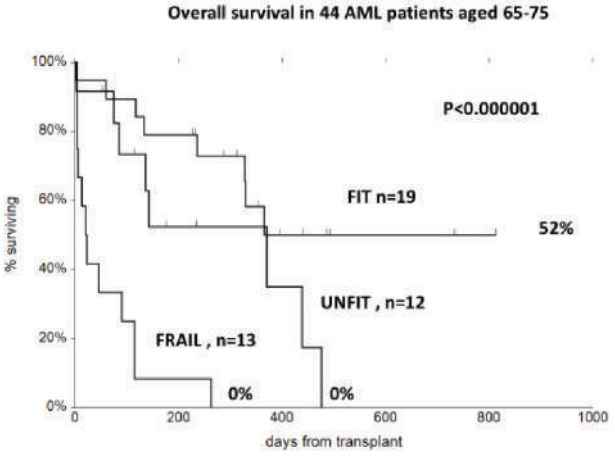

Figure 1.

P63

VITAMIN C DEFICIENCY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Vitamin C has been shown to play a significant role in suppressing progression of leukemia through epigenetic mechanisms, suggesting that administration of vitamin C may help to restore normal hematopoietic stem cell function and differentiation. We aimed to study the levels of vitamin C in plasma and within blasts in pts with AML, to characterize the role of this vitamin on AML biology and clinical course. To this aim, we studied the serum levels of vitamins A and E, and the plasma levels of vitamin C in 56 pts with AML, 9 with APL, and 7 with MDS, and in 15 healthy donors (HDs). Vitamin A, E and C levels were studied by high performance liquid chromatography at diagnosis, while vitamin C plasma levels were also studied in 14 pts in CR and 10 in progression. Intracellular vitamin C levels were also assessed in AML MNCs (n=9), blasts and lymphocytes (n=7), and MNCs from HDs (n=7). Expression of the main vitamin C transporters (encoded by SLC23A2, SLC2A1 and SLC2A3 genes) were assessed by QPCR in 22 AML and 16 HDs. There were no significant differences in vitamin A and E levels between pts and HDs.

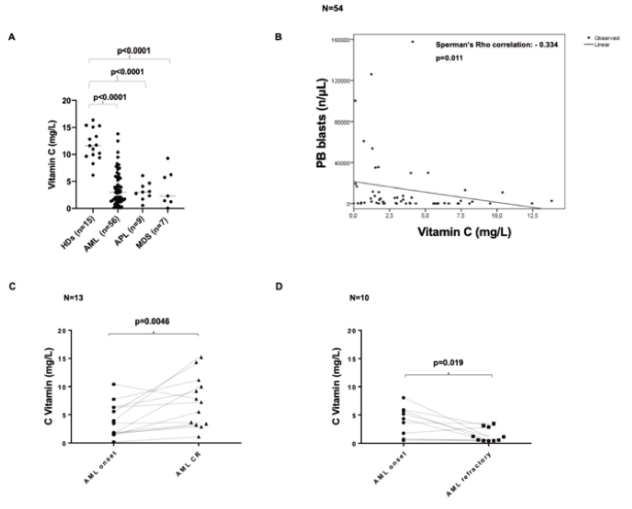

Figure 1. (A) Plasma levels of vitamin C in Myeloid Neoplasms and healthy donors. (B) Spearman's correlation between PB blasts count and plasma concentration of vitamin C. (C) Vitamin C plasma levels evaluated in 14 patients in CR and (D) in 10 cases in AML progression. HDs: healthy donors. AML: acute myeloid leukemia. APL: acute promyelocytic leukemia: MDS: myelodysplastic syndrome. PB: peripheral blood.

On the contrary, vitamin C concentration was significantly lower in pts as compared to controls (p<0.0001) (Figure 1A), was inversely correlated with peripheral blast counts (p=0.011) (Fig.1B), and significantly increased at the time of CR in 14 pts (p=0.0046) (Figure 1C). On the contrary, we observed a significant reduction of vitamin C concentration in 10 cases of refractory AML when compared to corresponding diagnostic samples (p=0.019) (Figure1D). Cytoplasmic vitamin C was also significantly reduced in MNCs purified from pts with AML versus HDs (p=0.0003), and when comparing sorted blasts to lymphocytes in individual pts. In this line, expression of the main vitamin C transporters SLC23A2, SLC2A1 and SLC2A3 was also significantly reduced in AML compared to HDs. There were no significant differences in vitamin C plasma level when grouping pts according to cytogenetics (p=0.367) or 2017 ELN risk stratification groups (p=0.855). Vitamin C levels did not play a predictive role for overall or relapse free survival.

In conclusion, our study shows that vitamin C levels are significantly decreased in pts with AML at the time of initial diagnosis, further decrease during disease progression and return to normal by achievement of CR. Correspondingly low intracellular levels may mirror increased Vitamin C metabolic consumption in proliferating AML cells.

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L-CARNITINE FOR ASPARAGINASE-INDUCED HEPATO-TOXICITY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: MULTICENTER OBSERVATIONAL STUDY OF ALL-CAMPUS GROUP

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Asparaginase is an important component of the multi-agent chemotherapy for treatment of adult acute lymphoblastic leukemia (ALL) patients. The toxicity profile includes allergy, pancreatitis, coagulopathy and liver toxicity that ranges from mild bilirubin and/or transaminase increase to fulminant hepatic failure. Risk factors for liver injury are age, previous liver disease, overweight and obesity conditions. L-Carnitine can help to overcome the mitochondrial dysfunction underlying asparaginase-induced liver toxicity. Up to date, there are only few published case reports about the use of carnitine for the treatment of hepato-toxicity after asparaginase therapy in patients with ALL. We retrospectively analysed 25 adult patients with ALL treated with L-Carnitine for liver toxicity after pediatric-like chemotherapy, including asparaginase, in 12 italian centers of the ALL-CAMPUS group. Briefly, 15 patients were males and 10 females, the median age at diagnosis was 43 years (range 22-67); 17/25 patients (68%) had B-ALL, 7/25 (28%) T-ALL and 1/25 (4%) MPAL; 14/25 patients (56%) were classified as standard risk, 2/25 (8%) as high risk, 8/25 (32%) as very high risk while in one patient the risk was not available. Assessing the risk factors for liver toxicity, 10/25 patients (40%) were of normal weight, 11/25 (44%) overweight and 4/25 (16%) obese; 2/25% (8%) were HBV positive, while 23 patients (92%) were HBV/HCV negative. None of the 25 patients had a previous liver disease. The hepato-toxicity appeared during the first course of chemotherapy in 23/25 patients (92%), while 2 patients showed liver toxicity after 3 and 5 chemotherapy courses, respectively. 21/25 patients (84%) received peg-asparaginase, while 4/25 (16%) were treated with levo-asparaginase. In 15/25 patients (60%) L-Carnitine was administered intravenously and in 10/25 (40%) orally. L-Carnitine was started after a median time of 3 days since the beginning of liver toxicity (range 0-51). The median time of administration was 16 days (range 2-61). The resolution of liver injury was achieved in 24/25 patients (96%), and it was complete in 18/24 patients (75%). No carnitine related side-effects were reported. Our study describes the largest serie of cases currently available and confirms that the overweight and obesity condition represents a predisposing conditions to the liver toxicity. We also confirm the efficacy and safety of L-Carnitine in the management of liver toxicity after asparaginase treatment.

P65

ENRICHMENT OF DOUBLE RUNX1 MUTATIONS IN ACUTE LEUKEMIAS OF AMBIGUOUS LINEAGE

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The WHO 2016 classification recognizes 7 entities under the category of acute leukemias of ambiguous lineage. The immunophenotypic heterogeneity of ALAL likely reflects a heterogeneous mutational profile that has been so far poorly characterized. To address the need of a better biological definition of ALAL, we conducted a mutational analysis using a targeted sequencing approach with a 54 myeloid and a 138 lymphoid gene panels on 10 patients diagnosed between 2008 and 2020 at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo of Pavia. Our study cohort consists of 5 AUL, 2 MPAL B/myeloid NOS, 1 MPAL T/myeloid NOS and 2 ALAL NOS. The most frequently mutated genes within the myeloid panel were NRAS (40%), RUNX1 (40%), ASXL1 (30%), DNMT3A (20%), BCOR (20%), EZH2 (20%), U2AF1 (20%). The only recurrently mutated lymphoid gene was KMT2C (25%), but mutations within this gene were mainly subclonal. The median number of mutations in myeloid genes was superior to the lymphoid ones (p 0.012). We then focused our attention on the RUNX1 gene, which is known to be essential for the development of lymphoid and myeloid lineages. Interestingly, all 4 RUNX1 mutated cases presented two mutations in the gene, mainly of founding type: 3 AUL and 1 patient with a MPAL B/myeloid NOS. All RUNX1 mutations resulted somatic. The allelic distribution of the RUNX1 mutations were in cis for one patient and in trans for two patients. In addition, the analysis of RUNX1 variants on RNA sequences in one patient revealed that virtually no functional RNA was present. This suggests that there is a complete loss of function of the RUNX1 protein which might influence blast phenotype. Sequencing of a case at relapse showed the loss of the double RUNX1 mutant asset; intriguingly, this change in the mutational profile was associated with the disappearance of the lineage ambiguity. Double RUNX1 mutations have been previously reported to be associated with AML with minimal differentiation, but not in ALAL.

In conclusion, we found that myeloid gene mutations are enriched in a cohort of ALAL cases strictly diagnosed according to WHO 2016 criteria. Moreover, our data seem to suggest that double RUNX1 mutation is a recurrent mutational pattern in leukemias with an undifferentiated phenotype and may support the hypothesis that AUL and AML with minimal differentiation represent a continuum of disease with a similar genetic background.

P66

RETROSPECTIVE ANALYSIS ON EFFICACY OF HYPOMETHYLATING AGENTS IN AML-MRC

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Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) is a well-defined subtype of AML characterized by a very poor prognosis. We retrospectively reviewed 132 elderly AML pts treated frontline with hypomethylating agents (HMAs); 70% and 30% of pts received azacitidine and decitabine, respectively. The median follow-up was 6.8 months (range 0.3-55.5). Sixty-five (49%) pts had an AML-MRC, 62 (47%) AML-NOS while 5 (4%) had therapy-related AML. Regarding AML-MRC pts, the median age was 75 years (range 58-86) and 87% had a Charlson Comorbidity Index score ≥3. The median BM blast count resulted significantly lower in pts with a previous history of MDS/MPN than pts with *de novo* AML-MRC (25% and 41%, p=0.001) and there was a male predominance for pts of the former group (82%, p=0.003). Cytogenetic assessment showed that 24% and 44% of pts carried a complex karyotype (CK) and adverse cytogenetic features, respectively. On the contrary, among AML-NOS, no cases of CK were reported. ELN-based risk assessment was globally available for 66 patients: 15 (71%) out of 21 high risk pts had an AML-MRC (p=0.039). AML MRC and AML-NOS pts differed only for the median bone marrow (BM) blast count that was significantly lower in AML-MRC group than AML-NOS (27% *vs* 44%, respectively, p<0.001). No other patient and disease-related differences, including the median number of HMA cycles received and overall response rates (AML-MRC 56%, AML-NOS 52%), were found. Median overall survival (OS) was significantly higher in AML-MRC than AML-NOS [9.6 months (95%CI 2.8-16.5) *vs* 7.6 months (95%CI 5.2-10.0) p=0.025]. A statistical trend toward significance was found for median progression free-survival (PFS) for AML-MRC compared to AML-NOS [(8.6 months (95%CI 6.0-11.3) *vs* 6 months (95%CI 2.3-9.7), p=0.076]. Pts with only multilineage dysplastic features (n=7) exhibited a trend of superior OS compared to other AML-MRC categories [26.9 months (95%CI 0.7-53.1) *vs* 9.4 months (95%CI 7.6-11.3) p=0.075]. BM blast count ≥30% was associated with inferior survival (p<0.001). Achieving partial remission or better (≥PR) or a stable disease (SD) after four HMA cycles did not confer different survival outcome (p=0.105). On the contrary, SD as best response was significantly associated with inferior survival than ≥PR (<0.001). The results of our retrospective analysis indicated that HMAs seems to be a valid option in unfit AML-MRC not suitable for intensive chemotherapies.

P67

HIGH RATE OF MINIMAL RESIDUAL DISEASE NEGATIVITY IN PATIENTS ACHIEVING COMPLETE REMISSION AFTER TREATMENT WITH VENETOCLAX-BASED REGIMENS FOR RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA

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Relapsed/refractory (R/R) acute myeloid leukemia (AML) is an unmet medical need. The only curative option is allogeneic hematopoietic stem cell transplantation (HSCT) which is only applicable in a fraction of patients due to the scarce efficacy and toxicity profiles of salvage regimens. Moreover, the efficacy of HSCT in disease control can be hampered by the persistence of residual leukemic populations, represented by minimal residual disease (MRD) positivity at the time of conditioning initiation. MRD persistence at the time of HSCT has been associated with high cumulative relapse incidence, comparable to those observed in the scenario of transplant with active disease; therefore, salvage approaches with potential for MRD eradication are needed. Between March 2018 and December 2020, 47 patients with R/R AML were treated at our institution with VEN-based regimens for primary induction failure (n=11), relapsed disease (n=25) or relapse after HSCT (n=11); 24 patients were treated with an intention-to-transplant (ITT). Reasons for the lack of ITT were age or precluding medical conditions. Partner drugs for VEN were azacitidine (n=29), LDAC (n=13) and decitabine (n=5). MRD evaluation was carried out for all CCR patients. MRD was measured at first CR achievement and as clinically indicated thereafter. Overall composite CR (CCR) rate was 55% (26/47). All CRs invariably occurred during the first six weeks of treatment. Of all patients in the CCR group, 16 patients (61%) achieved an MRD negative status evaluated by flow cytometry or RT-qPCR when applicable. MRD negativity status was invariably achieved by cycle 3. The only factor impacting on the likelihood of MRD negativity was the presence of a NPM1 mutation (p=0.014). In the CCR group, 16 patients were treated with on an ITT basis. Of those, 14 patients were ultimately able to proceed to HSCT without further treatment, while two patients experienced relapse before HSCT. With a median follow up of 11.7 months, overall survival for all CCR patients was 19.2 months (MRD-, not reached; MRD+, 11.9 months; non significant); disease-free survival was 10.6 months (MRD-, not reached; MRD+: 10.6 months, NS). Even if limited by the small number of patients and its retrospective nature, our study provides an interesting glimpse on the quality of responses achieved with VEN-based regimens in R/R AML and its clinical significance. Further validation in larger prospective studies is warranted.

P68

ALTERATION OF OSTEOGENIC DIFFERENTIATION INDUCED BY ACUTE MYELOID LEUKEMIA IN THE HEMATOPOIETIC NICHE: A POSSIBLE ROLE OF NOTCH SIGNALING

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Acute myeloid leukemia (AML) is characterized by the abnormal clonal proliferation of undifferentiated blasts and by a high relapse rate, associated with chemoresistance and bone marrow (BM) failure. These processes are supported by the alterations in the stromal component of the hematopoietic niche due to the interactions with leukemic cells. We have previously demonstrated that mesenchymal stromal cells (MSC) obtained from patients' BM presented intrinsic alterations in osteogenesis, characterized by a high presence of osteoblast precursors and a significant reduction of mature osteoblasts. We wondered whether the alteration of osteogenesis is specifically due to AML infiltration by the Notch signaling. We therefore established an *in vitro* co-culture system in which osteogenesis is induced in normal MSC in the presence of different AML cell lines, primary AML cells, or healthy $CD34^+$ cells. MSC cocultured for 3 days with AML cell lines showed an increased expression of Tissue Non-specific Alkaline Phosphatase (TNAP), a marker of early osteogenesis, and reduction of osteopontin and osteocalcin, markers of late osteogenesis. Of note, only direct cell contact, but not AML-conditioned medium affected TNAP expression on MSC. Furthermore, coculture of primary AML cells with MSC from healthy donors showed significant upregulation of TNAP expression in 13 of 20 AML samples, independent of either genetic or morphologic AML subtype. Coculture with normal $CD34^+$ cells from different healthy donors did not affect TNAP levels. Then we assessed the activity of the Notch pathway on MSC in the presence of different AML cell lines and we found that Notch1-2-3, Hes1 and Hey1 levels were markedly increased in cocultured MSC. To prove the association between increased Notch signaling and altered osteogenesis in MSC cocultured with AML, we added DAPT,

a γ-secretase inhibitor, which proved to efficiently reduce activation of Notch signaling in MSC. Adding DAPT successfully abrogated TNAP upregulation in MSC cocultured with AML cell lines and primary AML cells. Furthermore, stimulation with recombinant Jagged1 induced a strong upregulation of TNAP on MSC, which is abrogated in presence of DAPT. Overall, these results demonstrate that Notch signaling is activated by AML cells in MSC and induces early osteogenesis. These novel insights into the human AML BM microenvironment may help identify new targets which might pave the way for niche-targeted therapies in AML patients.

P69

A REAL LIFE STUDY OF ACTIVITY OF ATO PLUS ATRA REGIMEN IN TREATMENT OF ACUTE PROMYELOCITIC LEUKEMIA

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Background: All-trans retinoic acid (ATRA) with Arsenic Trioxide (ATO) has become standard of care for low-intermediate risk acute promyelocitic leukemia. Pilot APL0406 and NCRI AML17 trials have shown high efficacy and reduced hematologic toxicity with ATO and ATRA. However real-life studies confirming activity of this regimen in real life setting are lacking and required.

Methods: APL cases from fuor experienced hematological institution, treated with ATO and ATRA, were retrospectively collected. Analysis included APL with low/intermediate Sanz risk at first diagnosis, or APL relapsed after ATRA plus chemotherapy treatment. Primary end points were Overall Survival (OS) and Event-Free Survival (EFS). Secondary end-points included analysis of quality of response, factor affecting survival and toxicity.

Results: From 2014 to 2019, 77 patients treated with ATO and ATRA protocol were identified. Median 5y-OS was 97.4%, and median EFS was 96.1%. Complete remission was achieved in all 77 patients (100%), with persistent molecular remission in all but on patient, where a molecular relapse was observed. Survival analysis didn't show statistically significant differences among age categories (under 60 years old *vs* over 60 years old), risk stratification (low, intermediate, high) and frontline therapy *vs* salvage therapy. However epatoxicity and hyperleucocitosis was observed in 21% and 40% of patients respectively. QTc prolongation with needing for ATRA reduction was not observed. ATRA and ATO was associated with a good safety profile, with no treatment discontinuation.

Conclusions: Advances in the treatment of APL have changed the natural history from a highly fatal disease up to be definitely curable. Our real-life data confirm efficacy of ATO and ATRA regimen outside clinical trials. Furthermore, toxicity data show how this regimen could potentially be a curative strategy for all patients who are frail or unfit for age and comorbidities.

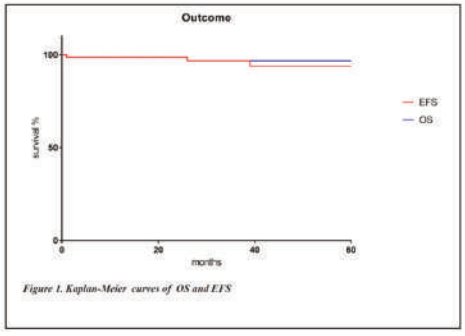

Figure 1.

P70

ROLE OF BMI AND COMORBIDITY IN SURVIVAL OF PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROME WITH DEL5Q DURING LENALIDOMIDE TREATMENT

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Introduction: Myelodysplastic syndrome (MDS) with del-5q represents a clinical and pathological entity recognized by WHO classification. Karyotype abnormalities involving isolated del-5q are the most frequently occurring in MDS (14%). Lenalidomide (LEN) is an immunomodulatory agent and represents the standard of treatment in patients with transfusion dependent del(5q)-MDS patients (pts). Achievement of TD and CCyR after LEN, presence of additional +2 cytogenetic abnormalities (with or without complex karyotype), biallelic TP53 mutations are consolidated disease specific predictors of overall survival. Individual pt variables have not been evaluated in this setting.

Aims and methods: We evaluated the impact of obesity (BMI) and comorbidity measured with Cumulative Illness Rating Scale (CIRS) on the outcome after LEN treatment. We analyzed 21 consecutive MDS-del5q pts treated with LEN as second line therapy after ESAs. Median age at start of LEN was 78 (44,8-98); M/F 11:10; Revised International Prognostic Scoring System (IPSS-R) before treatment was: 2 very low, 17 low, 1 intermediate and 1 high. Median time from diagnosis to treatment was 21 months, median follow up was 41.8 months. 2 pts underwent to allogeneic bone marrow transplantation, and survival was censored at time of transplant.

Results: Overall response rate was 95%, CCyR was 3/13. Median Overall Survival (OS) was 32 months. Disease progression was observed in 52% of cases (MDS-EB1 2 pts, MDS-EB2 4 pts and AML 5 pts). We observed a trend to better OS in pts who received 10 mg *vs* 5 mg (33.9 *vs* 18.1 months, p=0.054). The most prevalent baseline comorbidities included hypertension (28,6%), atrial fibrillation (19%), ischemic cardiac disease (9%), diabetes (19%), congestive heart failure (9%), renal insufficiency (9%) and thyroid disease (14,2%), prior solid tumor malignancy (23.8%) and prior venous thromboembolism (9%). Seven pts have a BMI >25. CIRS score was 0 in 7 pts, 1 in 5, 2 in 7 and 3 in only one case. A significant better OS was observed in pts with BMI<25 (49 *vs* 30 months, p=0.04). Median OS of pts with 0 CIRS was 30,9 *vs* 17 months in pts with CIRS ≥3 (p=0.02). We did not observe any correlation between CIRS and BMI (p=0.38).

Discussion: Our Cohort of elderly MDS del5q pts showed an OS consistent with data previously published. Beside disease specific variables, we evaluated individual variables and demonstrated the prognostic impact of BMI and elevated CIRS score on OS after LEN treatment.

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CD200 AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): MORE THAN A SURFACE MARKER. THE RELEVANCE OF ITS SERUM LEVELS IN PREDICTING PROGNOSIS

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The evaluation of CD200 expression has shown to be a useful tool to better classify chronic lymphoproliferative diseases. CLL overexpress CD200 with respect to other lymphoid leukemias. There is some evidence that serum levels of soluble CD200 (sCD200) could be related to disease progression in pts with CLL. However, very little is known about its prognostic significance. Serum samples were collected at diagnosis from 272 pts with CLL (median age 66 yrs, range 33-90) and from 78 age- and sex-matched healthy subjects (median age 63 yrs, range 42-100), as normal controls. Human CD200 (OX-2 membrane glycoprotein) ELISA kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China) was used to quantify sCD200 in serum samples. We found a significantly higher concentration of sCD200 in serum samples from CLL pts than in controls (median, 1281 pg/ml *vs* 799 pg/ml; p=0.0002). In pts with CLL, sCD200 was significantly higher in those ≥66 *vs* <66 yrs old (median, 1560 pg/ml *vs* 1193 pg/ml; p=0.0001), in those with Binet stage C *vs* A/B (2055 pg/ml *vs* 1274 pg/ml; p=0.0045), in those with unmutated *vs* mutated IgVH (1601 pg/ml *vs* 1131 pg/ml; p<0.0001), and in those with unfavorable (del11q or del17p) *vs* favorable (normal or del13q or tris12) FISH (1897 pg/ml *vs* 1239 pg/ml; p=0.0077). On the contrary, gender, bulky disease, whole blood cell or lymphocyte count, beta2-microglobulin serum levels and presence of autoimmune complications did not significantly correlate with sCD200. Time-to-first-treatment (TTFT) was shorter in pts with higher sCD200 levels (sCD200 >1281 pg/ml *vs* <1281 pg/ml, median TTFT, 61 *vs* 109 months; p<0.001). Baseline sCD200 values appear to have an impact on response to therapy (median in CR *vs* PR/NR pts, 1308 pg/ml *vs* 1590 pg/ml; p 0.0468), and this difference seems to increase if only pts who received chemotherapy or chemo-immunotherapy are considered (1244 pg/ml *vs* 1602 pg/ml; p=0.0193). On the contrary, an association between baseline sCD200 values and response to targeted agents was not found. Finally, sCD200 also had an impact on overall survival (OS) (sCD200 >1281 pg/ml *vs* <1281 pg/ml; median OS, 222 *vs* 299 months; p=0.005). Higher sCD200 correlated with a more aggressive behavior and was able to predict a worse prognosis. CD200 can be released from CD200+ neoplastic cells by ectodomain shedding and both surface and sCD200 are able to engage CD200 receptor, which in turn can result in increased tumor growth, by means of a negative control of immunosurveillance

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BASELINE HISTOPATHOLOGICAL FEATURES DID NOT CORRELATE WITH OUTCOME IN PATIENTS AFFECTED BY MYELOFIBROSIS TREATED WITH RUXOLITINIB

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Myelofibrosis (MF) is a myeloproliferative neoplasm associated with ineffective hematopoiesis, splenomegaly, and bone marrow (BM) fibrosis. Ruxolitinib (RUXO) has been shown to improve splenomegaly, symptom burden, and overall survival (OS) in pts with intermediate-2 or high-risk MF compared with placebo or best available therapy. It has previously been reported that the drug may reverse or markedly delay BM fibrosis with disease-modifying effect. The outcome of MF pts treated with RUXO was correlated with baseline histopathological features. We retrospectively reviewed initial trephine biopsy in 62 pts receiving RUXO outside clinical trials focusing on cellularity, megakaryocyte (MK) morphology and distribution, grade of fibrosis and vascular density. Sixty-two pts in 1:1 male/female ratio with a median age of 67 years received a diagnosis of primary MF (PMF) in 40.3% and secondary MF (SMF) in 59.7% of the cases. The 4-year estimated OS was 94.2%. An increased cellularity was observed in 87.1% of pts with a grade 2-3 fibrosis in 67% of patients. No histological differences are correlated with gender, age, hemoglobin and leucocytes. Prevalent MK bulbous nuclei were revealed in 93% in SMF and 7% in PMF (p=0.014). Pts with numerous giant MK had more frequently splenomegaly ≥5 cm from LCM (p=0.03). The median baseline ferritin was significantly higher in pts with homogeneous expression of CD3 (172.5 *vs* 123 ng/ml) (p=0.036), while, non-homogenous CD3 expression is more frequent in pts with constitutional symptoms (40% *vs* 8%) (p=0.009). Moreover, there was a tendency toward the presence of osteosclerosis in pts with constitutional symptoms (p=0.057). No differences in terms of median platelets count, thrombotic events and transfusion requirement were found according to MK distribution, dimension and nuclear features. The median age at diagnosis is increased in the presence of hypersegmented MK nuclei (p=0.031) and normal vascular density (p=0.045). No correlations in terms of splenic response (reduction of spleen volume of ≥35%) or symptoms reduction with vascular density (p=0.082, p=0.969), cellularity (p=0.402, p=0.716) or fibrosis (p=0.056, p=0.549) were observed. Unless not reaching a statistical significance, the absence of osteosclerosis was reported in 67% of pts who achieved a splenic response after RUXO. In conclusion, it seems that some histopathological features correlate with baseline features but the achievement of clinical responses with RUXO are not affected by these.

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IMPACT OF RELATIVE DOSE INTENSITY AND COMORBIDITIES ON OUTCOME OF FRONTLINE TREATMENT WITH OBINUTUZUMAB AND CHLORAMBUCIL IN CHRONIC LYMPHOCYTIC LEUKEMIA, A MULTICENTRIC ITALIAN STUDY

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Introduction: Since its approval in Italy in 2017, the use of Obinutuzumab (G) and Chlorambucil (Chl) as frontline treatment for chronic lymphocytic leukemia (CLL) patients (pts) has progressively increased. Whether the comorbidity burden and G-Chl relative dose intensity (RDI) may have an impact on outcome has not been investigated.

Aim: Our study aims to evaluate the impact of reduced RDI G-Chl treatment and identify pts at higher risk of dose reductions due to clinical or laboratory characteristics.

Methods: We conducted a retrospective multicenter study using secondary data involving 12 Italian centers, enrolling 130 pts with CLL treated with a frontline G-Chl regimen. For each patient we analyzed clinical and biological characteristics, focusing on comorbidities: we investigated the impact on RDI reduction of ECOG PS, each CIRS parameters individually, CIRS >6, CIRS >8, and at least one CIRS component ≥3 (CIRS 3+).

Impact of Obinutuzumab RDI reduction >20% on outcome

		RDI reduction of Obinutuzumab dose		
Characteristic	Overall, N = 130	>20%, N = 20	<20%, N = 110	p-value[1]
ORR, n (%)	111 (88%)	11 (61%)	100 (93%)	**0.001**
Median PFS	33 months	17.2 months	37.3 months	**0.001**
24-months PFS	68%	32%	74%	**<0.001**
36-months PFS	49%	32%	52%	**<0.001**
Median TTNT	40 months	24.4 months	NR	**0.001**
24-months TTNT	75%	57%	79%	**0.001**
36-months TTNT	54%	34%	58%	**0.001**

[1]Fisher's exact test

Impact of ECOG PS on RDI reduction >20%

		RDI reduction of Obinutuzumab dose		
Characteristic	Overall, N = 130	>20%, N = 20	<20%, N = 110	p-value[1]
ECOG PS in class, n (%)				**0.027**
0-1	94 (72%)	10 (50%)	84 (76%)	
>2	36 (28%)	10 (50%)	26 (24%)	

[1]Fisher's exact test

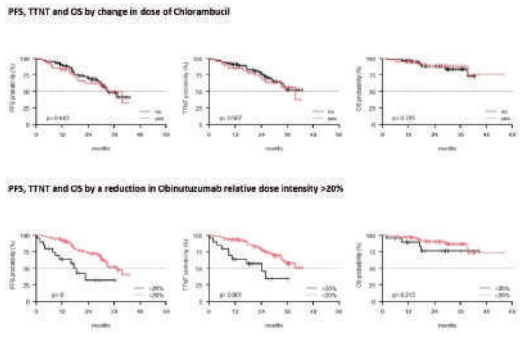

Figure 1.

Results: In our cohort, median age was 76 years (range 42-88); 91% of pts were aged over 65 years old. Median CIRS score was 7 (range 1-18) and 72% of pts had a CrCl <70 ml/min. At the end of treatment, overall response rate (ORR) was 88%, with 26% of CR and 62% of PR. Median follow-up was 29.1 months (range 1.8-55.7). The only factor that independently impacted outcome in terms of PFS and TTNT was G RDI reduction >20%: hazard ratios were 3.03 (range 1.49-6.25, p=0.002) and 2.94 (range 1.37-6.25, p=0.006) respectively. ECOG ≥2 showed a trend towards significance in influencing both PFS (HR 1.73, range 0.98-3.16, p=0.078) and TTNT (HR 1.91, range 0.94-3.49, p=0.076). While dose modifications of Chlorambucil did not show to have an impact, pts who received a decrease <20% of RDI in Obinutuzumab showed a better outcome in terms of ORR, PFS and TTNT, but not OS (ORR 93% *vs* 61%, median PFS 37.3 *vs* 17.2 months, median TTNT not reached *vs* 24.4 months, p=0.001, Figure 1). These results are similar to those obtained with a 100% RDI. ECOG PS ≥2 was the clinical factor with an impact on reducing RDI >20% (50% *vs* 24%, p=0.027).

Conclusions: Our study shows that a decrease >20% of G RDI results in a worse outcome in terms of ORR, PFS and TTNT. A <20% dose reduction, in contrast, showed no difference when compared with 100% RDI. High comorbidity burden had an impact on the RDI reduction >20%, not as single CIRS variable, but as a whole, focusing our attention on pts with ECOG PS ≥2, who are at higher risk of recurrences that will need earlier treatment.

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CHOICE OF FRONTLINE TYROSINE KINASE INHIBITOR IN VERY ELDERLY CML PATIENTS: A "CAMPUS CML" STUDY

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Treatment of chronic phase (CP) chronic myeloid leukemia (CML) with tyrosine kinase inhibitors (TKIs) proved to be almost equally effective in young and elderly patients. Three TKIs, imatinib (IM), dasatinib (DAS) and nilotinib (NIL), are approved for frontline therapy in Italy. Choice of frontline TKI is based on a combined evaluation of patient's characteristics and expectations, with age usually playing a prominent role. However, to date, few data are available on patterns of TKI selection in very elderly patients. To analyse the use of frontline TKI therapy in a large and unselected cohort of very elderly CP-CML patients we retrospectively evaluated 300 patients aged ≥75 year diagnosed from 1/2012 to 12/2019 at 31 Hematology Centres participating at the "Cam-

pus CML" project. Clinical features at diagnosis for the whole cohort and according to frontline TKI are reported in Table 1. As to frontline TKI, 260 patients (86.7%) received IM and 40 (13.3%) a 2G-TKI (DAS n=26, 65%; NIL n=14, 35%). Of the 260 IM-treated patients, 179 (68.9%) started with standard dose (400 mg/day) and 81 (31.1%) with a reduced dose (300 mg/day n=58, 22.3%; <300 mg/day n=21, 8.8%). Among the 40 patients starting a 2G-TKIs, 30 (75%) received standard dose and 10 (25%) a reduced dose (NIL <600 mg/day n=2; DAS 80 mg/day n=4 and 50 mg/day n=4). There were no differences between patients treated with imatinib or 2G-TKI (Table 1); only a previous cerebrovascular event had a trend in favour of IM. It is however evident that the distinct toxicity profiles of NIL and DAS had an impact on TKI choice as, for example, no patient with diabetes or ischemic heart disease received NIL. Following widespread introduction of generic IM in Italy in early 2018, patients were divided in 2 groups: among 219 patients diagnosed from 2012 to 2017, 184 (84%) received IM and 35 (16%) a 2G-TKI, while patients diagnosed in 2018-2019 were treated with IM in 76/81 (93.8%) cases and with a 2G-TKI in 5 (6.2%) cases only (p=0.026). IM remains the frontline drug of choice in very elderly CML patients, and this trend seems to increase after the introduction of the generic formulation. However, 2G-TKI are used in a small but sizeable group of patients, without a clear correlation with baseline CML features, thus probably reflecting a physician's evaluation of patient's fitness and/or expectation. Efficacy and safety of initial reduced TKIs doses in the setting of very elderly patients warrant further analyses.

Table 1. Clinical features of the whole cohort and according frontline TKI treatment.

	All patients (300)	Frontline imatinib (260)	Frontline 2G-TKI (40)	p
Gender, M/F (%)	172/128 (57.3 – 42.7)	150/110 (57.7 – 42.3)	22/18 (55.0 – 45.0)	0.749
Median age (years) (IQR)	79.4 (77.1 – 82.7)	79.6 (77.2 – 82.9)	78.9 (76.9 – 81.1)	0.234
Hb, g/dl (IQR)	12.3 (10.9 – 13.7)	12.2 (10.7 – 13.7)	12.9 (11.3 – 14.8)	0.118
WBC, x 10^9/l (IQR)	43.1 (26.7 – 86.7)	46.7 (26.3 – 91.1)	40.8 (30.7 – 63.8)	0.224
PLTS, x 10^9/l (IQR)	360 (222 – 580)	354 (227 – 555)	418 (197 – 676)	0.602
Spleen, n° evaluable (%):	291	254	37	
Not palpable	182 (62.5)	161 (63.4)	21 (56.8)	
< 5 cm below costal margin	88 (30.2)	75 (29.5)	13 (35.1)	0.737
≥ 5 cm below costal margin	21 (7.3)	18 (7.1)	3 (8.1)	
Sokal score, n° evaluable (%):	289	251	38	
Low	13 (4.5)	12 (4.8)	1 (2.6)	
Intermediate	198 (68.5)	173 (68.9)	25 (65.8)	0.695
High	78 (27.0)	66 (26.3)	12 (31.6)	
ELTS score, n° evaluable (%):	279	244	35	
Low	38 (13.6)	30 (12.3)	8 (22.9)	
Intermediate	147 (52.7)	128 (52.5)	19 (54.2)	0.141
High	94 (33.7)	86 (35.2)	8 (22.9)	
Arterial hypertension, n° (%)	202 (67.3)	176 (67.7)	26 (65.0)	0.710
Diabetes, n° (%)	60 (20.0)	52 (20.1)	8 (20.0)	0.991
Previous neoplasm, n° (%)	70 (23.3)	59 (22.8)	11 (27.5)	0.512
COPD, n° (%)	54 (18.0)	45 (7.4)	9 (22.5)	0.440
Ischemic heart disease, n° (%)	40 (13.3)	37 (14.3)	3 (7.5)	0.241
Cerebrovascular events, n° (%)	22 (7.3)	22 (8.5)	0	**0.055**
Concomitant drugs, n° evaluable (%):	284	245	39	
0 - 2	88 (31.0)	72 (29.4)	16 (41.2)	
3 – 5	118 (41.5)	105 (42.9)	13 (33.3)	0.429
> 5	78 (27.5)	68 (27.7)	10 (25.5)	

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IMPACT OF COMORBIDITIES ON THE CHOICE OF FIRST-LINE TYROSINE KINASE INHIBITOR IN CHRONIC PHASE CML: A "CAMPUS CML" ANALYSIS ON OVER 1700 PATIENTS

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The choice of frontline tyrosine kinase inhibitor (TKI) in chronic phase (CP) chronic myeloid leukemia (CML) is based on a combined evaluation of disease and patient's characteristics. The presence of comorbidities is of pivotal importance, as incidence of toxicities of different TKIs are prevalent among patients with specific clinical condition. However, the weight of comorbidities on TKI selection has not been specifically investigated. To analyse the use of frontline TKIs according to concomitant diseases and drug burden we retrospectively evaluated 1752 CP-CML patients that started imatinib (IM), dasatinib (DAS) or nilotinib (NIL) between 1/2012 and 12/2019 at 31 Hematology Centres involved in the national "Campus CML" project. For all patients we recorded comorbidities at the time of CML diagnosis and the number of concomitant drugs taken. Frontline TKI was IM in 964 (55%), DAS in 297 (17%) and NIL in 491 (28%) patients, respectively. Incidence of 6 main comorbidities was recorded: arterial hypertension (AH) (n=692, 39.5%), diabetes (n=199, 11.4%), chronic obstructive pulmonary disease (COPD) (n=137, 7.8%), previous neoplasm (n=239, 13.6%), ischemic heart disease (IHD) (n=123,7%) and cerebrovascular events (CE) (n=49, 2.8%). The relative rates of comorbidities according to the three TKIs are reported in Table 1.

Table 1. Frontline TKI choice according to main comorbidities and number of concomitant drugs.

	Imatinib 964 (55%)	Dasatinib 297 (17%)	Nilotinib 491 (28%)	P (I vs D)	P (I vs N)	P (D vs N)
Arterial hypertension, n° (%):						
YES	475 (49.3)	99 (33.3)	117 (23.8)	**<0.001**	**<0.001**	**0.004**
NO	489 (50.7)	198 (66.7)	374 (76.2)			
Diabetes, n° (%):						
YES	138 (14.3)	51 (17.2)	10 (2.0)	0.446	**<0.001**	**<0.001**
NO	826 (85.7)	246 (82.8)	481 (98.0)			
Chronic obstructive pulmonary disease, n° (%):						
YES	100 (10.4)	18 (6.1)	19 (3.9)	0.076	**<0.001**	0.159
NO	864 (89.6)	279 (93.9)	472 (96.1)			
Previous neoplasm, n° (%):						
YES	172 (17.8)	32 (10.8)	35 (7.1)	**0.014**	**<0.001**	0.073
NO	792 (82.2)	264 (89.2)	456 (92.9)			
Ischemic heart disease, n° (%):						
YES	106 (11.0)	13 (4.4)	4 (0.8)	**0.003**	**<0.001**	**0.001**
NO	858 (89.0)	284 (95.6)	487 (99.2)			
Cerebrovascular events, n° (%):						
YES	45 (4.7)	2 (0.7)	2 (0.4)	**0.006**	**<0.001**	0.611
NO	919 (45.3)	295 (99.3)	489 (99.6)			
Concomitant drugs, n° evaluable (%):	885	292	484			
0	222 (25.1)	139 (47.6)	282 (58.2)			
1 – 2	241 (27.2)	75 (25.7)	144 (29.8)	**<0.001**	**<0.001**	**<0.001**
3 – 5	255 (28.8)	50 (17.1)	50 (10.3)			
>5	167 (18.9)	28 (9.6)	8 (1.7)			

Compared to NIL, all comorbidities were significantly more frequent in patients receiving IM; compared to DAS, rates of AH, previous neoplasm, IHD and CE were higher for IM, while no difference was reported for diabetes and COPD. Compared to NIL, the rates of AH, diabetes and IHD were significantly higher for DAS. Among 1663 patients evaluable for concomitant medications, 644 (38.7%) took no drugs, 460 (27.7%) took 1-2 drugs, 356 (21.4%) took 3-5 drugs and 203 (12.2%) took ≥6 drugs. A higher burden of co-medications was recorded in the IM cohort compared to 2G-TKIs (p<0.001), but also for DAS compared to NIL (p<0.001). Our data highlight the role of comorbidities and concomitant

drugs on frontline TKI choice in the real-world. Patients treated with 2G-TKIs had significantly fewer comorbidities and concomitant therapies than those receiving IM, thus making comparisons on toxicity and efficacy difficult. Among 2G-TKIs, burden of concomitant diseases and medications was bigger in patients receiving DAS than NIL. Further correlations with other features (e.g. age) are warranted to define the relative weight of comorbidities on TKI choice.

P76

GENETIC AND EPIGENETIC MECHANISMS REGULATING CATALASE EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is an incurable disease characterized by an extremely variable clinical course. Along with the understanding of the molecular heterogeneity of the disease, growing interest is emerging in redox metabolism in CLL. We have recently documented a differential catalase expression in CLL associated with divergent clinical behaviors. However, the mechanisms controlling the transcription of catalase gene are poorly understood. The main objective of this study is to investigate regulatory mechanisms underlying differential expression of catalase in CLL. We investigated the role of the rs1001179 SNP and methylation levels of the catalase promoter on catalase expression in primary CLL cells, using RFLP-PCR and pyrosequencing. Catalase expression has been assessed using qPCR and flow cytometry. The rs1001179 SNP genotyping shows that CLL cells harboring the T allele exhibit a significantly higher catalase expression compared with cells bearing the CC genotype. Moreover, we show that methylation of catalase promoter influences catalase expression. First, CLL cells exhibit lower methylation levels compared with healthy donor (HD) B cells, in line with the higher catalase mRNA and protein levels expressed by CLL in comparison with HD B cells. Then, the methylation levels at specific CpG sites negatively correlate with the catalase gene expression level in CLL cells. Remarkably, the role of promoter methylation in regulating catalase expression was functionally validated inhibiting the activity of methyltransferase in primary CLL cells, using 2'-deoxy-5-azacitidine (DAC). Treatment of leukemic cells with DAC induces a significant increase in catalase gene expression, thus showing that DNA methylation controls catalase expression in CLL. Finally, we investigated the relationship between the genetic and epigenetic regulatory levels, in controlling catalase expression using a mathematical linear model. Remarkably, the rs1001179 T allele and methylation interact in regulating catalase gene expression, thus indicating that the CT/TT genotypes show a lower methylation levels and a higher catalase gene expression level. The key result of this study is to provide new insights into the knowledge of genetic and epigenetic mechanisms at the basis of differential expression of catalase in CLL, which could be of crucial relevance for the development of therapies targeting redox pathways. We thank Gilead for funding support.

P77

PREGNANCY OUTCOME IN WOMEN WITH ESSENTIAL THROMBOCYTHEMIA: A 30 YEAR SINGLE-CENTER EXPERIENCE

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Background: Obstetric complications are common in ET women. There is a higher risk than in the general population of early pregnancy loss and fetal growth retardation (FGR) (respectively 10–15% and 5%). Furthermore, the live birth rate ranged from 50% to 70%. A variety of therapeutic strategies has been proposed, with a risk-driven intensity of treatment. Still, there is a lack of an international agreement for the management of women during pregnancy.

Aim: This study aims to describe our monocenter experience rate of obstetrics complications in ET patients.

Patients and Methods: We recorded 110 pregnancies in 61 ET women (median age 34.5 years) from 1990 to 2020 (1.8 pregnancies per woman). Eight pregnancies were excluded from our analysis for lack of data. We detected JAK2 mutation in 30 women (49.2%), CALR in 7 (11.5%), CMPL in 4 (6.5%). Antepartum prophylaxis consisted of aspirin (ASA) in 25 pregnancies, low molecular weight heparin (LMWH) in 22, both in 37; 18 pregnancies were untreated. Cytoreduction with interferon was needed only in 17 pregnancies.

Results: Among the evaluable pregnancies, 74 pregnancies (72.5%) ended up with live childbirth. The rate of miscarriages was 24.5% (n=25), and stillbirth 1.9% (n=2). In 1 case (0.9%), a newborn died few hours after birth. Other obstetric complications occurred in 23 pregnancies (22.5%). FGR was the most frequent (8.8%). We registered 3 thrombotic events, 1 during pregnancy (TIA during ASA) and 2 during puerperium (1 DVT of the leg during LMWH, and 1 cerebral vein thrombosis without). The rate of live births using for at least 6 months ASA or LMWH or both was 80% (20/25), 77% (17/22), and 84% (31/37), respectively. Overall the rate of live births was 81% (68/84) using antithrombotic prophylaxis and 33% (6/18) without (odds ratio, OR, 0.11, 95%CI 0.03-0.36, p=0.0002). Logistic regression showed that the OR of live birth was 3.49-fold (95%CI 1.37-8.89, p=0.008) using ASA and 2.92 (95%CI 1.14-7.43, p=0.02) using LMWH; age >35 years, JAK2 V617F mutation, a history of thrombosis, and use of interferon did not affect the probability of live birth. We recorded 5 non-fatal peripartum bleeding events during LMWH and 1 without (OR 1.07, 95%CI 0.11-9.8, p=0.98).

Conclusions: Antithrombotic prophylaxis in ET pregnant women reduces the probability of fetal loss by 89% without a significant increase in bleeding. The pregnancy outcome is not influenced by the JAK2 mutational status either by the use of interferon.

P78

TREATMENT OF CLL RELAPSED/REFRACTORY PATIENTS IN COVID-19 PANDEMIC: A REAL-LIFE EXPERIENCE WITH VENETOCLAX-RITUXIMAB COMBINATION IN SOUTHERN ITALY

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To evaluate incidence and severity of COVID-19 cases in a well-defined cohort of patients with CLL receiving venetoclax-based combinations a questionnaire was sent to a cohort of CLL treating hematologists from hematological centers in southern Italy. Participants were asked: a) to indicate whether they had offered a test for detection of COVID-19 infection (mainly nasopharyngeal swabs) only to CLL patients who reported symptoms or universally; b) to provide information on the incidence of COVID-19 infection and its severity; c) to specify reasons of possible treatment modifications. The survey was restricted to R/R CLL patients treated from Feb 1st to Dec 31th 2020 with time-limited venetoclax/rituximab (VR) combination as recommended by MURANO protocol (venetoclax for up to 2 years plus rituximab for the first 6 months), within their clinical practice. A specific questionnaire was sent to 30 CLL hematologists. Twenty-six responded but only 24 declared to have treated at least one patient with VR combination in the observation period. Of those, 20.8% worked in academic hospitals. Overall, the survey allowed to collect data on 124 patients. The median number of patients treated in each center was 5 (range,1-15). COVID-19 surveillance based on viral RNA RT-PCR on nasopharyngeal swabs was performed in most patients before beginning the ramp-up with venetoclax (66.9%) and before each rituximab infusion (53.2%). Adherence to treatment was relatively high (70.8%). Only 29.1% physicians modified the therapeutic program mainly because of grade 3 neutropenia. Changes consisted of transient interruption of venetoclax, reduction of doses, and delay of rituximab infusion. Only 2/124 patients (1.6%) had a symptomatic RT-PCR proven diagnosis of COVID-19 infection and required hospitalization. Both patients needed oxygen therapy and admission into an intensive care unit. Of those, 1 patient who was receiving VR combination at the time of COVID infection, eventually died. The second patient developed COVID-19 infection while receiving venetoclax monotherapy. He recovered from COVID-19 infection and after 21 days of treatment interruption, he was able to restart venetoclax. This survey, performed on a large number of CLL patients treated with VR combination in real-world clinical practice provides relevant information on safe treatment of CLL with a venetoclax-based regimen during the COVID19 pandemic.

P79

MANAGEMENT OF ANEMIA IN MYELOFIBROSIS: A MULTICENTER REAL-LIFE EXPERIENCE WITH BIOSIMILAR ERYTHROPOIESIS STIMULANTING AGENTS

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Background: Erythropoiesis stimulating agents (ESA) are a useful treatment for anemia in many hematological malignancies. However, the role of ESA, especially biosimilar ESA, in myelofibrosis (MF) is not well established.

Aims: To evaluate efficacy and safety of biosimilar epoetin (B-ESA) alpha and zeta in management of anemia in MF patients (pts).

Methods: We retrospectively evaluated pts with MF from 4 Italian Centres who received B-ESA for at least 1 month to treat anemia. Anemia response (AR) was defined as Complete Response (CR) according to the International Working Group criteria (Tefferi et al,2013) or as Partial Response (PR) in case of transfusion decrease of >50% or sustained Hb increase between 1-2 g/dL in transfusion independent pts (Cervantes et al,2004). All other cases were included in Non Responder (NR) group.

Results; We included 79 pts (41 males, 38 females) affected by primary (44) or secondary (35) MF treated with B-ESA (50 alpha, 29 zeta) from 2009 to 2021. At B-ESA start (baseline) median age was 75 years (range 39-92), median endogenous erythropoietin (EPO) level was 44 U/L (range 7-1742), median Hb was 9 g/dL (range 7-10) and 15 pts (19%) were transfusion dependent. AR was observed in 62 pts (78%) with 49 CR (62%) and 13 PR (16%), 17 pts were NR (22%), 6 pts (9.7%) lost response after a median time of 12.3 months (range 4-17). Median time to response and median exposition time to B-ESA were 2.2 months (range 1-17) and 15.4 months (range 1-107), respectively. In univariate analysis significant predictors of response at baseline were transfusion independency (Fisher's exact test, p<0.001), EPO<50 U/L (p=0.028), ferritin<200 ng/mL (p=0.002) and Hb>8.5 g/dL (p=0.004). After a median follow-up of 40.3 months (range 4-338) from diagnosis and 19.1 months (range 1-107) from baseline, 33 pts (42%) died, 10 of them (13%) for leukemic evolution. Only 2 pts (2.5%) stopped B-ESA for toxicity, 1 of whom for pulmonary embolism. Median survival from baseline was significantly affected by transfusion dependency (59.4 *vs* 14.9 months in transfusion independent *vs* dependent pts at baseline, p=0.0014, Figure 1) and response to B-ESA (58.4 months in AR *vs* 14.4 in NR group, p=0.07).

Conclusion: B-ESA seem to be an effective and well-tolerated option for anemia treatment in MF setting. Transfusion independent pts show a significant survival advantage compared to transfusion dependent pts, suggesting the possibility of better outcome with an early B-ESA treatment.

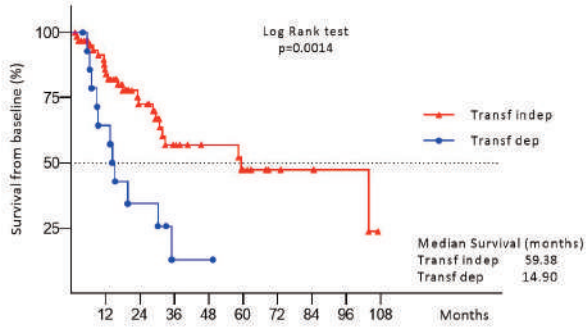

Figure 1 – Survival from baseline in pts treated with B-ESA who were transfusion independent vs dependent at start of B-ESA

Figure 1.

P80

TYROSINE KINASE INHIBITORS (TKIS) DOSE REDUCTION IN CHRONIC PHASE–CHRONIC MYELOID LEUKEMIA (CP-CML) PATIENTS (PTS) CAN BE SAFE AND DOES NOT PRECLUDE THE POSSIBILITY OF ACHIEVING A MAJOR MOLECULAR RESPONSE (MMR) AND ENTERING TREATMENT FREE REMISSION (TFR)

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TKIs in CP-CML pts allow a near normal life expectancy; hence it becomes crucial to avoid adverse events due to therapy with potential significant morbidity and mortality. In this landscape, TKI dose reduction may be considered to optimize treatment strategy. We analyzed the impact of TKI dose reduction in 195 CP-CML pts with a minimum follow up (f.u.) of 12 months (mos), treated in our center, divided into 2 groups: pts receiving full dose TKI (FDT) and pts receiving reduced dose TKI (RDT) at least in the 6 mos before the last f.u. or, for pts in TFR, in the 6 mos preceding TKI discontinuation. RDT pts received imatinib < 400 mg/d, nilotinib < 300 mg BID (or < 400 BID for 2nd line therapy), bosutinib < 500 mg/d, ponatinib < 45 mg/d.

Results: 148 pts, including 31 in molecular relapse after TFR, are currently on TKI, with a median f.u. of 91 (12-397) mos from diagnosis. Of them 74 receive FDT (22/74 after TFR failure), 74 RDT (9/74 after TKI failure), mostly due to side effects during FDT. Forty six pts are in TFR, with a median f.u. of 58 (3-86) mos from TKI discontinuation: 13/46 (28%) pts received RDT for at least 6 mos before TKI stop. One patient didn't resume TKI after TFR failure because of renal failure. We analyzed different parameters in the 2 groups (including in each group both pts currently treated and pts in TFR): in the RDT group, age at the last f.u. is significantly higher (71 *vs* 58 years, p 0.00018) while median f.u., type of transcript, Sokal risk score, type of 1st line TKI (imatinib *vs* 2nd generationTKI) don't differ in the 2 groups. Considering the 148 pts currently on TKI, the number of pts in deep molecular response (DMR) at the last f.u. is significantly lower (p 0.01) in RDT group but the number of pts with MMR and with molecular response < MMR don't differ between RDT and FDT pts. Overall, 77 pts followed in our center attempted TKI discontinuation, median f.u. 56 (3-123) mos from discontinuation, and 46/77 (59%) are presently in TFR. Of these 77 pts, 22 (28%) assumed RDT for at least 6 mos before TKI stop. The reduced TKI dosage didn't significantly influence TFR duration.

In conclusion, reduced TKI dose treatment in selected pts, chiefly in the elderly, can be safe and seems not to influence the MMR achievement that is the goal to offer a normal life expectancy. A RDT could reduce, but not preclude, a DMR achievement as well as the possibility of a TKI discontinuation but doesn't seem to influence the TFR duration.

PUBLISHED ONLY

Anemia and erythrocyte disorders

D001

ACTIVATE: A PHASE 3, RANDOMIZED, MULTICENTER, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF MITAPIVAT IN ADULTS WITH PYRUVATE KINASE DEFICIENCY WHO ARE NOT REGULARLY TRANSFUSED

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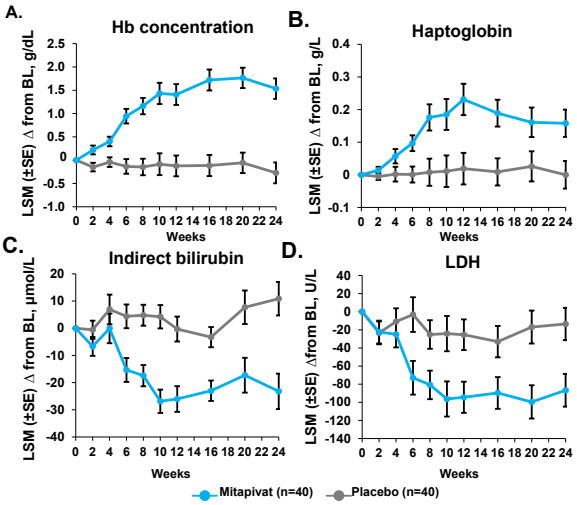
Pyruvate kinase (PK) deficiency is a rare hereditary disease caused by reduced red blood cell PK (PKR) enzyme activity leading to defective glycolysis and hemolytic anemia. ACTIVATE (NCT03548220) evaluated the efficacy and safety of mitapivat, an investigational, first-in-class, oral, allosteric activator of PKR, in adult patients (pts) with PK deficiency who were not regularly transfused. It was a phase 3, randomized, double-blind, placebo (PBO)-controlled study. A 12-week (wk) dose escalation (5, 20, 50 mg BID) period was followed by a 12-wk fixed-dose period.

Figure: Hb and hemolysis marker outcomes for mitapivat vs placebo[a]



[a]Difference in LSM for secondary endpoints comparing BL to average change at Weeks 16, 20, and 24; p-values are based on the MMRM method: Hb concentration, p<0.0001; haptoglobin, p=0.0079; indirect bilirubin, p<0.0001; LDH, p=0.0027;. BL=baseline; Hb=hemoglobin; LDH=lactate dehydrogenase; LSM=least squares mean; MMRM=mixed-effect model repeated measure; SE=standard error

Figure 1.

Primary endpoint was hemoglobin (Hb) response, defined as ≥1.5 g/dL increase from baseline (BL), sustained at ≥2 scheduled visits at Wks 16, 20, or 24. Secondary endpoints were prespecified: change from BL in Hb concentration, indirect bilirubin, reticulocyte %, lactate dehydrogenase (LDH), haptoglobin, and PK deficiency diary (PKDD) and PK deficiency impact assessment (PKDIA). 80 pts were randomized (mitapivat n=40; PBO n=40); mean age 36 *vs* 37, 40% *vs* 40% male, mean BL Hb 8.6 *vs* 8.5 g/dL. The primary endpoint was met, with 16 mitapivat pts (40%) achieving a sustained Hb response *vs* 0 PBO pts (p<0.0001). Secondary endpoints were met, including significant improvements with mitapivat compared with PBO in BL Hb, hemolysis (figure), and PROs: average change from BL (difference in least squares mean [95% CI]) in Hb concentration, 1.8 g/dL (1.2, 2.4; p<0.0001), indirect bilirubin, –26.26 µmol/L (–37.82, –14.70; p<0.0001), reticulocyte %, –0.1011 (–0.1391, –0.0632; p<0.0001), LDH, –70.81 U/L (–115.88, –25.74; p=0.0027), haptoglobin 0.158 g/L (0.043, 0.273; p=0.0079), and PKDD –3.11 (–5.80, –0.41; p=0.0247) and PKDIA –3.25 (–6.39, –0.12; p=0.0421). Treatment-emergent adverse events (TEAEs) occurred in 35 pts in each arm. The most common TEAEs with mitapivat were nausea and headache, which were less frequent for mitapivat *vs* PBO (n=7; 17.5% *vs* n=9; 23.1% and n=6; 15.0% *vs* n=13; 33.3%). TEAEs grade ≥3 occurred in 10 (25.0%) mitapivat *vs* 5 (12.8%) PBO pts. The most common TEAEs grade ≥3 with mitapivat were hypertriglyceridemia and hypertension (both n=2; 5.0%). No TEAEs led to discontinuation. ACTIVATE is the first PBO-controlled study in PK deficiency. Primary and secondary endpoints were met, indicating clinically meaningful benefit for pts treated with mitapivat. No new safety signals were identified. Mitapivat has the potential to be the first disease-modifying drug therapy approved for PK deficiency.

D002

ABSTRACT WITHDRAWN

D003

THE ORAL COMPLEMENT FACTOR B INHIBITOR IPTACOPAN IS SAFE AND EFFECTIVE IN IMPROVING HEMATOLOGICAL RESPONSE IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS WITH POOR RESPONSE TO ECULIZUMAB, EVEN IN MONOTHERAPY

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Background: The hematological benefit of paroxysmal nocturnal hemoglobinuria (PNH) with anti-C5 treatment is limited by residual intravascular hemolysis (IVH) and/or emerging C3-mediated extravascular hemolysis (EVH). Therefore, the aim of this phase 2 study was to assess the safety, tolerability, pharmacokinetic/-dynamic and efficacy of the new complement factor B inhibitor (iptacopan) in PNH patients with active hemolysis despite anti-C5 therapy.

Methods: This is a multi-center, open-label phase 2 trial [NCT03439839] enrolling adult PNH patients who showed signs of active hemolysis despite receiving eculizumab treatment. For enrollment, patients were required to demonstrate lactate dehydrogenase (LDH) >1.5 ULN and a PNH Type III erythrocyte or granulocyte clone size >10%. Iptacopan was given orally as add-on therapy at a dose level of 200 mg BID. The primary endpoint was the effect of iptacopan on the reduction of hemolysis measured as change in LDH from baseline (BL) value to Week 13. At 13 weeks patients could enter into a long-term study extension (ongoing), allowing discontinuation of eculizumab.

Findings: In the ten patients enrolled, iptacopan was well tolerated. There were no fatal events and no treatment-related serious adverse events (SAE) during the core study. At Week 13, iptacopan resulted in

marked reduction of LDH (mean ±SD 539±263 *vs* 235±44 IU/L; p=0.008), associated with significant improvement of Hb levels (mean ±SD 97.7±10.5 *vs* 129.5±18.3 g/L; p<0.001). All but 2 patients achieved Hb levels >12 g/L. All biomarkers of hemolysis improved on iptacopan treatment, including bilirubin (mean±SD 12.6±4.8 *vs* 36.3±14.7 µmol/L; p<0.001), and reticulocyte count (mean±SD 80.1±32.9 *vs* 194.1±71.2 x10^9/L; p<0.001). All patients experienced complete abrogation of C3 deposition during iptacopan treatment (mean±SD 0.18±0.12% *vs* 20.5±15.4%; p=0.007) and marked increase of the size of PNH erythrocyte population (mean±SD 79.8±41.3% *vs* 37.7±24.8), consistent with a full prevention of EVH. Seven patients stopped eculizumab and continued iptacopan as monotherapy for at least 3 months, with no change in any laboratory value (LDH, Hb, bilirubin, reticulocyte count, C3 deposition and PNH erythrocyte population).

Conclusions: Iptacopan is a new oral factor B inhibitor that blocks both, intra- and extra-vascular hemolysis in patients with hemolytic PNH; current phase 3 trials may establish it as new standard of care for PNH.

D004

INHIBITION OF COMPLEMENT C1S WITH SUTIMLIMAB IN PATIENTS WITH COLD AGGLUTININ DISEASE (CAD): INTERIM RESULTS OF THE PHASE 3 CARDINAL STUDY LONG -TERM FOLLOW-UP

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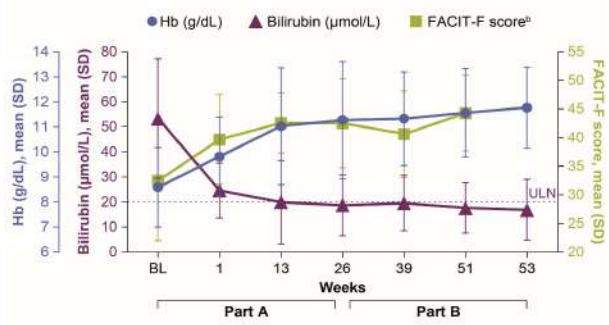
Introduction: CAD is a rare autoimmune hemolytic anemia characterized by classical complement pathway–mediated chronic hemolysis, anemia and fatigue as well as increased risk for thromboembolism and early mortality. Sutimlimab is a first-in-class humanized monoclonal antibody that selectively targets the classical complement pathway at C1s, while leaving the lectin and alternative pathways intact. The CARDINAL study (NCT03347396) evaluated the efficacy and safety of sutimlimab in adults with CAD and a recent history of transfusion. Here we present the interim 1-year long-term follow-up data from CARDINAL.

Methods: CARDINAL is a Phase 3, open-label, single-arm, multicenter study with a 26-week treatment period (Part A) and an ongoing 2-year extension (Part B). Key inclusion criteria included confirmed CAD diagnosis, baseline hemoglobin (Hb) ≤10 g/dL, and ≥1 red blood cell transfusion in the previous 6 months. Sutimlimab (<75 kg or ≥75 kg with a dose of 6.5 g or 7.5 g) was administered intravenously on days 0 and 7, then every two weeks. This interim analysis up to 1 year (data cut: January 16, 2020) evaluates safety and tolerability (primary objectives), with change in hemolytic anemia markers and the FACIT-Fatigue scale score (efficacy endpoints).

Results: At Part A baseline, 24 patients (mean age 71.3 years; 62.5% female) had a mean Hb of 8.6 g/dL and received a median of 2 transfusions <6 months prior to enrollment. All patients who completed Part A (n=22) enrolled in Part B. Sutimlimab treatment lead to rapid and sustained improvements in hemolytic anemia (mean Hb >11 g/dL from Weeks 5–53; mean bilirubin <20 µmol/L from Weeks 3–53), and improvement in QOL (FACIT-Fatigue score >40 from Weeks 3–51), all of which coincided with near-complete classical pathway inhibition. From Week 5 to 26 and Week 26 to 53, 17 (70.8%) and 19 (86.4%) patients remained transfusion-free, respectively. Across the entire study period, all 24 patients experienced ≥1 treatment-emergent adverse event (TEAE) and 12 patients experienced a serious TEAE. One serious TEAE (viral infection) was assessed as sutimlimab-related by the investigator. No meningococcal infections were reported.

Conclusions: The 1-year interim results of the ongoing CARDINAL long-term study demonstrates that continued inhibition of the classical complement pathway upstream at C1s with sutimlimab provides sustained and durable treatment effects in patients with chronic CAD.

Figure 1. Mean (SD) for Hb, bilirubin, and FACIT-F following sutimlimab treatment[a]. The main treatment period (Part A) was from Week 1 to Week 26 and continued into the long-term follow-up period (Part B)



	Number of patients at visit (%)						
	Baseline	Week 1	Week 13	Week 26	Week 39	Week 51	Week 53
Hb (n=24)	24 (100.0)	24 (100.0)	21 (87.5)	20 (83.3)	22 (91.7)	19 (79.2)	16 (66.7)
Bilirubin (n=21)	21 (100.0)	20 (95.2)	19 (90.5)	17 (81.0)	13 (61.9)	16 (76.2)	19 (90.5)
FACIT-F (n=24)	22 (91.7)	21 (87.5)	21 (87.5)	17 (70.8)	20 (83.3)	18 (75.0)	NR

Dotted line indicates the ULN for bilirubin (20 µmol/L).
BL, baseline; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; Hb, hemoglobin; NR, not reported; SD, standard deviation; ULN, upper limit of normal.
[a]BL (Week 0) is defined as the last non-missing value prior to the first administration of the study drug.
[b]FACIT-F data were not reported at Week 53.

Figure 1.

D005

BASELINE CHARACTERISTICS BY AGE OF A GLOBAL COHORT OF PATIENTS DIAGNOSED WITH PYRUVATE KINASE DEFICIENCY – A DESCRIPTIVE ANALYSIS FROM THE PEAK REGISTRY

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Pyruvate kinase (PK) deficiency is the most common hereditary red cell glycolytic enzyme defect leading to lifelong hemolytic anemia. This descriptive analysis aimed to characterize the clinical manifestations and disease management strategies for the pediatric (<18 years [yrs]) and adult cohort (≥18 yrs) with PK deficiency enrolled in the Peak Registry (NCT03481738). The Peak Registry is an ongoing, global prospective and retrospective study of adult and pediatric patients (pts) diagnosed with PK deficiency. Demographic, medical history, laboratory, and treatment data were collected from eligible pts enrolled in the Registry as of the latest data cut. A total of 140 pts (56 pediatric and 84 adult), with non-missing data at time of enrollment, were included in this analysis. Mean age of participants at enrollment was 7.8 yrs (SD 4.6) for the pediatric cohort *vs* 37.4 yrs (SD 15.5) for adults. Hemoglobin levels (median [range]) were 8.4 g/dL (5.8–12.3) in the pediatric cohort and 9.5 g/dL (6.7–12.9) in adults. Ferritin levels (median [range]) in the pediatric cohort were 772 ng/mL (78–2499) and 404 ng/mL (19–2263) in adults. History of chelation therapy was 50.0% (0–5 yrs), 54.5% (6–11 yrs), and 63.6% (12–17 yrs) in pediatric subgroups and 30.6% in adults. The median age at splenectomy for pediatric and the adult groups were at 5 yrs (2–12 yrs) and 6 yrs (1–27 yrs), respectively. The higher frequency of splenectomy (0% [0–5 yrs], 52.2% [6–11 yrs], 61.5% [12–17 yrs]) with increasing age across pediatric cohorts matches a decreased frequency in those who were treated with regular transfusions (≥6 transfusions within 1 yr prior to enrollment), 46.7% (0–5 yrs), 14.3% (6–11 yrs), and 10.0% (12–17 yrs). The frequency of splenectomy and regular transfusions was similar between the 6–17 yrs old cohort (55.6% and 12.9%) and the adult cohort (51.3% and 9.4%). This analysis provides early insight into PK deficiency disease manifestations, confirming that complications start early on, with pediatric pts experiencing significant anemia and treatment with transfusions and chelation before age 6. Despite the high rate of splenectomy in this cohort, many children and adults continue to have substantial anemia and disease burden. The Registry will continue to collect data in pts to better understand the clinical manifestations and complications of PK deficiency over time.

Table 1.

	Overall Population		Pediatric Subgroups		
	Adult, ≥18 yrs N=84	Pediatric, <18 yrs N=56	0–5 yrs N=19	6–11 yrs N=24	12–17 yrs N=13
Demographic and Medical History					
Gender, N	84	56	19	24	13
Female, n (%)	48 (57.1)	30 (53.6)	11 (57.9)	11 (45.8)	8 (61.5)
Age at diagnosis, N	76	51	18	22	11
Median (range), yrs*	16 (0–68)	1 (–1–11)	0 (–1–2)	0.5 (–1–11)	4 (–1–11)
Genotype, N	65	27	8	11	8
Missense/Missense, n (%)	41 (63.1)	11 (40.7)	2 (25.0)	5 (45.5)	4 (50.0)
Missense/Non-missense, n (%)	21 (32.3)	11 (40.7)	5 (62.5)	4 (36.4)	2 (25.0)
Non-missense/Non-missense, n (%)	3 (4.6)	5 (18.5)	1 (12.5)	2 (18.2)	2 (25.0)
Splenectomy history available, N	80	54	18	23	13
Ever received splenectomy n (%)	41 (51.3)	20 (37.0)	0 (0)	12 (52.2)	8 (61.5)
Age at splenectomy, N	38	19	0	12	7
Median (range), yrs	6 (1–27)	5 (2–12)	–	5 (2–10)	6 (4–12)
Chelation therapy history available, N	72	51	18	22	11
Ever had chelation therapy, n (%)	22 (30.6)	28 (54.9)	9 (50.0)	12 (54.5)	7 (63.6)
Transfusions history available, N	76	54	18	23	13
Ever transfused, n (%)	48 (63.2)	50 (92.6)	17 (94.4)	22 (95.7)	11 (84.6)
Transfusion history 12 months prior to enrollment, N	84	46	15	21	10
Regularly transfused pts (≥6 transfusions), n (%)	6 (9.4)	11 (23.9)	7 (46.7)	3 (14.3)	1 (10.0)
Number of transfusions, mean (SD)	9.2 (2.8)	9.5 (3.1)	10.0 (3.6)	8.7 (3.1)	9.0 (–)
Non-regularly transfused pts (never or 0–5), n (%)	58 (90.6)	35 (76.1)	8 (53.3)	18 (85.7)	9 (90.0)
Number of transfusions, mean (SD)	0.4 (1.1)	0.9 (1.5)	1.1 (1.9)	1.0 (1.5)	0.7 (1.3)
Lab Parameters					
Hemoglobin, N	38	38	12	16	8
Median (range), g/dL	9.5 (6.7–12.9)	8.4 (5.8–12.3)	8.6 (5.8–12.3)	8.3 (7.1–10.9)	8.2 (6.8–11.4)
Percent reticulocyte count, N†	17	13	5	6	2
Median (range), %	5.3 (2.6–40.7)	9.3 (2.2–42.5)	3.4 (2.2–29.1)	25.3 (3.6–42.5)	24.1 (13.4–34.8)
Indirect bilirubin, N	25	18	6	9	3
Median (range), mg/dL	3.3 (0.8–23.1)	3.1 (1.4–12.9)	3.4 (1.4–3.9)	2.9 (1.5–12.9)	3.9 (2.9–8.2)
Lactate dehydrogenase, N†	22	12	3	5	4
Median (range), U/L	225 (120–849)	654 (135–2049)	710 (552–2949)	566 (206–1551)	677 (135–1798)
Ferritin, N	26	16	6	6	4
Median (range), ng/mL	404 (19–2263)	772 (78–2499)	847 (123–2000)	430 (78–905)	1474 (264–2499)

N, represents number of patients with data available
*If age at diagnosis denotes 'presumably diagnosed in utero' then entered '-1' to indicated duration: yrs-year

D006

EARLY-ONSET OSTEOPENIA AND OSTEOPOROSIS IN PATIENTS WITH PYRUVATE KINASE DEFICIENCY

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Hereditary pyruvate kinase (PK) deficiency results in lifelong hemolytic anemia and several significant comorbidities. Among these is reduced bone mineral density (BMD), which can result in premature osteopenia, osteoporosis, and fractures. This study evaluated the prevalence of BMD abnormalities in pooled pre-treatment baseline data from 3 clinical trials involving patients (pts) with PK deficiency investigating mitapivat, an allosteric activator of PK: DRIVE-PK (NCT02476916), ACTIVATE (NCT03548220), and ACTIVATE-T (NCT03559699). This is the first large PK deficiency cohort in which dual-energy x-ray absorptiometry (DXA) scores were systematically assessed. All 3 studies included pts ≥18 years (yrs) of age with a confirmed diagnosis of PK deficiency. Pts were eligible for DRIVE-PK and ACTIVATE if they were not regularly transfused and ACTIVATE-T if they were regularly transfused. BMD was measured using DXA at baseline, and osteopenia and osteoporosis were identified on DXA according to standard definitions. Of 159 pts evaluated (DRIVE-PK, n=52; ACTIVATE, n=80; ACTIVATE-T, n=27), median age was 34 yrs and majority were female (55.3%). Of 155 pts who had baseline T-scores for total femur, spine, and femoral neck, 38 (24.5%) had a T-score of ≥–1.0 at all locations, indicating normal BMD; 91 (58.7%) had a T-score of >–2.5 to <–1.0 at ≥1 locations, indicating osteopenia; and 26 (16.8%) had a T-score of ≤–2.5 at ≥1 locations, indicating osteoporosis. The proportion of pts in each T-score range for the 3 locations is shown in the Figure 1. In contrast to the DXA findings, only 28 (17.6%) pts had a known medical history of osteopenia and 23 (14.5%) had a known medical history of osteoporosis. Taking together DXA results and medical history for all 159 pts, 85 (53.5%) had osteopenia and 33 (20.8%) had osteoporosis. Median age for pts with osteopenia or osteoporosis (n=118) was 36 yrs (range, 18–78). Of these, 20 pts (16.9%) were regularly transfused and 98 pts (83.1%) were not regularly transfused. In this cohort, universal DXA revealed that >75% of adults with PK deficiency had osteopenia or osteoporosis, irrespective of transfusion requirements. Given the young median age of the cohort (34 yrs), findings have considerable significance and implications for the screening and care of pts with PK deficiency throughout their adult lives. Early monitoring with DXA to ensure a prompt diagnosis of bone density abnormalities and indicated treatment may be warranted.

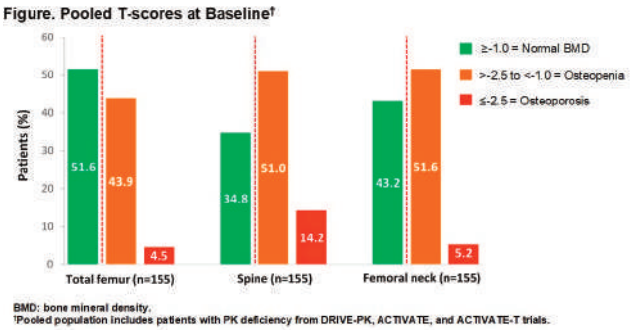

Figure 1.

D007

A NEW SUSPECTED CAUSE OF ERYTHROCYTOSIS: EPAS1 MUTATIONS ASSOCIATED WITH MUTATIONS IN OTHER GENES

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Introduction: Patients with Idiopathic Erythrocytosis (IE) exhibit persistently elevated hemoglobin (Hb) and hematocrit (Ht) and variable serum erythropoietin (EPO) levels. Mutations of the genes of the oxygen sensing pathway may cause an erythrocytosis. While mutations in *VHL* and *EGLN1* are relatively common, *EPAS1* mutations are rarely found in erythrocytotic patients: in two families, carrying *EPAS1* G537R mutation, erythrocytosis was present, associated with normal EPO level. Other mutations in *EPAS1* were described but at present not correlated to erythrocytosis.bRecent studies underlined that mutations in *HFE* gene are present in some IE patients, even if its relation with erythrocytosis is not clearly defined. We report *EPAS1* mutations associated with other molecular alterations in erythrocytotic patients evaluated with an ad hoc Next Generation Sequencing (NGS) panel.

Methods: We studied 118 sporadic patients with IE in whom primary and secondary acquired causes of erythrocytosis were excluded. Our NGS panel evaluate all the exonic parts of fifteen genes: *JAK2*, *EGLN1*, *EPOR*, *FTL*, *FTH*, *ASXL1*, *HFE*, *HFE2*, *TFR2*, *HAMP*, *SLC40A1*, *SLC11A2*, *VHL*, *BPMG*, and *EPAS1*. Bioinformatics tools analyzed data and all the mutations found were validated with Sanger Sequencing.

Results: In 80 (67.8%) patients (Hb 148-191 g/L, Ht 50-54%) we found at least one germline mutation: 55 patients have only 1 mutation and 25 have 2 to 4 mutations. Six males (7.4%) carry a missense mutation in the *EPAS1* gene (4 F374Y, 1 T766P and 1 R550W) (Table 1); 2 of these patients have associated *EGLN1* C127S, 3 heterozygous *HFE* H63D mutation and 1 compound *HFE* H63D/C282Y. Finally, one has a germline *JAK2* G48E mutation. All these patients had normal serum EPO levels.

Conclusions: Our NGS panel investigates a number of genes and found patients with more than one gene mutated. The *EPAS1* mutations have yet been described but their pathogenic effect is still now unknown. We speculate that *EPAS1* molecular alterations may become clinically significant in association with another one, being in 2/3 of cases a *HFE* mutation. Interestingly, in two cases the *EPAS1* associated mutation is Tibetan *EGLN1* considered a key condition of Tibetans adaptation to altitude, but whose functional role is unclear still now. We conclude that NGS study of larger number of patients with erythrocytosis will help in identifying the molecular causes of more patients with IE.

Table 1.

PATIENT	SEX	AGE [Years]	EPO [mU/mL]	MUTATIONS
A	Male	43	5.5	*EPAS1* F374Y + *JAK2* G48E
B	Male	41	22.1	*EPAS1* T766P + *EGLN1* C127S
C	Male	58	4.7	*EPAS1* R550W + *HFE* C282Y
D	Male	20	33.5	EPAS1 F374Y + *HFE* H63D
E	Male	60	-	*EPAS1* F374Y + *HFE* H63D + *EPOR* N487S
F	Male	64	7.4	*EPAS1* F374Y + *EGLN1* C127S + *HFE* H63D/C282Y

D008

EPIDEMIOLOGY OF SICKLE CELL DISEASE IN ITALY: FINDINGS FROM THE GREATALYS (GENERATING REAL WORLD EVIDENCE ACROSS ITALY IN SCD) STUDY

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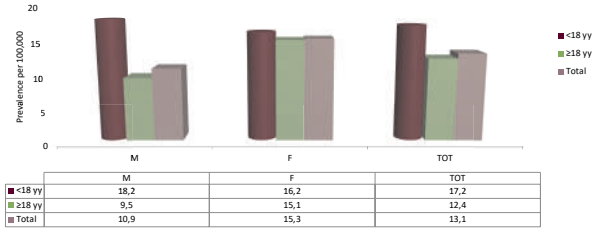
Aim: The epidemiologic profile of sickle cell disease (SCD) in Italy is rapidly evolving. The study aims to evaluate the prevalence of SCD in clinical practice settings, and to estimate the number of SCD patients currently living in Italy.

Methods: Within the GREATalyS study, an observational retrospective analysis was conducted based on administrative databases from 2 Regions and 15 Local Health Units geographically distributed across Italy, covering around 15.3 million individuals (about 25% of the whole Italian population). Patients with ≥1 hospitalization discharge diagnosis for SCD (primary or secondary) between January 2010-December 2018 were included. Prevalence was stratified by age, sex, presence of crisis (based on diagnosis at inclusion) and geographic area. Data were re-proportioned to the total Italian population.

Results: Prevalence of SCD diagnosis in 2018 was calculated of 13.1/100,000 individuals (10.9/100,000 males, 15.3/100,000 females). Stratification by age showed a prevalence of 17.2/100,000 cases in young (<18 years old) and 12.4/100,000 cases in adult (≥18 years old) individuals. Data reproportioned to the Italian population estimated a total of 7,977 patients (1,690 young, 6,287 adult) with SCD in Italy in 2018. When the number of SCD patients with/without crisis were projected to the Italian population, 1,279 SCD patients with crisis and 5,894 without crisis were estimated (804 unspecified). Among adult patients, prevalence of SCD diagnosis with crisis was higher in those with <45years, from 2.45/100,000 (age group 35-44) to 3.04/100,000 (age group 25-29) while a significant descending trend was observed after 54 years down to 0.34 in age group 75-84 years. Higher prevalence of SCD diagnosis without crisis was observed in Southern (12.44/100,000) compared to the North (6.93/100,000) and Center (4.34/100,000) areas.

Conclusion: The GREATalys study provided up-to-date insights into the epidemiologic burden and overall distribution of SCD in Italy. When prevalence calculated for year 2018 (13/100,000) was reproportioned to national population, 7,977 SCD patients were estimated, three-fourth of which without a concomitant diagnosis of crisis, suggesting a high disease burden beyond severe crisis. The real-world settings could have considered patients not referred to specialistic centers potentially underreported in previous SCD epidemiology analysis, thus providing a more realistic scenario of the highly variable presentation of SCD.



	M	F	TOT
<18 yy	18,2	16,2	17,2
≥18 yy	9,5	15,1	12,4
Total	10,9	15,3	13,1

Figure 1.

D009

A MULTICENTRIC GIMEMA LABNET VALIDATION AND STANDARDIZATION NON-TRANSFERRIN-BOUND-IRON (NTBI) STUDY. FIRST ROUND ROBIN

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Under normal conditions, iron circulates in the body bound to serum transferrin. However, in iron overload conditions, the ability of transferrin to bind iron is exceeded, forming "free" or "unbound" iron: non-transferrin-bound-iron (NTBI). Free iron is a toxic reactive oxygen species, capable of causing oxidative stress and cellular structures damage finally leading to cell necrosis. Of relevance NTBI is chelable by available chelators. Iron overload markers are: transferrin saturation, serum ferritin, number and quantity of red blood cell transfusions, liver iron concentration. However, all these parameters reflect iron burden and not iron toxicity that is highly variable and dependent by different factors. The trigger of iron induced damage is NTBI and summary of NTBI level over time is the most important parameter included in the Coates formula. Therefore, once available, NTBI dosage can be a fundamental factor in predicting iron-related damage and the target for iron chelation therapy. Unfortunately, NTBI measure is still not standardized and not widely available. Therefore, the GIMEMA LABNET started a national project to homogenize NTBI level. To this end, a standard protocol based on HPLC, developed at the Lab. Med. Gen. Policlinico Milano, was adopted for the determination of NTBI. Serum samples from 14 patients with or without iron disturbance were collected, stored and blindly sent to 3 Italian laboratories (Milano, Orbassano, Verona). Each center worked blindly and independently. Data analysis reveal a discrepancy in the NTBI values, with a wide quantitative inter laboratory difference possibly operator dependent (mean: MI 0.45±0.83; ORB 1.31±1.29; VR 1.70±3.06 microM/L) (Figure 1). However, all values agreed in the detection of free iron in samples with pathological percentage of transferrin saturation (> 70%). The goodness of the detection technique used is therefore evident, but also the need to identify the internal cut-offs in each laboratory to establish the pathogenicity ranges of the NTBI values. After extensive discussion and procedure adjustment of the processes steps a second-round robin has been completed. Results will be reported at the study presentation. The possibility of directly determining iron reactive species without resorting to indirect techniques would lead acting on the direct, chelable, iron related damage triggering factor improving diagnostic and therapeutic capability.

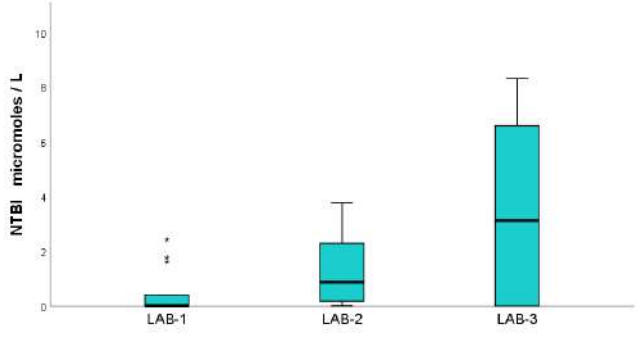

Figure 1. Box-plots graph-The mean of the measurement and relative dispersion of NTBI values by3 laboratories presented.

D010

USE OF CYCLOSPORINE IN THE TREATMENT OF AUTOIMMUNE CYTOPENIAS: EFFICACY AND SAFETY

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Background: Autommune cytopenias (autoimmune hemolytic anemia AIHA, autoimmune thrombocytopenia ITP, and chronic idiopathic neutropenia CIN) are a heterogeneous group of diseases characterized by the presence of autoantibodies directed against erythrocytes, platelets (PLT) and neutrophils (ANC). Available therapies are based on the use of frontline steroids, followed by different treatments different depending on the disease considered (*i.e.* splenectomy, rituximab and thrombopoietin-receptor agonists). Cyclosporine (CyA) is an immunosuppressant widely used in post transplant settings and aplastic anemia.

Aim: The aim of this study was to evaluate the efficacy and safety of cyclosporine in a cohort of patients with AIHA, ITP, and CIN, followed at a reference hematologic center in Milan.

Methods: All patients treated with CyA 3-5 mg/ kg day in the last 20 years were evaluated. Responses were evaluated at 3, 6 and 12 months, and divided into partial (PR, for Hb> 10 g/dL; PLT> 30x 10^9/L and ANC> 0.8x10^9/L) and complete (CR, for Hb> 12 g/dL; PLT> 100x10^9/L; ANC>1x10^9/L). Adverse events were recorded according to CTCAE criteria.

Results: 23 patients, 9 had ITP (39%), 11 AIHA (48%) and 3 CIN (13%), were included, 9 men (39%) and 14 women (61%), with a median age of 60 year (21-81). The median time from diagnosis to CyA was of 10 years (5-15), and patients had required a median of 3 (1-6) previous therapy lines. On the whole, 16 patients (69.5%) responded (table1): 34% CR, 44% PR at month+3; 39% CR and 39% PR at month+6; and 26%CR and 43%PR at month+12. A progressive increase in PLT, Hb and ANC was observed along the study period, and median duration of therapy was 5 years (1-9). Interestingly, better responses were observed in patients with baseline bone marrow hypocellularity by age (p=0.01). Adverse events were mainly G1-2, occurring in 52% of patients, and included asthenia, dyspnea, myalgia, nausea, vomiting, diarrhea and abdominal pain, epistaxis, petechiae and an Escherichia Coli cystitis. Only 3 patients developed a G3 event: 1 TEP, 1 Aspergillus lung infection and 1 bronchitis.

Conclusion: Cyclosporine was effective in about 70% of pretreated patients with ITP, AIHA, and CIN, particularly in those with hypocellular bone marrow at diagnosis. The occurrence of infectious episodes, including a fungal pneumonia, warrants careful surveillance in this heavily pretreated patient population.

Table 1.

Table1	Month+3	Month+6	Month+12
ITP (N=9)	CR 5 PR 8	CR 7 PR 7	CR 4 PR 9
AIHA (N=11)	CR 1 PR 2	CR 1 PR 2	CR 1 PR 1
CIN (N=3)	CR 2 PR 0	CR 1 PR 0	CR 1 PR 0
Tot = 23	**CR: 8 (34)** **PR: 10 (44)**	**CR: 9 (39)** **PR: 9 (39)**	**CR: 6 (26)** **PR: 10 (43)**
Hb g/dL, PLTx10^9/L, and ANCx10^9/L values during CyA therapy			
Baseline	Plt x 10^9/L baseline, median (range) Hb g/dL baseline, median (range) ANC x 10^9/L baseline, median (range)	8 (4-12) 9 (8-10) 1.65 (0.1-3.2)	
Month+3	Plt x 10^9/L, median (range) Hb g/dL, median (range) ANC x 10^9/L, median (range)	40 (14-67) 9,5 (9-10.7) 0.954 (0.18-1.7)	
Month+6	Plt x 10^9/L, median (range) Hb g/dL, median (range) ANC x 10^9/L, median (range)	130 (109-150) 10 (9,4-10,7) 1.7 (0.21-3.2)	
Month+12	Plt x 10^9/L, median (range) Hb g/dL, median (range) ANC x 10^9/L, median (range)	52 (45- 58) 11,6 (9,3-14) 1.7 (0.13-3.4)	

D011

ENERGIZE AND ENERGIZE-T: TWO PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDIES OF MITAPIVAT IN ADULTS WITH NON–TRANSFUSION-DEPENDENT OR TRANSFUSION-DEPENDENT ALPHA- OR BETA-THALASSEMIA

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Background: Thalassemias are characterized by ineffective erythropoiesis and hemolysis due to imbalanced α- and β-globin-chain production and precipitation. Adenosine triphosphate (ATP) levels are reduced in thalassemic red blood cells (RBCs), despite increased energy demands. Mitapivat is an oral activator of RBC pyruvate kinase (PKR), a glycolytic enzyme that regulates ATP production. In a phase 2 study of patients (pts) with α- or β-non–transfusion-dependent thalassemia (NTDT), twice-daily (BID) dosing with mitapivat increased hemoglobin (Hb) levels by ≥1.0 g/dL in 80% of pts, supporting the broadening of mitapivat's development in thalassemia.

Aims: To report the study designs of ENERGIZE (2021-000211-23) and ENERGIZE-T (2021-000212-34), two phase 3 trials to assess the efficacy and safety of mitapivat in adults with α- or β-NTDT or transfusion-dependent thalassemia (TDT), respectively.

Methods: Both studies are multicenter, randomized, double-blind, placebo-controlled trials (Figure 1). In ENERGIZE, ~171 pts with NTDT will be randomized to receive 100 mg mitapivat BID or placebo for 24 weeks (wks). Pts then have the option to transition to a 5-year, open-label extension. Key inclusion criteria: documented diagnosis of thalassemia (β-thalassemia ± α-globin mutations, Hb E β-thalassemia, or α-thalassemia), Hb concentration ≤10.0 g/dL, and NTDT. Primary endpoint: Hb response defined as a ≥1.0 g/dL increase in average Hb concentration from Wk 12 through 24 compared with baseline (BL). Secondary endpoints: pt-reported outcomes, changes in Hb, markers of hemolysis and erythropoiesis, and safety. In ENERGIZE-T, ~240 pts with TDT will be randomized to receive 100 mg mitapivat BID or placebo for 48 wks. Pts can then transition to a 5-year, open-label extension. Key inclusion criteria: documented diagnosis of thalassemia (same genotypes as detailed for ENERGIZE), and TDT. Primary endpoint: transfusion reduction response, defined as a ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-wk period through Wk 48 compared with BL. Secondary endpoints: additional measures of transfusion burden, changes in iron markers, and safety.

Results: Not yet available.

Conclusions: ENERGIZE and ENERGIZE-T are the first pivotal studies to assess the efficacy and safety of mitapivat across a broad spectrum of pts with thalassemia (ie, pts with TDT and NTDT; α- and β-thalassemias). Both studies will start enrollment in 2021.

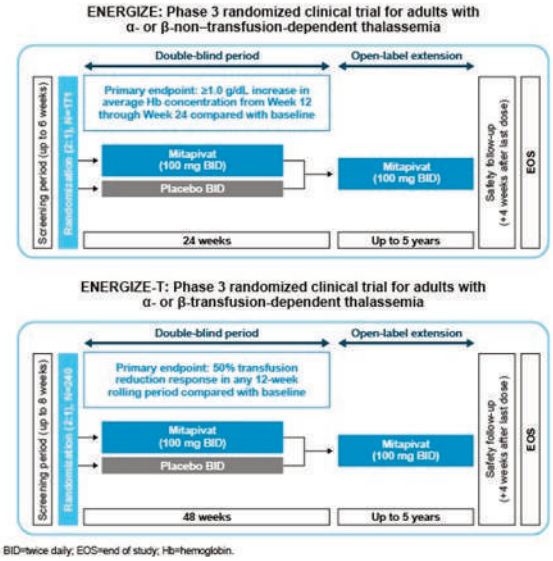

Figure 1.

D012

SUTIMLIMAB, A COMPLEMENT C1S INHIBITOR, IMPROVES QUALITY OF LIFE IN PATIENTS WITH COLD AGGLUTININ DISEASE: PATIENT-REPORTED OUTCOMES RESULTS OF THE PHASE 3 CARDINAL STUDY

W. Barcellini[1a], A. Röth[2], T.H. Anderson Tvedt[3], Y. Miyakawa[4], D.J. Kuter[5], W. Hobbs[6b], J. Su[6b], X. Jiang[6], J. Morales Arias[6b], I.C. Weitz[7]

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Introduction: Cold agglutinin disease (CAD) is a rare autoimmune hemolytic anemia characterized by classical complement pathway (CP)-mediated hemolysis. Patients with CAD also experience profound fatigue and poor quality of life (QoL). Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) and other QoL measures are unevaluated in CAD. Sutimlimab, a humanized monoclonal antibody that prevents CP activation by selectively targeting C1s, was shown to rapidly stop hemolysis and significantly improve anemia in CAD (Röth et al. New Engl J Med 2021). Here we present the effect of sutimlimab on patient-reported outcomes (PRO) from Part A of the Phase 3 Cardinal study (NCT03347396).

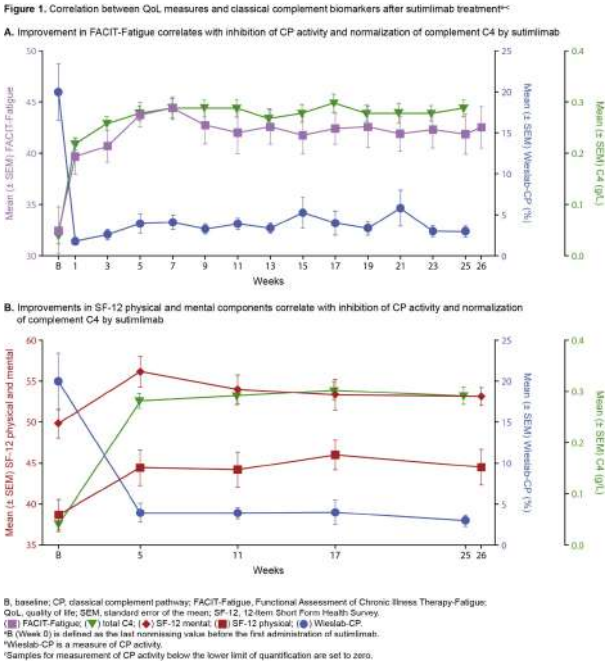

Figure 1.

Methods: Cardinal Part A is a 26-week, open-label, single-arm study of sutimlimab efficacy and safety in patients with CAD who had ≥1 red blood cell transfusion in the prior 6 months. Intravenous sutimlimab (dosed 6.5 g if <75 kg or 7.5 g if ≥75 kg) was given on Days 0 and 7, then biweekly. Mean change from baseline for fatigue was a secondary endpoint using FACIT-Fatigue scores at the treatment assessment timepoint (TAT; average of values from Weeks 23, 25, and 26). Exploratory QoL endpoints were assessed using the EuroQol 5-dimension 5-level (EQ-5D-5L) questionnaire and the 12-Item Short Form Health Survey

(SF-12). Results were reported using descriptive statistics.

Results: Enrolled patients at baseline (n=24) had abnormal QoL consistent with cancer or autoimmune disease. Mean (standard deviation [SD]) FACIT-Fatigue increased from 32.5 (10.6) at baseline to 44.3 (6.5) at the TAT (n=17), with an estimated mean (standard error) increase of 10.9 (1.4). Clinically meaningful FACIT-Fatigue improvements (≥3-point increases) occurred in ≥75% of patients (interquartile range: 5.0–15.5 points). For EQ-5D-5L (n=16), the mean (SD) increases in index and visual analog scale scores from baseline to Week 26 were 0.074 (0.185) and 16.8 (16.9), respectively. Mean (SD) increases in SF-12 physical and mental component scores (n=16) from baseline to Week 26 were 5.37 (7.60) and 4.37 (10.02) points, respectively. QoL improvements correlated with resolution of hemolysis, near-complete inhibition of CP activity, and rapid normalization of complement C4 (Figure 1 A/B).

Conclusions: CP activation with subsequent hemolysis plays a critical role driving fatigue and poor QoL in patients with CAD. Treatment with sutimlimab resulted in rapid, clinically meaningful improvements in all PROs measured.

D013

A CASE OF EVANS SYNDROME SECONDARY TO COVID-19 VACCINATION

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Evan's syndrome (ES) is a rare condition, defined as the concomitant immune thrombocytopenia (ITP) and autoimmune haemolytic anaemia, due to warm antibodies, usually of IgG isotype. Course is chronic in more than 80%, with multiple relapses 2. it is associated to other diseases in 30-40% cases, most particularly haematologic malignancies and systemic lupus erythematosus3. We describe, at our knowledge, the first case of ES induced by mRNA COVID-19 vaccine.

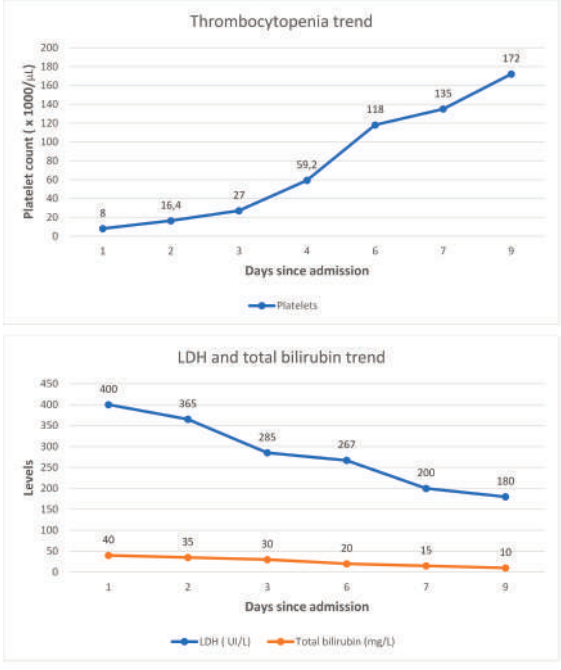

Figure 1.

Case Report: A 84-year-old male patient presented at the Emergency Department of our Institution on March 31th 2020, for appearance of a large hematoma extending from the left deltoid to the omolateral forearm, and evidence of ecchymosis at counterlateral arm and legs. He practiced mRNA COVID-19 vaccine thirteen days before and detected first local ecchymosis after 48 hours. Blood counts showed severe thrombocytopenia (platelets = $8x10^3$ /mL), normochromic normocytic anaemia (haemoglobin= 10.0 g/dl). Haemolysis labs were positive with positive direct Coombs test. SARS-COVID 2 antibodies was negative. Peripheral blood smear highlighted reticulocytes and nucleated RBCs, in the absence of schistocytes. An osteomedullary biopsy highlighted normal cellularity, slight notes of erytroid iprplasia, slightly increased megakaryocyte series with some elements in terminal thrombocytopoiesis and dismorphic elements. This clinical picture raised concern for post-vaccination Evans Syndrome. We immediately began treatment with methylprednisolone 1 mg/kg every 12 hours to be continued for 4 weeks and IVIG 400 µg/kg for 5 days. Gradually we obtained a normalization of platelet count (Figure 1, upper level), and LDH and serum bilirubin levels (Figure 1 lower level). At third week haemoglobin value has risen to 12.4 g/dl, while the platelets have stabilized at 170 x 103/µL. So we started steroid tapering. Unfortunately platelets drop to 65 x 103/mmc and hemoglobin to 10.5 gr/dl with a concomitant increase of total bilirubine and LDH. We decided to begin a new cycle of IVIG. Interestingly SARS-COVID 2 antibodies continue to be negative. ES has been reported in association to COVID-19 infection. To our knowledge, this is the first evidence of association with SARS-CoV-2 vaccination. Patients who do not develop antibodies against spike protein may be more at risk of developing autoimmune diseases due to a predisposition for a heterologous immunological response.

Cytogenetics, Molecular Genetics

D014

HIGH RESOLUTION GENOME-WIDE NGS METHODOLOGY FOR CHROMOSOMAL COPY NUMBER ABERRATIONS IN ACUTE MYELOID LEUKEMIA

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Introduction: Chromosomal aberrations have deeply impact on diagnosis, risk stratification and treatment of acute myeloid leukemia (AML). Complex karyotype (CK) represents an adverse prognostic factor associated with inferior outcomes in patients with AML. Cariogram reconstruction is laborious and difficult to interpret, above all in complex cases or when the quality of the cytogenetic preparation is not optimal.

Methods: Bone marrow from 25 AML and 15 bone marrow donor were provided for chromosomal aneuploidy (copy number variation analysis-CNV) and conventional cytogenetic. We developed a high-resolution genome-wide NGS-based analysis providing >98% coverage of the genome with a resolution of 500 kb. For CNV 100 ngr of genomic DNA was employed for an fragment-based library preparation and analysis was performed by Ion Torrent Chef-S5 platform. CNV was performed according a modificated-protocols based on Ion Express Plus gDNA Fragment Library Preparation. Analyses is performed with a specific workflow (Low-pass aneuploidy) built to individuate CNV with >30% and 500Kb resolution. 15 male donor samples are used to create a comparative baseline. At least 1 million reads were evaluated for CNV

Results: We studied 25 AML patients for karyotipe and CNV. We identified 8 normal karyotipes by conventional cytogenetic, 7 were confirmed by CNV, 1 sample show a deletion of chromosome 9 not identifiable to standard cytogenetic resolution. In 3 cases we found one isolated aberration by standard cytogenetic while CNV analysis identified additional aberration which change cytogenetic risk assessment. For 2 samples, culture preparation don't allowed a cytogenetic analysis, NGS-CNV identified an abnormal kariotype with cytogenetic adverse prognosis. Conventional cytogenetic identified 7 complex karyotypes difficult to interpret, in these cases CNV adding more accurate identification of chromosomal abnormalities (3 or >5)

Conclusions: We have developed genome-wide methodology to identify chromosomal aneuploidy. NGS approach shows good concordance with standard cytogenetic, excellent intra-laboratory reproducibility and reduction of labour time consuming. NGS improve resolution and cariogram reconstruction to lead a gain in cases of difficult interpretation, in failed or not optimal cytogenetic analysis. NGS methodology represent an aid in cytogenetic analyses to improve patient stratification and optimize therapeutic decisions in AML

Supported by PSN 2016 Sicilia

D015

CLINICAL VALIDATION OF A NEW MYELOID NEXT GENERATION SEQUENCING PANEL FOR DETECTION OF SINGLE NUCLEOTIDE VARIANTS AND INSERTIONS/DELETIONS

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Myeloid neoplasms are a heterogeneous group of neoplasms including acute myeloid leukemia (AML), myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms/myelodysplastic syndrome. Genetic abnormalities are used as diagnostic, prognostic, and predictive biomarkers in patients with these diseases. Next-generation sequencing (NGS) enables reliable detection of patient-specific mutations covering complete genes in molecularly heterogeneous diseases such as AML, MPN and MDS. NGS should, therefore, be incorporated in the routine work-up of preferably bone marrow specimens for accurate risk stratification in AML, MDS and MPN. Mutations in several genes, such as TP53, EZH2, ETV6, RUNX1, and ASXL1, are independent prognostic predictors of reduced overall survival in MDS. Currently, the European Leukemia Network (ELN) and National Comprehensive Cancer Network (NCCN) recommends genetic testing for all patients with newly diagnosed AML. This includes: conventional cytogenetics, screening for nine gene mutations including NPM1, CEBPA, RUNX1, FLT3, TP53, ASXL1, IDH1, IDH2 and c-KIT and screening for gene rearrangements including PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1 and BCR-ABL1. Both institutions acknowledge that the recommended mutational testing has to be interpreted as a "minimum" for daily clinical practice in order to accurately assess genomic risk and use targeted therapy where appropriate. Herein, we describe the clinical validation of the Archer VariantPlex® NGS panel that interrogates for 75 genes commonly seen in myeloid neoplasms and some Lymphoid malignancy markers. Our validation set of 50 DNA samples included acute and chronic myeloid neoplasms, with single-nucleotide variants and small insertions/deletions. The Archer VariantPlex® on the NextSeq® 550Dx platform shows good performance in terms of depth of coverage, on-target reads, and uniformity. The panel achieved 98% and 100% concordance with reference DNA and DNA samples, respectively, with a clinical sensitivity and specificity of 99% and 100% for DNA respectively. Precision and reproducibility were 100%, and the lower limit of detection was generally 5% variant allele fraction for DNA. In conclusion, the Archer VariantPlex® panel is a highly accurate and reproducible next-generation sequencing panel for the detection of common genetic alterations in myeloid neoplasms.

D016

A NEW PCR-BASED SENSITIVE MOLECULAR TOOL FOR DETECTION OF FLT3-TKD MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background: FLT3 mutations characterize 20-30% of AML patients; their detection is today crucial after the recent approval of FLT3 inhibitors (midostaurin for upfront treatment and gilteritinib for relapsed/refractory AML).

Aim: We retrospectively employed a new accurate and more sensitive PCR-based molecular technique for detecting FLT3-TKD mutationsand we compared it to the "classic" assay.

Patients: We assessed76 AML patients; 45male and 31 female, median age was 56 (19-75). In 22% of cases AML was classified as "with recurrent genetic abnormalities", in 22% as "with MRC", in 11% "therapy-related" and in 45% as "NOS". According to the ELN risk stratification, 16% of subjects were at favorable, 50% at intermediate, and 34% at adverse risk.

Methods: We checked both FLT3 mutation types at diagnosis and at the eventual relapse using GeneScan PCR for FLT3-ITD and PCR followed by EcoRV digestion and agarose gel run (in case of mutated samples, the PCR product is not digested). The GeneScan method reaches the sensitivity of $5x10^{-5}$, while the second one of $5x10^{-2}$.As new method, we adopted the ARMS-PCR technique (qBiomarker Somatic Mutation PCR Assays, Qiagen),with a sensitivity>$1x10^{-2}$ (half than a log higher than the classic technique).

Results: At diagnosis, FLT3-ITD and -TKD mutations were detected in 15 (20%) and 3 (4%) patients, respectively. When patients were re-assessed by the new PCR method, FLT3-TKD mutations were detected in 12 patients (15.8%). No patient simultaneously carried FLT3-ITD and

-TKD mutations. In 15 cases, FLT3 mutations occurred in NPM1-mutated patients; 2 subjects were mutated also for CBF/MYH11, other 2 for JAK2V617F, 2 for c-KIT, 2 for N-RAS and 1 for BCR-ABL1. Nine FLT3-mutated patients received FLT3 inhibitors;CR was achieved by 36 patients (47.3%), but 18 of them (50%) relapsed, after a median time of 7.6 months.In our series, OS was significantly conditioned by CR, age (>65y), ELN score. Presence of FLT3-ITD did not change prognosis, whereas FLT3-TKD mutated patients had a poorer outcome (2y-OS, 34% for FLT3-TKD wild-type *vs* 11% for mutated cases; p=0.003) (Figure1). The prognostic value of FLT3-TKD remained also in multivariate analysis, independently from age.

Conclusions: Our study presents a new PCR-based technique, with higher sensitivity, able to increase the identification of FLT3-TKD-mutated cases. Since these patients showed a poorer prognosis, these cases are candidate to receive FLT3 inhibitors.

D017

DOES THE SAME DOSE FIT FOR ALL? A NEW METHOD TO DETERMINE PONATINIB PLASMATIC CONCENTRATION

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Background: Ponatinib is a 3rd generation tyrosine kinase inhibitor (TKI)very effective in Chronic Myeloid Leukemia (CML);nevertheless, cardiovascular (CV) adverse events represent a major issue during therapy and some groups proposed to reduce the drug dose for avoiding CV complications. It has been established that the therapeutic plasmatic concentration of Ponatinib must be ≥ 20 ng/mL, but little is known about the relationship between drug dose, plasmatic concentration and toxicity or response to treatment.

Aim: We present here a new high resolution mass spectrometry-based method to determine Ponatinib plasma concentration.

Method: We collected 15 peripheral blood samples from 11 CML patients (6 males and 5 female, mean age 44) in treatment with Ponatinib at different doses (from 45 mg daily to 30 mg weekly). The reason for switch to Ponatinib was toxicity in 4 and resistance in 7 cases. In the resistant cohort, Ponatinib allowed to reach major (MMR) and deep molecular response (DMR) in 28.5% and 71.5% of cases, respectively. Samples were analyzed by LC/HRMS (Ultimate 3000 LC system with TurboFlow technology coupled to a Q-Exactive system). After deproteinization with an acetonitrile/methanol solution (75/25), the samples were injected directly into the system, using Tracefinder® software for quantification analysis. The procedure was validated and successfully applied to the blood samples in routine laboratory analyses and can be considered fast and easy.

Results: The method proves to be reliable with both precision and accuracy higher than 85% and showing a very good specificity and sensitivity. The therapeutic Ponatinib concentration of 20.0 ng/mL was reached in 73.3% of tested samples, regardless of the dosage scheme (15, 30 or 45 mg daily), except for 2 samples from the same patient treated with a very-low dose (30 mg weekly) because of a very-high cardiovascular risk.

Conclusions: 1) even if on a small series, our data confirm the high response rate achievable with Ponatinib; 2) the achievement of the minimal therapeutic concentration regardless of the dosing regimen can explain the dose-independent Ponatinib efficacy also reported in the OPTIC Trial; 3) the method resulted to be accurate and easily applicable for a patient-tailored treatment in clinical practice. In the further steps, we'll investigate if the cytochromeP450 or efflux-pump polymorphisms might condition the Ponatinib plasma levels.

D018

IDENTIFICATION OF ATYPICAL PML/RARA FUSION TRANSCRIPT BY MOLECULAR, CYTOGENETICS AND FLOW CYTOMETRY ANALYSIS

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Acute promyelocytic leukemia (APL) is characterized by the PML-RARA fusion gene, as a consequence of the t(15;17)(q22;q21)translocation. Depending on the PML breakpoint,usually located within intron6,exon6,or intron 3, different PML/RARA transcript isoforms may be generated: long (bcr1), variant(bcr2), and short (bcr3),respectively.We report molecular characterization of a APL case with atypical PML/RARA transcript , not clearly detectable using standard molecular procedure. Differential detection of PML-RARA bcr1, bcr2, bcr3 fusion transcripts were analyzed using a LAMP technology based kit (DiaSorin Molecular) and no amplification was detected. Subsequently a Real time PCR was performed using HemaVision-28Q kit (DNA Diagnostic), no detecting amplification. Given the strong clinical and morphological suspicion, we tested the sample for RT-PCR (Biomed protocol) using PML-A2 and RARA-B primers. Analysis of PCR product showed one specific band of 440 bp, a size that does not correspond with the recognized size of the typical transcripts. The immunophenotipic characterization carried out on FACS CANTO II BD, showed 90 % of abnormal mononuclear cells 45% of which expressed CD34 and a myeloid phenotype (CD117 CD13 CD33 partial CD2 MPO). Apart from this population with the help of logical gate, we found a population that didn't express CD34 and HLA DR and had cytometric characteristics of APL. Bone marrow aspirate demonstrated hypercellular marrow with 40% blasts, characterized by medium to large cells with altered nucleus/cytoplasm ratio, and 30% abnormal medium-sized promyelocytes, with bilobed nuclei, hypergranulated cytoplasm and rare Auer bodies. The traditional cytogenetic analysis at the diagnosis, assayed by the R-banding, revealed the following karyotype 46,XY,t(15;16;17) (q24;p13;q21)[18]/47,XY,+8, t(15;16;17)(q24;p13;q2). This is the first description of specific translocation involving three chromosome: 15, 17 and 16. In order to get a correct identification of transcript and perform sensitive and specific quantitative evaluation of MRD during the follow up we are now performing direct sequencing of PCR product. The final diagnosis made was APL and the patient started therapy with arsenic trioxide and all-trans retinoic acid. Nowadays he is responding to therapy and the clinical picture improved. This case indicates that the use of different experimental approaches and different techniques is fundamental to confirm the diagnosis of difficult cases of APL.

Hemostasis, Thrombosis, Thrombocytopenia and Platelet Diseases

D019

A CASE WITH VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTOPENIA (VITT) AFTER CHADOX1 NCOV-19 VACCINATION WITH UNDETERMINED ANTIBODIES INDUCING ATYPICAL PLATELET ACTIVATION DEMONSTRATED BY FLOW CITOMETRY: CLINICAL AND BIOLOGICAL CONSIDERATIONS

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A 34 yo woman with headache 1 week after vaccination with ChAdOx1 nCoV-19 went to Emergency. She take contraceptive pill from 12 years, without thromboembolic events, platelets were 30.000/mm3. Computed Tomography (CT) and magnetic resonance showed thrombosis of superior sagittal sinus. High dose corticosteroid and a reduced dose of Fondaparinux was started. Platelets were transfused to maintain a value >30.000/mm^3. For the D-dimer increase (>80 mg/L) and the fibrinogen decrease (74 mg/dl), fresh frozen plasma and intravenous immunoglobulins (IvIg) 2 g/kg were administered. Worsening of clinical conditions associated to portal vein thrombosis complicated by bleeding tendency, forced the stop of IvIg and the start of plasma-exchange (PEX). After 12 hours a CT documented a massive cerebral hemorrhage in parietal-temporo-occipital and a worsening of thrombosis. PEX and Fondaparinux were stopped and IvIg were resumed to complete the total dose of 120 gr. The first increase in platelet at 42000/mm^3 and the initial improvement of coagulative tests were observed just after the PEX. At day +6 the platelets increased to 60.000/mm^3 and a clinical improvement was noticed. Full dosage of Fondaparinux was resumed. At day +10 platelets count reached the normal value. She was discharged at day 40 with thrombosis resolution.

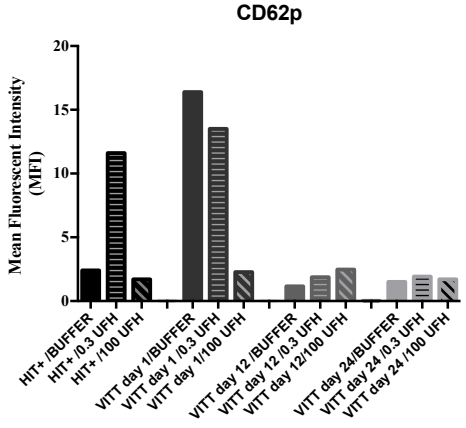

Figure 1.

Laboratory results:patient was negative for antiPF4-Ab (AccuStar method), but platelets activating antibodies were detected by a flow cytometric measuring P-selectin exposure of control platelets incubated with patient's plasma for 1 hour at room temperature in the presence of buffer, 0.3UI/uL UFH, 100 UI/uL UFH. PE-conjugated P-selectin CD62p and FITC-conjugated CD42 antibodies were added to the mixture. The sample from a HIT+ patient was used as control. At day+1 plasma from our VITT patient induced activation of platelets in absence of heparin. Heparin addition inhibited platelet activation by VITT plasma. Stimulating activity became undetectable at d+12 and +24 after treatment (Figure 1). Present report shows a negative AbantiPF4 VITT case in whom undetermined antibodies inducing atypical platelet activation were documented by a functional flow cytometric test. The observation that high concentration of heparin exposure inhibits platelet activation may support the heparin use in these cases. Finally, the sudden increase in platelets immediately after PEX may suggest its efficacy together with the immunosuppression in removing platelets antibodies.

D020

CLINICAL PRESENTATION, DIAGNOSIS, TREATMENT AND OUTCOME OF A SEVERE CASE OF VITT (VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTOPENIA) POST VAXZEVRIA VACCINE EXPOSURE, MIMICKING HEPARIN-INDUCED THROMBOCYTOPENIA (HIT)

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A healthy smoker 57 year-old female went to Emergency Unit for headache and petechiae 11 days after her first Vaxzevria vaccine dose. Blood tests showed severe thrombocytopenia (10x10^9/L) and increased d-dimer (5.09 mg/L). A CT scan showed a presumably longstanding thrombotic apposition in the aorta. Brain angio MR resulted negative. During the hospitalization in absence of other cytopenias or morphological findings immunoglobulins (Ig) 1 g/kg on the first day and methylprednisolone 0.8 mg/kg/day were started; at discharging platelets were 70x10^9/L and d-dimer furtherly rose. The patient was prescribed oral prednisone 1 mg/kg/day at home. Five days later she was hospitalized for abdominal pain. CT showed pulmonary embolism, portal vein thrombosis, increased thrombus in aorta, splenic artery thrombosis and splenic infarcts. Platelets were 55x10^9/L. Despite negative rapid test for HIT available in Pescara Hospital, heparin was avoided; after replacement therapy for hypofibrinogenemia (86 mg/dL) the patient was treated with Ig 1 g/kg for 2 days, dexamethasone 40 mg/die for 4 days, acetylsalicylic acid 100 mg/die and fondaparinux 7.5 mg/die (this latter replaced with dabigatran 150 mg twice a day once liver function tests improved). A complete acquired and congenital thrombophilia screening, paroxysmal nocturnal hemoglobinuria phenotype, ADAMTS-13 (activity and antibodies), Jak2V617F, CALR, MPL mutations were also performed, resulting negative. VITT diagnosis was confirmed based on positive results of functional HIPA test (heparin-induced platelet activation) at low dose and also in absence of heparin (despite negativity of immunoassay), all performed at Careggi Hospital in Florence. Platelets progressively and firmly increased to normal range. Last CT scan showed the resolution of pulmonary embolism and portal vein thrombosis. Despite the plausible functional asplenism there aren't indications for splenectomy. Dabigatran dosage at peak time and through was in the recommended range, with no evidence of bleeding. The patient was discharged without severe complications thanks to early diagnosis and treatment, in absence of cerebral thrombosis. A clinical and laboratory monitoring should be suggested in patients receiving viral vectors vaccines becoming symptomatic for

headache, abdominal pain, dyspnea or leg pain at 4-28 days post exposure. In suspected case of VITT, even before confirming tests, HIT-compatible anticoagulants should be used, associated with Ig.

	Day 6	Day 11	Day 16	Day 20	Day 21	Day 23	Day 28	Day 34	Day 40	Day 49	Day 53
Hemoglobin (g/L) (NV 12-16)	14.9	13.4	11.6	13.3	12	9.1	9.8	8.7	9.7	11.2	10
WBC x10³/ul (NV 4.000-10.000)	7.300	11.700	12.300	21.200	18.200	29.900	23.400	18.740	8650	10.210	9.490
Platelets x10³/ul (NV 130-400)	120	10	79	55	48	145	364	309	109	513	385
Fibrinogen (mg/dl) (NV 180-400)	458	345	265	86	240	202	190	603	610	517	427
D-Dimer (mg/l) (NV 0,0-0,5)	1,31	5.09	37.8	7.10	4.82	4.55	3.82	3.47	3.21	2.4	1.74
PT (%) (NV 70-120)	79	76	82	79.9	74	75	74	64	75	76	75
PTT (sec) (NV 28-40)	36.3	33	38	26.2	30.9	30	48	49	41	41	40
AT III (%) (NV 80-120)	112	109	113	124	129	114	111	97	100	106	102
AST (U/L) (NV 5-34)	30	66	221	66	1782	330	45	46	46	33	29
ALT (U/L) (NV 0-55)	25	43	103	68	636	394	68	20	13	8	8
LDH (U/L) (NV 125-220)	227	264	284	635	2126	677	368	476	380	372	315

Table 1. Laboratory findings. Results of blood exams performed in the days indicates. ***Day 0 = Vaccine day.**

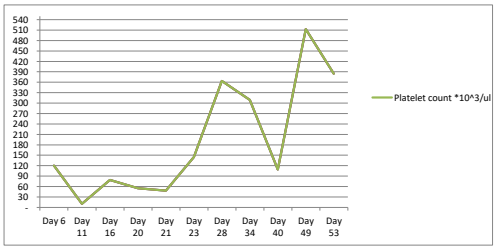

Figure 1.

D021

RISK ASSESSMENT MODELS (RAM) OF VENOUS THROMBOEMBOLISM (VTE) IN PATIENTS WITH BLOOD CANCERS: A SYSTEMATIC REVIEW

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Blood cancer patients face a relative risk of VTE 28fold higher than the general population. Nevertheless, they also face a significantly higher risk of bleeding than overall cancer patients, thus prevention of VTE is not systematically implemented in this population. A more detailed estimation of VTE risk might support personalized primary prevention decisions. We therefore aimed at reviewing the VTE risk score that have been validated in blood cancer patients. According to GRADE-18 guidelines, we conducted at Nov 2020 a systematic search of literature databases (Cochrane Library, EMBASE, PubMed/MEDLINE) including the studies reported at meetings since Jan 2010. The search was limited to the following blood cancers: multiple myeloma (MM), lymphoma, acute leukemia (AL). Six-month VTE rates for risk categories, sensitivity, specificity, AUC or C-statistics and predictive values of the retrieved scores were recorded. Moreover, PROBASt score was applied for quality appriasal. We retrieved 82 records which resulted in 9 VTE RAMs: 3 ones were devloped in patients with MM, 5 ones in patients with lymphoma and only one was retrieved for AL. Sensitivity of lymphoma RAMs ranged from <50% to 93% and specificity from 54% to 90%. AUC ranged from 0.579 to 0.783. The negative predictive vale of the 5 RAMs was consistently higher than 95% except for the Khorana score (88%). The positive predictive value, however, was very low, ranging from 20% to 35%. Also C-statistics was higher for RAMs specifically developed in lymphoma patients (0.76-0.88) rather than Khorana score (0.60). The expected rate of VTE in high-risk patients ranged from 16% to 25% in lymphoma-specific RAMs, while it was lower than 15% for Khorana score. The expected rate of VTE in low-risk patients was lower than 3.8% in lymphoma specific RAMs. As a result, prophylaxis decisions based on lymphoma specific RAMw achieved a number need to treat lower than 10, namely less than 10 high-VTE-risk lymphoma patients need to receive primary prophylaxis in order to avert 1 VTE. Throly score reported the highest quality. In MM patients, four RAMS were validated: sensitivity ranged from 40% to 85% and specificity from 43% to 72%. C-statistics of the RAMs was poor, ranging from 0.51 to 0.66. Only the IMPEDE-VTE score allowed to discriminate risk categories differing by >10% VTE risk at 6 months.

In conclusion, disease-specific VTE RAMs outperform general RAMs, however they still have suboptimal positive predictive values. Specific bleeding RAMs are also awaited.

D022

ATYPICAL SITE VEIN THROMBOSIS ASSOCIATED WITH THROMBOCYTOPENIA AFTER THE FIRST DOSE OF CHADOX1 NCOV19 VACCINE: REPORT OF TWO CASES WITH DIFFERENT CLINICAL OUTCOMES

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Introduction: Following the first administration of the ChAdOx1 nCoV19 vaccine, rare adverse events characterized by VTE in typical and atypical sites accompanied by variable degrees of thrombocytopenia were recorded, generally occurring from 1w to 3w after inoculation, especially in young women. Most involved sites were cerebral and splanchnic veins. Aim of the current work is to report on the diagnosis and management of atypical site vein thrombosis associated with severe thrombocytopenia after exposure to the first dose in two women presenting with different clinical scenarios and outcomes.

Case 1: 45-year-old woman, obese, mute medical history. Ten days after the inoculation of the first dose, she was admitted to the emergency room for abdominal pain unresponsive to analgesics. Blood tests showed severe thrombocytopenia and D-dimer increase. Contrast CT scan of abdomen showed extensive thrombosis of the spleno-mesenteric-portal axis. Continuous i.v. infusion of HMW heparin therapy was initiated with target range aPTT ratio 1.4-1.7. Severe hematemesis with acute anemia occurred about 36h after the start of therapy. Endoscopy didn't detect gastrointestinal bleeding, but haemorrhagic foci from the pharynx without any detectable lesions. Due to severe secondary hypotension, the patients was transferred to ICU. The latest blood tests suggested a pattern of DIC. A new CT scan confirmed the known thrombosis plus early signs of arterial distress, and abdominal free effusion. Rescue therapy with HD IVIG and off-label use of the anti-IL-6 Ab Tocilizumab was undertaken. Despite the therapy death occurred in 4 days after admission.

Case 2: 61-year-old woman with endometriosis. 14 days after the first dose, she had pain in the right lower limb and headache. The echo-color-doppler showed evidence of popliteal and tibial vein DVT. CT scan showed left internal jugular DVT and bilateral PE. Blood tests found thrombocytopenia and D-dimer increase. She began therapy with corticosteroids, HD IVIG and Fondaparinux. Five days after treatment she had a clinical-laboratory improvement. The patient was discharged asymptomatic with a DOAC therapy.

Conclusions: ChAdOx1 nCoV19 vaccination can result in rare cases of thrombotic thrombocytopenia, a mechanism still being studied as it is mainly attributed to the production of anti PF-4 Ab. Our deeper awareness of the disease after the first case allowed us to act more effectively in the accurate management of the second patient.

D023

SAFETY AND EFFICACY OF DOACS IN CANCER PATIENTS UNDERGOING IMMUNOTHERAPY

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Direct oral anticoagulants (DOACs) are increasingly used for venous

thromboembolism (VTE) treatment in patients with solid tumors. Recently, Moik *et al*. reported on Blood that lung cancer patients treated with immune checkpoint inhibitors (ICIs) have a high risk of developing both venous (VTE) and arterial thromboembolic (ATE) complications. However, whether the increased risk of VTEs under ICIs depends on the treatment itself or reflects baseline patient risk has not yet been established. Moreover, the role of DOACs in advanced NSCLC patients treated with ICIs has never been studied. We reviewed advanced NSCLC treated with ICIs and DOACs in our Institution and we identified 10 patients with locally advanced or metastatic NSCLC. The median duration of DOAC treatment was 17 months. Reason for starting anticoagulant treatment was pulmonary embolism (PE) in five patients (50%), deep vein thrombosis (DVT) in one patient (10%), and prophylaxis in four patients (40%). In the latter group, 3 received DOAC for previous PE and 1 one after diagnosis of NVAF. All PEs were occasionally found during cancer staging and asymptomatic, while DVTs were symptomatic. Six out of ten patients (60%) experienced VTEs under immunotherapy, and four of them showed PD-L1 tumor proportion score (TPS) >50%. In two out of six cases VTE occurred within the first three months of starting immunotherapy. One of these six patients was already on fondaparinux full dose treatment due to previous DVT. All patients received edoxaban 60 mg daily dose. None of our patients discontinued DOAC during immunotherapy and any VTE recurrence was described during DOAC treatment. No major or minor bleeding complications were observed. Our retrospective series suggests these considerations. First, none of our patients developed major or minor bleedings on DOACs, and no delays or changes in planned cancer treatment occurred. Second, none of our patients experienced VTEs recurrence after starting DOAC. In contrast, a patient developed a recurrence of VTEs despite being on a full dose of fondaparinux even if we can't exclude poor compliance with injective therapy. Type and timing of prophylactic or anticoagulant treatment in long-term responder NSCLC patients treated with ICIs currently represents an unmet need. Further studies are warranted to better defined the role of DOACs in this subgroup of patients.

D024

EFFICACY OF TREATMENT OF RHEUMATOLOGICAL DISEASE IN A CASE OF THROMBOTIC THROMBOCYTOPENIC PURPURA ASSOCIATED TO SYSTEMIC LUPUS ERYTHEMATOSUS

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Thrombotic microangiopathies (TMA) are a group of disorders caused by multiple etiologies. Thrombotic Thrombocytopenic Purpura(TTP) is due to congenital or acquired deficiency of ADAMTS 13 (including immune inhibition); secondary Hemolytic Uremic Syndrome (s-HUS) is linked to complement pathway activation by precipitating cause, including rheumatological diseases. Systemic Lupus Erythematosus(SLE) is an autoimmune disease potentially affecting each organ. One of its pathogenetic pathways involves a defect in B cell suppression leading to the production of many autoantibodies(Ab).

Case report: We describe the case of a 68-year-old woman affected by SLE. She was hospitalized due to anemia (Hb 7,7 g/dl), thrombocytopenia (PLTs 75000/mmc), left arm hypostenia and distal paraesthesia. We found high CRP and SLE specific Ab levels, hypocomplementemia, mild proteinuria and schistocytes in blood smears. She showed sensorymotor polyneuropathy and microhaemorragic and vasculitic abnormalities on brain MRI. We excluded immune hemolysis, disseminated intravascular coagulation, infections and bone marrow neoplastic infiltration. We hypotized a SLE flare with polyneuropathy, vasculitis, nephritis and sHUS, and so we started steroid boluses and plasmapheresis. We marvilled of admission ADAMTS13 tests showing presence of specific Ab and enzymatic activity < 5% as in TTP. In the meantime patient's neurological state and biochemical parameters had improved and blood count normalized. A prompt ADAMTS13 reassessment showed its normalization.So we decided to continue SLE treatment adding mycophenolate, strictly monitoring exams and clinical state. 3 months after the flare, the patient is still asymptomatic, persisting only mild left arm hypostenia. Lab tests show satisfactory Hb and normalization of CRP, PLTs, proteinuria, SLE Ab, ADAMTs 13 inhibitor and activity.

Conclusions: Scientific recommendations suggest to suspect TMA in patients showing schistocytosis associated with thrombocytopenia and non-immune hemolytic anemia. Rarely TMAs may present without peripheral hemolysis or significative thrombocytopenia, as in our case.

We think that in our patient TTP resolved thanks to treatment of underlying SLE, because it eliminated ADAMTs13 Ab production restoring normal enzymatic activity. This suggests that, in TMAs associated to SLE, treatment of underlying disease is mandatory because it could be decisive even on its own and even in case of associated TTP.

D025

SUCCESSFUL TREATMENT OF SEVERE ACQUIRED HEMOPHILIA (AHA) WITH SERIOUS BLEEDING IN AN ELDERLY PATIENT (PT)

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AHA is a rare but life threatening disorder resulting in appearance of spontaneous bleeding in individuals without past medical history or family health history of bleeding disorders. It is induced by autoantibodies targeting and inhibiting endogenous FVIII. Half of the cases are idiopathic (iAHA), whereas other recognised causes include autoimmune disorders, malignancies, pregnancy, infections, dermatologic conditions or medications. Laboratory hallmark is prolongation of the aPTT with normal PT. Treatment of AHA is based on two goals: administration of a clotting factor to reduce bleeding and use of immunomodulatory agents in order to obtain blood clearance of the inhibitor.

Clinical Case: In October 2020, a 76 years old man was admitted to hospital due to symptomatic and severe anaemia and appearance of significant right upper-extremity, axillary, and breast hematoma without previous trauma or anticoagulant use. His medical past history reported hypertension. He did not report any family health history of bleeding or thrombosis. At presentation, complete blood count showed WBC $8.27x10^9$ /L, Hb 69 g/L and PLTs $263x10^9$ /L. Coagulation tests showed normal PT and INR but aPTT was unmeasurable. Hepatic and renal function tests were normal. Mixing study revealed presence of inhibitors. FVIII activity was <0.25%, with high titre of FVIIIi (16.2 Bethesda units BU). FIX, XI, XII, XIII, and vWF activities were normal. LAC testing was negative. Secondary causes of AHA were excluded so patient was diagnosed with iAHA. Treatment with recombinant FVIIa (NovoSeven®) 90 mcg/kg every 4 hours, methylprednisolone (1 mg/kg) and rituximab 375 mg/m^2 IV weekly for 4 doses was started. After the first administration of rituximab, patient experienced a severe bleeding from femoral artery and urgent angio-embolization was performed. Following such complication and as a result of persistent low F VIII activity level of 1,4% with FVIII 3.9 BU, Cyclophosphamide 1 mg/Kg daily was added to treatment. One month after the first dose of rituximab, FVIII titre increased to 52% and inhibitor titre decreased to 0.9 BU. Coagulation tests showed normal PT and aPTT. Pt did not experience any bleeding after discontinuation of immunosuppressive therapy. FU at 3 months showed absence of clinical symptoms and normal coagulation tests with an increase in Hb levels (119 g/L). FVIII activity level was 55% and FVIIIi was 0.5 BU. Pt continues monthly FU showing normal complete blood count and aPTT.

Infections

D026

SCREENING WITH ANTI SARS COV2 NOSOPHARYNGAL SWAB BEFORE HOSPITALIZATION AND/OR ADMINISTRATION OF CHEMOTHERAPY: EXPERIENCE OF A SINGLE INSTITUTION ON 765 ONCOLOGICAL AND HEMATOLOGICAL PATIENTS

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Cancer patients (Pts), particularly those with hematological or lung cancers and metastatic disease, are at an increased risk of developing a severe COVID-19 infection. A meta-analysis from the CCC19 registry (Covid-19 and Cancer Consortium) and other cohorts showed a mortality risk in this subgroup of pts about 10 times higher (26% *vs* 2-3%) than in the general population. In addition, a reduced protective effect of the vaccine has been shown in pts treated with immunosuppressive and/or B-cell-depleting drugs. Usually, the best technique used for early detection of viral infection is RT-PCR performed on nasopharyngeal swab (NPS) samples, but it is time-consuming (takes several hours) and requires specialized laboratory personnel. Rapid antigen tests on NPS are also available recently, which give a response in about 20-30 minutes. We explored the use of SARS COV2 rapid antigen testing in pts before hospitalization and/or starting chemotherapy.

Patients and Methods: A total of 765 pts, 250 affected by haematological diseases and 515 by advanced solid tumors, were enrolled. NPS samples were collected by trained medical staff for both RT-PCR and SARS-CoV-2 Rapid Antigen Test (Roche Diagnostics Gmbh), according with manufacturer's instructions. A first validation of the techniques was performed on 361 patients who received both RT-PCR and rapid antigen tests. PPV and NPV were calculated, and correlation data were analysed with Chi Square test, using the SPSS statistical package. Subsequently, 3768 additional rapid antigenic tests were carried out.

Results: In the validation cohort, 336 pts tested negative for both techniques, 2 pts were false negative (antigenic test negative and RT-PCR positive), 22 pts tested positive for both techniques and 1 was false positive (antigenic test positive and RT-PCR negative). Statistical analysis showed a very high correlation ($p<0.0001$) between tests. NPV and PPV were 99.4% and 95.7%, respectively. A series of 3768 negative antigen tests belonged to patients who showed no clinical signs of SARS COV2 infection.

Conclusion: NPS antigen test is a rapid, reproducible, high-sensitive and inexpensive tool able to identify a SARS COV2 infection. It allows to select pts for a safe administration of chemotherapeutic drugs and, if routinely used, it could avoid the risk of admitting COVID-19 positive pts into the ward. In our hands, results totally overlap RT-PCR data and are obtained quickly without any lab support.

D027

ADENOVIRUS INFECTION IN ADULT PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT: INCIDENCE, CLINICAL MANAGEMENT AND OUTCOME

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Background: Adenovirus infection (ADVi) is a known complication in paediatric patients (pts) undergoing allogeneic hematopoietic stem cell transplant (alloHSCT). In adult alloHSCT pts incidence of ADVi is lower (6% *vs* 32%), but with reported mortality rate up to 80%. Guidelines for diagnosis and management of ADVi in alloHSCT come mainly from paediatric studies including the established cut-off to start pre-emptive treatment at ADV viremia (ADVv) > 10^3 cp/mL.

Aim of the study was to describe incidence and outcome of ADVi with particular attention to timing and progression to systemic ADV disease (sADVd) in adult alloHSCT.

Methods: We performed a retrospective study in alloHSCT performed between 01/01/2014 and 31/12/2019 at the Bone Marrow Transplant Centre of the San Martino Hospital in Genoa, Italy. ADVi and ADVd were defined according to ECIL criteria.

Results: Overall, 445 pts underwent alloHSCT during the study period. Median age was 52 years (rage, 18-73), 54% were male and 51% had an acute myeloproliferative disease. Most patients (75%) received transplant from a haploidentical donor with post-transplant cyclophosphamide GVHD prophylaxis. The ADVv monitoring increased from 35% in 2014 to 91% in 2019. Any ADVi occurred in 59 pts, including 37 ADVv (Figure 1). None of the patients developed > 1 ADVv episode. At day +180 after HSCT, the incidence of ADVv was 6% and 3.1% for ADV-DNA > 10^3 cp/mL. The median time to first positive ADVv was day +55 in all, and +111 in patients who later developed sADVd. The rate of sADVd in viremic pts was 38%. No case of sADVd occurred in patients with maximum ADVv < 10^3 cp/mL (Figure 1). Antiviral treatments were cidofovir in 9 cases and brincidofovir in 2. ADV-related death was 1.6% in the whole cohort, 18.9% among those with ADVv, and 53.8% among those with max ADVv > 10^5 cp/mL. During the study period, AdV-DNA testing was also performed in 132 blood samples from non-HSCT pts with haematological malignancies. None of the patients had ADVv > 250 cp/ml.

Conclusions: There was a progressive increase in the rate of ADVv testing from 2014 to 2019. The overall incidence of ADVv was similar to what reported for other adult HSCT centres (6%). Most cases of sADVd had a late onset. ADVv > 10^5 cp/mL was associated with high mortality, despite antiviral treatment. AdV viremia was not detected in haematological patients without HSCT. Unlike for conventional hematological malignancies, AdV is a major problem for HSCT.

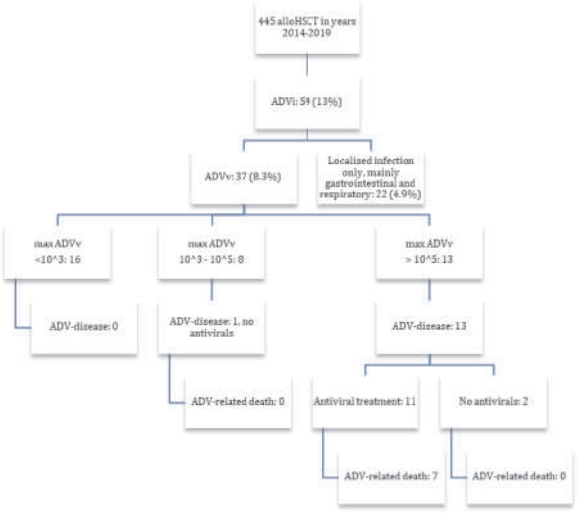

Figure 1. ADV-infections in alloHSCT adult patients.

D028

SERUM IGA RATHER THAN IGG LEVELS CORRELATE WITH LONGER SARS-COV2 VIRAL CLEARANCE AND SHORTER SURVIVAL OF ONCOHEMATOLOGIC PATIENTS

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Introduction: Recent works demonstrated that in cancer patients, severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) shedding can persist for many weeks after disease onset. IgA protect mucosal surfaces against pathogens by neutralizing respiratory viruses or impairing their attachment to epithelial cells. However, a relevant number of patients with hematological malignancies have low serum IgA levels and it is unknown whether they have a higher risk of severe coronavirus disease (COVID) or delayed viral clearance.

Aim: The aim of this study was to correlate immunological parameters with SARS-CoV2 clearance and COVID outcome in onco-hematologic patients.

Methods: Electronic medical charts of patients followed at the Hematology unit of Azienda Ospedale - Università di Padova were retrospectively reviewed to identify cases with COVID since March 2020. SARS-CoV2 infection was assessed by real-time reverse transcription PCR from a nasopharyngeal swab. Categorical variables were compared with Chi-square test. Time to viral clearance (TTVC) and overall survival (OS) were calculated from SARS-CoV2 swab positivity to negativity and last available follow-up, respectively. Survival curves where compared with Log-rank test.

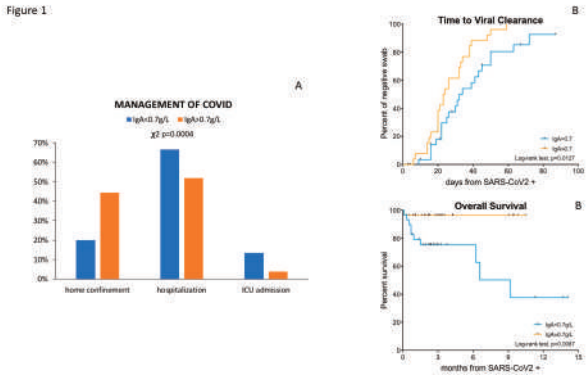

Figure 1.

Results: Eighty-three patients had SARS-CoV2 infection till February 2021. The median age was 71 years, 72% were male, and 41% were under treatment at COVID. Overall 53% of the patients were hospitalized and 9.6% required intensive care unit (ICU) admission. The median absolute lymphocyte count was 1,419/uL of which CD3+ were 48%, 37% CD8+ T cells and 9% normal B cells. Mean CD4+ were 1,160/uL, serum IgG level 8.46g/L, IgA 1.10g/L and IgM 0.81g/L. After a median follow-up of 4 months 88% patients were alive and the median TTVC was 28 days. We observed that patients with serum IgA<0.7g/L, more commonly were hospitalized (67% *vs* 52%) and required ICU admission (13% *vs* 4%, p=0.004, Figure 1A). In addition, patients with low IgA had a longer TTVC (34 *vs* 23 days, p=0.0127, Figure 1B) and shorter OS (9.2months *vs* not reached, p=0.0087, Figure 1C). Conversely serum IgG or IgM, CD4 levels did not correlate with TTVC and OS. These data were confirmed in multivariate analysis.

Conclusions: We herein compared the outcome of SARS-CoV2 infection in patients with hematological malignancies with immunological parameters. Serum IgA emerged as a key variable in limiting SARS-CoV2 shedding and patients' survival, that deserves further investigation.

D029

HEPATITIS E IN PATIENS WITH LYMPHOPROLIFERATIVE DISORDERS: A PROSPECTIVE OBSERVATIONAL STUDY

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Hepatitis E virus (HEV) infection is an emerging disease in industrialized countries. HEV is a positive-sense single-stranded RNA virus, whose infection is not limited to the liver but may affect other organs. Indeed, HEV infection shares with hepatitis C virus (HCV) several extrahepatic manifestations, such as glomerulonephritis, pancreatitis, thyroiditis and thrombocytopenia, among others. There have been claims that HEV might reactivate and induce liver toxicity in patients treated for hematological malignancies (HM). For this reason, since 1st of June 2019 to 1st of April 2021 we tested for HEV antibodies all consecutive patients with HM presenting to our out-patient department in need of therapy. Serological tests for HEV infection for IgM and IgG were performed by enzyme-linked fluorescent assay (bioMerieux SA), and confirmed by WANTAI test. Serum HEV-RNA was determined by RT-PCR (Cobas 6800-HEV-Roche). Liver function was assessed by means of AST, ALT and bilirubin levels, which were monitored at every cycle, while upper abdomen echography was performed at baseline and at the end of treatment. Four-hundred and twenty-four patients were included. HEV serology was positive in 23 patients (5.42%). All positive patients had IgG antibodies, with no patient either IgM- or RNA-positive. Interestingly, all IgG+ patients had lymphoproliferative disorders (7 DLBCL, 5 FL, 1 HL, 2 NHL T, 3 MM, 4 NHL different from FL and DLBCL), except one myelodysplastic syndrome. At baseline no patient had signs or symptoms related to liver disfunction (maximum level of ALT 47 U/L). All patients received antineoplastic treatment: immunochemotherapy (*i.e.* rituximab-chemotherapy) in 22, BTK inhibitors in 1, and 3 proceeded to autologous transplant. Liver function remained in the range of normality in all patients, except two. These were both affected by FL in second relapse, and had sudden but reversible increase of ALT (x3) after a cycle of R-DHAOX. No further sign of liver dysfunction (bilirubin level, AST, echography) was noted in the other patients. No extrahepatic manifestations were registered. In conclusion, patients with HM and HEV previous infection can reliably undergo standard therapy, inclusive of immunotherapy, with reversible hepatic flares not exceeding 10% of them. Epidemiological studies are ongoing to address the impact of the virus in HM.

D030

CLINICAL CHARACTERISTICS AND OUTCOME OF INVASIVE INFECTIONS DUE TO SAPROCHAETE SPECIES IN PATIENTS AFFECTED BY HEMATOLOGICAL MALIGNANCIES. A MULTICENTER STUDY ON BEHALF OF SEIFEM/FUNGISCOPE REGISTRY

M.I. Del Principe[1], M. Criscuolo[2], D. Seidel[3,21], M. Dargenio[4], Z. Ráčil[5], M. Piedimonte[6], F. Marchesi[7], G. Nadali[8], P. Koehler[3,21], N. Fracchiolla[9], C. Cattaneo[10], N. Klimko[11], A. Spolzino[12], D. Yilmaz Karapinar[13], H. Demiraslan[14], R. Duarte[15], J. Demeter[16], M. Stanziani[17], L. Melillo[18], C.M. Basilico[19], S. Cesaro[20], G. Paterno[1], O.A. Cornely[3,21,22], C. Califano[23], M. Delia[24], A. Busca[25], L. Pagano[2] on behalf of the SEIFEMSorveglianza Epidemiologica Infezioni nelle Emopatie and FUNGISCOPEGlobal Emerging Fungal Infection Registry

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Invasive Saprochaeta species (*S.spp.*) infections are an emerging threat in patients with hematological malignancies (HM). Owing the difficulty of isolation and the intrinsic resistance to echinocandins, these infections are associated with high mortality rates. To identify baseline factors and provide a basis for therapeutic decisions, we conducted a retrospective multicenter study. All cases of proven *S. capitata* and *S. clavata* infection, observed from January 2010 to December 2020 in HM patients, were collected from SEIFEM (Sorveglianza Epidemiologica Infezioni nelle Emopatie) group and from FUNGISCOPE (Global Emerging Fungal Infection Registry) database. The characteristics of our patients were compared with those of a group of HM patients with *Candida* (*C.*) *spp*. infection, matched for age and treatment. We recorded 88 *S.spp* cases, median age 54 years (range 2-78), 44 patients (50%) were female and 65(74%) had a diagnosis of acute leukemia. Of these, 63(72%) were classified as *S. capitata* infections and 25(28%) as *S. clavata*. In univariate analysis, the infection of *S. clavata* was associated with age <60 years (21/25 patients, p= .01). Overall, 86% cases presented fungemia. Focal or disseminated organ involvement was observed in 36% of cases. Antifungal prophylaxis (AP) and the central venous catheter (CVC) correlated with *S.spp* (p=.000) and *C. spp* infections (p=.004), respectively. Thirty-six (40%) *S.spp* cases were breakthrough infections as occurring during AP, mainly anti- mold AP. Two patients didn't receive antifungal therapy (AT). The AT was liposomal amphotericin B (L-AMB) in 37(42%), azoles in 25 (29%), echinocandins in 24(27%) and combination (azoles plus L-AMB) in 7 (8%) patients. The efficacy of first AT was observed in 8/37 (22%), 15/25 (60%) and 1/24(4%) of patients who received L-AMB, azoles and echinocandins, respectively (p=ns). CVC was removed after fungal isolation in 42/77 (54%). Mortality rate at 30 days was 39%. Parameters that influenced outcome were the age>60years (p=.007), the septic shock (p=.01), the duration of steroid treatment (p=.03) and the neutrophil recovery (p=.000). In multivariate analysis, only parameter influencing the outcome was the neutrophil recovery (OR: 8.18, 95%CI 1.942-33,112, p=.004). In conclusion, *S.spp* infections are often breakthrough infections, the most effective treatment for which has not yet been established, but neutrophil recovery appears to play an important role in the favorable outcome.

D031

SARS-COV2 INFECTION AND HAEMATOLOGICAL MALIGNANCIES: A PROSPECTIVE OBSERVATIONAL SINGLE CENTRE 1-YEAR-LONG EXPERIENCE

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SARS-CoV-2 infection represents a major threat for frail patient populations. Case series suggested that cancer patients have a poor outcome following COVID-19, due to their underlying conditions and cytotoxic treatments. We prospectively enrolled all consecutive patients with hematological malignancy (HM) and RT-PCR positive nasopharyngeal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) at the hematology department of Verona, Italy, since March 1st 2020 to March 1st 2021. The aim of this study was to evaluate overall survival (OS) and infection fatality rate (IFR) according to patients' clinical and laboratory characteristics. One-hundred and ten patients were included. Median age was 69 years (range 24-93), and 70 were males (64%). Median Charlson comorbidity index was 3 (0-13). Fifty-eight percent of patients were admitted to the hospital, while the remaining were followed-up as outpatient. Overall, 34% had a diagnosis of lymphoproliferative disorder (13% chronic lymphocytic leukemia), 32% multiple myeloma, 27% myeloproliferative diseases (10% MDS/LAM), and 8% Hodgkin lymphoma. Median time from HM diagnosis to SARS-CoV2 infection was 29 months (1-300). Among admitted patients, the severity of disease was mild in 32%, and severe or critical in 68%, with 21% necessitating intensive care procedures. 61% of patients had active HM at the time of SARS-COV2 diagnosis, and 58% were on active therapy. Overall, IFR was 35.8%. Three-months OS was 62%+/-6% (Figure 1A). As expected, admitted patients had significantly worse OS than not admitted (p<0.0001). No significant difference for OS was observed between different histologies (p=0.91), and active ongoing treatment for HM at the time of COVID infection also did not impact on OS (p=0.69). Univariate analysis revealed that age, male gender, anemia, thrombocytopenia, active smokers, active HM, and COVID severity at the time of the positive swab were associated with impaired OS. In multivariate analysis anemia (Hb<10 g/dL; HR 3.6) and COVID severity (severe/critical; HR 4.3) retained independent significance. OS curves stratified for the number of these 2 independent risk factors are shown in Figure 1B.

In conclusion, this unicentric series confirmed the high IFR of patients with SARS-CoV2 and HM. Our study highlights the importance of not postponing life-saving therapies in HM patients in need of therapy and emphasizes the importance of early vaccination strategies.

Figure 1.
A) OS of all patients with HM
B) OS of all patients by number of risk factors

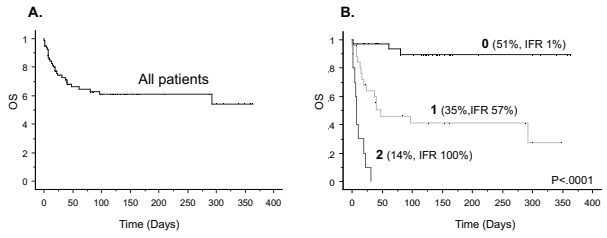

Figure 1.

D032

ABSTRACT WITHDRAWN

D033

BLOODSTREAM INFECTIONS CAUSED BY STRONG BIOFILM-PRODUCING BACTERIA INCREASE THE RISK OF END-ORGAN DISEASE AND MORTALITY IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Bacterial bloodstream infection (BSI) represents a significant complication in patients with hematological malignancies (HM). However, factors leading to BSI and progression to end-organ disease and death are only partially understood. The study analyzes host and microbial risk factors and assesses their predicted impact on the development of BSI and mortality. A total of 96 patients with HM and BSI were included in the study. Host-associated risk factors and all-causes of mortality were analyzed by multivariable logistic regression at 30 days after the onset of the first BSI in the first neutropenic episode. The level of biofilm production of bacterial isolates was analyzed by the clinical BioFilm Ring Test. The median age was 60 years (range 20-77 years). The underlying diagnoses were acute leukemia n=53 (55%), lymphoma n=30 (31%) and myeloma n=13 (14%). Bacterial isolates from BSI were 96. Escherichia coli was the most common isolate (n=28, 29.2%), followed by Pseudomonas aeruginosa (n=16, 16.7%). MDR (n=10) caused 10.4% of bacteremia episodes. Weak biofilm producers were significantly (p<0.0001) more abundant (72.2%) than strong (27.8%) biofilm-producers. Specifically, strong biofilm-producers were 9.6% for *E. coli,* 100% for *P. aeruginosa*, 50% for *K. pneumoniae*, and 23.3% for Coagulase-negative Staphylococcus spp. (CoNS). Mortality at day 30 was 8.3% (8/96), and all deaths were attributable to Gram-negatives. About 22% of all BSI were catheter-related (CRBSI). The mortality rate (p=0.62) and biofilm production level (p=0.75) were not correlated with CRBSI. Notably, strong biofilm-producing bacteria were an independent risk factor (p=0.018) associated with the end-organ disease. Besides, multivariate analysis indicated that the presence of strong biofilm-producing bacteria (p=0.013) and MDR strains (p=0.006) were independent risk factors associated with 30-day mortality. Strong biofilm-producing bacteria and MDR strains caused a limited fraction of BSI in patients with HM. Strong biofilm-producing bacteria present a high risk of end-organ disease and that, together with an MDR phenotype, are significantly and independently associated with an increased risk of death. The rapid identification of biofilm-producing bacteria from BSI can offer a key biomarker to predict the clinical and therapeutic outcomes in patients with HM.

D034

OUTCOMES OF COVID-19 IN CELLULAR THERAPY RECIPIENTS: REAL-LIFE APPLICATION OF CIBMTR RISK FACTORS

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Patients with haematological malignancies and COVID-19 have worse outcomes than both the general population with COVID-19 and patients with haematological malignancies without COVID-19. According to a CIMBTR analysis, Allo-HSCT patients who develop COVID-19 showed poor overall survival (OS), with age 50 years or older, male sex and development of COVID-19 within 12 months of transplantation as factors associated with a higher risk of mortality. We analyzed data from 40 consecutive patients (38 allo-HSCT and 2 patients treated with CAR-T cell) with COVID-19 (inclusion criteria: SARS-CoV-2 positivity, aged >/= 18 years) with available data on outcome who were followed at our Hematology and Bone-Marrow Transplantation Unit as previously reported between 23 February 2020 and 15 March 2021. The median time from allo-HSCT to COVID-19 diagnosis was 34 months (range 2-209 months). The median follow-up of survivors after COVID-19 was 115 days (range 15-379). 15 (37,5%) allo-HSCT recipients were receiving immunosuppression within 6 months of COVID-19 diagnosis, active GvHD was reported in 16 (40%) allo-HSCT recipients. COVID-19 severity was mild in 30 (75%), while severe disease requiring mechanical ventilation occurred in 4 (10%). At 30 days after the diagnosis of COVID-19, OS was 95% (95% CI 81-99%). Patients' stratification according to risk-factors (age >/= 50 years, male sex, time from transplantation <12 months) underlined the intrinsic impact on time to clearance of viremia: 40 days (median, range 7-87) in patients with 2-3 risk factors (11 patients), 21 days (median, range 7-63) in patients with 0-1 risk factors (25 patients) - p 0.05 (Mann-Whitney U test). The need for stringent surveillance and aggressive treatment measures in allo-HSCT recipients who develop COVID-19 is widely acknowledged. We presented better OS data than other reports, even though time to resolution of infection is influenced by the number of risk factors. The massive campaign of vaccination will hopefully reshape this scenario, improving the possibility of resolution of COVID-19 in a high-vulnerable population. Moreover, the awareness of the impact of the three risk factors is crucial for a patient tailored counseling.

D035

REGULAR SCREENING FOR SARS-COV-2 IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES ON ACTIVE ANTICANCER TREATMENT IN THE OUTPATIENT SETTING

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Introduction: SARS-CoV-2 infection has a heterogeneous and unpredictable course, ranging from asymptomatic patients to fatal cases. Sev-

eral studies demonstrated that patients with cancer have higher morbidity and mortality from COVID-19 as compared with the general population. During the epidemic outbreaks, the delivery of anticancer treatments in outpatient facilities has been preferred over in-patient treatment to reduce both the burden on healthcare system and the exposure of patients to infection. Early recognition and management of suspected cases are also determinant to improve patients' outcome and to reduce the in-hospital spread of virus. Here we report the results of systematic screening for SARS-CoV2 infection in asymptomatic patients with hematologic malignancies on active anticancer treatment in the outpatient setting.

Patients and Methods: Patients with hematological malignancies treated with chemotherapy and/or immunotherapy in the outpatient facility of the Division of Hematology of Fondazione IRCCS Policlinico San Matteo between November 15th 2020 and April 15th 2021 were tested for SARS-CoV-2 infection before each cycle of therapy. SARS-CoV-2 infection was ascertained on nasopharyngeal swab specimens by means of reverse transcriptase-polymerase chain reaction (RT-PCR) assay.

Results. We analyzed 846 nasopharyngeal swabs from 253 consecutive patients. The median number of swabs per patient was 3 (range: 1-7). The diagnosis and type of treatment are shown in Table 1. Eleven of 253 patients (4%) tested positive to screening swab, corresponding to 11 out of 846 swabs (1.3%). One of 11 patients (9%) died, 10 (91%) recovered from infection and 8 could restart treatment. Characteristics and outcome of patients positive to a screening swab are reported in Table 2. Outside screening, SARS-CoV-2 infection was diagnosed in 10 additional patients (4%), who were tested for fever (n=6), contact with a positive subject (n=2), hospitalization for other reasons (n=2). Six of 10 patients developed interstitial pneumonia and 3/10 (30%) died.

Conclusions: Over a 5-month period, 4% of asymptomatic patients on active anticancer treatment tested positive to screening swab. Most of them had a good outcome and could successfully resume therapy. The early identification of these asymptomatic cases lead to prompt interruption of immunosuppressive therapy and immediate isolation of patients, likely improving their outcome and preventing in-hospital spread of virus.

Tables 1 and 2.

Table 1 - Characteristics of patients with hematologic malignancies on active anticancer treatment screened for SARS-CoV2

Characteristic	
Sex, n. of patients (%)	
male	145 (57%)
female	108 (43%)
Age (years), median (IQR)	68 (59-75)
Diagnosis	
Multiple myeloma	92 (36%)
Indolent lymphoma	61 (24%)
Aggressive lymphoma	42 (17%)
Hodgkin lymphoma	20 (8%)
Acute myeloid leukemia/myelodysplastic syndrome	15 (6%)
Acute lymphoblastic leukemia	9 (4%)
Chronic lymphoid leukemias	8 (3%)
Other	6 (2%)
Type of therapy	
Including monoclonal antibodies	168 (66%)
Without monoclonal antibodies	85 (34%)

Table 2 - Characteristics and outcome of patients positive at screening for SARS-CoV-2

Patient, sex, age	Diagnosis	Treatment	Onset of symptoms after screening	Hospitalization	Outcome	Treatment resumed
LC, F 62y	HL	AVD	no	no	resolved	no
ADL, F 23y	HL	Brentuximab	fatigue	no	resolved	yes
LC, F 60y	Myeloma	Dara-RD	no	no	resolved	yes
VDA, M 83y	Myeloma	Elo-RD	no	no	resolved	yes
NL, M 66y	Myeloma	Carfilzomib Dexamethasone	no	no	resolved	yes
ZM, M 48y	HL	Nivolumab	no	no	resolved	yes
RM, M 66y	Follicular lymphoma	Obinutuzumab	interstitial pneumonia	yes	resolved	no
GD, M 66y	Follicular lymphoma	Rituximab Bendamustine	interstitial pneumonia	yes	died	NA
ER, M 77y	Follicular lymphoma	Rituximab Bendamustine	no	no	resolved	yes
LM, M 70y	Follicular lymphoma	R-CHOP	interstitial pneumonia	no	resolved	yes
AR, F 70y	Myeloma	Dara-RD	no	no	resolved	yes

D036

PRO-ADRENOMEDULLIN IN PREDICTING SEVERE INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Despite progresses in treatment, infections remain an important cause of morbidity and mortality, in patients with hematological malignancies (HM). However, no biochemical marker has yet been identified that can direct the diagnosis of infection in febrile HM patients and predict its severity and outcome. This study evaluates the ability of two biochemical markers, pro-calcitonin (PCT) and pro-adrenomedullin (proADM) to identify the development of sepsis or septic shock in febrile HM patients, neutropenic (FN, 81%) and non-neutropenic (FNN, 19%), as well as the ability of these markers to predict patient mortality at 30 days. Between January 2019 and August 2020 73 cases of febrile episodes were registered from 53 patients followed at the Hematology Unit of Policlinico Tor Vergata in Rome, Italy. The median age was 59 years (range 18 to 79 years) and 62% of patients were male. Thirty-six patients (68%) had acute leukemia. Febrile episodes were: fever (18%), pneumonia (52%), sepsis (47%) and septic shock (23%). (Table 1). PCT and proADM were assessed at different endpoints (day 1, 3, and 5 from the onset of fever). We observed that PCT was able to predict sepsis both in FNN patients from day 3 ($p<0,001$, cut-off 0,32 ng/mL) and in FN patients from day 5 ($p<0,03$, cut-off 0,13 ng/mL). ProADM was not useful in diagnosing sepsis in FN patients, but was able to identify sepsis in FNN patients already from the day 1 ($p<0,001$, cut-off 0,80 nmol/L). With regard to the ability to predict septic shock, both markers were effective from day 1 both in FN (PCT $p<0,001$, cut-off 0,33 ng/mL; proADM $p<0,001$, cut-off 2,07 nmol/L) and in FNN patients (PCT $p<0,001$, cut-off 1,03 ng/mL; proADM $p<0,001$, cut-off 2,07 nmol/L). While PCT was able to predict mortality at 30 days only in FNN patients ($p<0,001$, cut-off 1,03 ng/mL), proADM predicted 30 days mortality in both groups even when using the sample collected at fever onset ($p<0,002$, cut-off 0,87 nmol/L in FN; $p<0,0001$, cut-off 1,08 nmol/L in FNN). Moreover, Pro-ADM levels were significantly lower in FN ($p<0,044$, cut-off 0,8 nmol/L) than in the FNN group ($p<0,001$, cut-off 1,7 nmol/L) possibly indicating a likelihood of poor prognosis. Given the characteristics of proADM as a marker of organ dysfunction and our results in FNN, larger prospective studies are warranted to clarify the role of proADM in identifying FN patients with HM at risk of developing severe infections and, therefore, in need of specific and timely therapeutic interventions.

Table 1. Patients and Febrile Episode Characteristics.

Characteristic	N° (%)
Total n° patients	53
AML[a]	30 (57%)
ALL[b]	6 (11%)
CLL[c]	1 (2%)
CMML[d]	1 (2%)
NHL[e]	12 (22%)
HL[f]	2 (4%)
MM[g]	1 (2%)
Age (years)	
Median (range)	59 (18-79)
Gender	
Male	33 (62%)
Female	20 (38%)
Total n° of febrile episodes	73
Sepsis	34 (47%)
Septic Shock	17 (23%)
Fever	13 (18%)
Pneumonia	38 (52%)
Total n° of dead patients	16 (30%)
N° of dead patients at 30 days	11 (21%)

[a]AML Acute myeloid leukemia; [b]ALL Acute lymphoblastic leukemia ; [c]CLL Chronic lymphotic leukemia; [d]CMML Chronic myelomonocytic leukemia; [e]NHL Non-Hodgkin lymphoma; [f]HL Hodgkin lymphoma; [g]MM Multiple myeloma.

D037

INCIDENCE OF PNEUMOCYSTIS JIROVECII PNEUMONIA IN PATIENTS WITH PREVUOUSLY UNTREATED ACUTE MYELOID LEUKEMIA AND DURING INDUCTION THERAPY

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Background: Several studies in immunocompromised patients (pts),

such as those with HIV infection, undergoing cancer chemotherapy or organ transplant, have led to the development of guidelines for the use of prophylaxis to prevent Pneumocystis jirovecii pneumonia (PJP) in these specific categories. Instead, since the association between PJP and acute myeloid leukemia (AML) is not clearly defined, the role of prophylaxis in pts with AML is not yet established.

Methods: We retrospectively analyzed all consecutive pts with newly diagnosed non-M3 AML, admitted to the Hematology Department of University Tor Vergata in Rome, during the period 2010-2020.

Table 1.

N. of patients	251
Sex	
Male	153 (61%)
Female	98 (39%)
Age	median 62 yrs
<65 yrs	145 (57,8%)
>65 yrs	106 (42,2%)
BAL performed:	67
prior first line therapy	39 (58,2%)
after first line therapy	28 (43,3%)
AML treatment	
intensive chemotherapy	179 (71,3%)
non intensive treatment	36 (14,3%)
supportive care	36 (14,3%)
PJ+ BAL	11/67 (16,4%)
prior first line therapy	2/39 (5,1%)
after first line therapy	9/28 (32%)
Comorbidities	
none	105 (41,8%)
one or more	146 (58,2%)
Co-present lung infections	
Aspergillosis	5/11 (45,5%)
Bacterial pneumonia	1/11 (9%)
CMV	1/11 (9%)
None	3/11 (27,3%)
Lung HRTC scan findings	
Typical	6/11 (54,5%)
Atypical	5/11 (45,5%)

BAL, bronchoalveolar lavage; AML, Acute Myeloid Leukemia; PJ, Pneumocystis jirovecii; CMV, Cytomegalovirus; HRTC, High-Resolution Computed Tomography

Results: Among 251 consecutive pts with non M3-AML (61% males, median age 62 years), 179 were submitted to intensive chemotherapy (IC), 36 to non-intensive treatment (NIT) and 36 received only supportive care. Bronchoalveolar lavage (BAL) was performed in 67 patients, in 39 (58,2%) of them before starting any antineoplastic treatment, in 28 (43,3%) during aplasia induced by the first cycle of antineoplastic therapy-(20 after IC and 8 after NIT). PJ infection was demonstrated in 11/67 (16,7%) of BAL (11 males, median age 71 years), with an incidence of 4,3% among our series of pts. Two PJP (18,2%) occurred in untreated pts, 7/11 (63,6%) in pts submitted to IC and 2/11 (18,2%) after the first cycle of NIT. A chest Computed Tomography in all pts with PJP revealed ground-glass opacities, 5/11 (45%) pts showed also atypical features as consolidations and nodules (Table 1). Eight pts (73%) presented fever before BAL. Following PJP diagnosis, all pts were treated with trimethoprim/sulfamethoxazole intravenously. Nine (82%) pts developed severe hypoxemia, requiring high-flow oxygen with at least 50% FiO2. One patient died because of PJP, while two pts (18%) died because of AML progression with active PJP. Eight pts (73%) survived until discharge from hospital. In univariate analysis, being older than 65 years (OR 15, 95%CI 1,89-119,08; p=0.001), the presence of one or more comorbidities (OR 7,65, 95%CI 0,96-60,69; p=0.028), smoke habits (OR 14, 95%CI 1,76-111,12; p=0.001) were significantly associated with PJP. In multivariate analysis older age and smoking habit remain significant as independent factors associated with PJP (p=0.021 and 0.017 respectively).

Conclusion. In our experience, PJP is not uncommon among pts with AML. In clinical care of AML, awareness of PJP should be heightened and prophylaxis should be considered, particularly in older pts.

D038

SEROLOGICAL RESPONSE TO SARS-COV2 VACCINATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: PRELIMINARY DATA OF A PROSPECTIVE, MULTICENTER, OBSERVATIONAL STUDY "CERVAX"

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Patients (pts) with hematological malignancies are, usually, poor responder to vaccinations due to the immune incompetence induced by the disease and/or the treatments received, resulting in profound and lasting suppression of B, CD4-T lymphocytes, and hypogammaglobulinemia. In the SARS-COV2 pandemic era, their vulnerability exposes them to a fatal outcome, due to COVID19 infection, in up to 40% of cases. Previous studies with mRNA SARS-COV2 vaccination, did not test this formulation on hematologic pts. Therefore, a prospective, multicenter, observational study (CERVAX) to assess the post-vaccination serological response (time of acquisition and maintenance of immunity) in a cohort of pts with hematological malignancies, negative for COVID19 infection, undergoing mRNA SARS-COV2 vaccination, was developed. Subjects with lymphomas/chronic lymphoproliferative disorders (NHL/CLL) and, multiple myeloma (MM), off therapy for at least 3 months, in watch-and-wait or, in treatment with BTK inhibitors, BCL-2 inhibitors, IMIDs are included.

Table 1. Clinical and biologic features of patients evaluable at T1 (N=19)

	Pts	Age (yrs)	HM	Ongoing treatment	Baseline T0											IgG anti SARS-COV2* (AU/ml) T1
					WBC (x10³/L)	Neutr (x10³/L)	Lymph (x10³/L)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	CD3 (x10³/L)	CD4 (x10³/L)	CD8 (x10³/L)	CD19 (x10³/L)	IgG anti SARS-COV2* (AU/ml)	
NHL/CLL	1	81	CLL	Ibrutinib	6,8	3,3	2,8	492	46	17	2,55	0,93	1,70	0,33	1	1
	2	80	CLL	Venetoclax	541,5	11,4	488,1	1489	10	18	7,23	3,37	3,86	472,87	0,5	0,5
	3	88	NHL	None	24,1	2,2	21,2	1421	731	568	18,67	1,15	0,23	0,242	0	1,3
	4	86	NHL	None	4	2,1	1,2	866	52	7736	0,77	0,60	0,15	0,02	1,3	7,7
	5	84	NHL	None	6,9	3,3	2,5	167	20	138	1,71	0,58	1,17	0,43	0	0,5
	6	87	NHL	None	6,1	4,1	1,2	1385	263	72	0,88	0,63	0,25	0,04	10,3	50,5
	7	86	NHL	None	6,4	4,2	1,5	1296	258	61	0,77	0,52	0,27	0,28	0	82,7
	8	84	NHL	None	8,6	5,8	2	1081	538	56	0,87	0,35	0,47	0,47	1,6	79,9
	9	75	NHL	Ibrutinib	5,6	2,6	2	766	156	818	1,84	0,94	0,92	0,002	2,4	1,2
	10	73	NHL	None	5,6	3,3	1,6	631	129	192	0,93	0,60	0,34	295	0,3	110,2
	11	63	NHL/CLL	None	3,3	2,1	0,79	1077	134	95	0,45	0,29	0,11	0,03	0,9	71,4
	12	72	NHL	None	3,7	2,1	0,73	667	58	17	0,48	0,19	0,32	0	3,3	2,4
	13	68	NHL	None	3,6	2,6	0,62	272	10	23	0,36	0,18	0,16	0,14	1	39,7
	14	80	CLL	Venetoclax	5,5	3,6	1,03	374	15	42	0,96	0,31	0,65	0,001	1,2	0,2
MM	15	86	MM	None	6	3,5	1,8	603	3170	30	1,47	0,59	0,93	0,12	4	259,9
	16	80	MM	None	5,7	2,9	2,1	631	2315	37	1,78	0,79	0,71	0,18	0,9	424,7
	17	81	MM	None	6,4	4,2	1,47	461	2765	19	1,01	0,83	0,17	0,19	1,2	122,6
	18	83	MM	Lenalidomide	2,5	0,8	1,3	1619	388	32	1,11	0,28	0,79	0,004	0	1,8
	19	64	MM	None	6,5	3,2	2,3	2513	157	26	1,56	0,84	0,73	0,15	2,6	211,8

HM: hematological malignancies; WBC: white blood cell; NHL: non-hodgkin lymphoma; CLL: chronic lymphocytic leukemia; MM: multiple myeloma; *IgG anti SARS-COV2 positive if >50 AU/ml.

Complete blood count (CBC), IgG, IgA, IgM, B/T lymphocyte subpopulations and IgG anti-SARS-COV2 are evaluated as baseline (T0). The assessment of serological response to vaccination will be performed at different time points: before the second dose (T1), and then at 3-6-12 months after first dose (T2-3-4, respectively). The SARS-CoV2 IgG II Quant Assay (Abbott Core Laboratory) will be used. Two hundred pts are expected to be enrolled. Since March 2021, 36 pts have been included in the study: 24 (67%) with NHL/CLL and 12 (33%) with MM. Median age was 80 years (range 63-91). In 19/36 (53%), T1 is available. Clinical and biologic features of patients evaluable at T1 are shown in Table 1. A positive response (IgG anti-SARS-COV2 >50 AU/ml) to first dose (T1) was observed in 9/19 pts: 5/14 (36%) with NHL/CLL and 4/5 (80%) with MM. Current data are preliminary, as enrollment is still ongoing. More consistent data will be available in the upcoming months.

D039

CLINICAL CHARACTERISTICS AND OUTCOME OF 125 POLYMICROBIAL BLOODSTREAM INFECTIONS IN HEMATOLOGIC PATIENTS. AN 11 YEARS EPIDEMIOLOGIC SURVEY

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Background: Polymicrobial-Bloodstream-Infections (pBSI) occurring in hematological patients are still poorly understood and specific information are very limited.

Objectives and methods: in this epidemiologic survey we describe clinical characteristics and outcome of 125 consecutive p-BSI occurred in onco-hematological patients. Polymicrobial-Bloodstream-Infections (pBSI) was defined with the isolation of 2 or more bacteria from blood culture specimens obtained within 72h.

Results: over an 11-years period we documented 500 bacterial-bloodstream-infections (BSI) in 4542 hospital admissions and 25% (125) of these were pBSI (Figure 1). Most common underlying hematological disease was acute myeloid leukemia and 89% of patients had severe neutropenia. Fifty pBSI (40%) occurred in SCT-patients, mostly within 30 days from SCT (42/50-84%). Principal bacterial association was Gram-positive plus Gram-negative (57%). Resolution rate of pBSI was 82%, without differences between SCT and non-SCT cases. pBSI-related mortality was 15% (6% in SCT-cases). Septic shock occurred in 16% of cases and septic shock-related mortality was 65% (75% in SCT-cases and 63% in non-SCT-cases; p=0,6). Multidrug-Resistant (MDR) bacteria were involved in 22% of pBSI and the MDR-pBSI-related mortality was significantly higher in SCT-patients (p=0,007).

Conclusions: this observational study highlights that pBSI is not a rare bloodstream infectious complication in onco-hematological patients. pBSI-related mortality is lower than 20% but, if septic shock occurs, mortality reaches 65%. MDR-bacteria were involved in 22% of cases and pBSI-MDR-related mortality was significantly higher in SCT patients.

FIGURE 1. Distribution of Bloodstream Infections (BSI) and Infection Related Mortality (IRM).

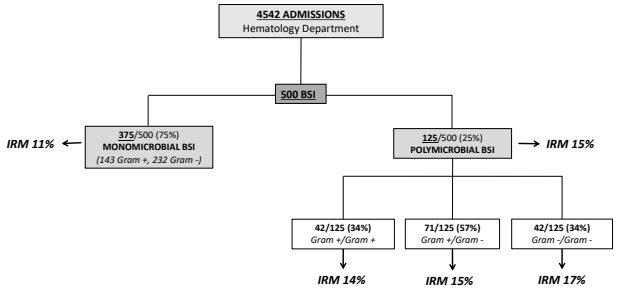

Figure 1.

D040

CLINICAL FEATURES AND OUTCOME OF SARS-COV2 INFECTION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. A REAL-LIFE STUDY FROM PADUA UNIVERSITY HOSPITAL

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) causes a heterogeneous coronavirus disease (COVID19), ranging from an asymptomatic infection to a life-threatening inflammatory syndrome. Recent data suggest that the illness from COVID19 is more severe among patients with hematologic malignancies. However, the median follow-up in most studies is short (range 30-60days).

Aim: The aim of this study is to describe the clinical features and outcome of COVID19 in onco-hematologic patients followed at Padova University Hospital.

Methods: Medical charts of patients followed at the Hematology Unit of Padova University were retrospectively reviewed to identify cases with COVID19 till 28 Feb 2021. SARS-CoV2 infection was assessed by real-time reverse transcription polymerase chain reaction from nasopharyngeal swabs. Continuous variables were compared with Wilcoxon sum test, while categorical variables were compared with Fisher's exact or Chi-square test. Overall survival (OS) was calculated from SARS-CoV2 swab positivity to last available follow-up or death. Survival curves were compared with Log-rank test.

Results: RESULTS. Eighty-three patients had SARS-CoV2 infection between March 2020 and February 2021. Their median age was 71 years (range 40-93), 36% ≥75years, 72% were male, 84% had comorbidities and in 29% CIRS was ≥6. Sixty-six % of patients received therapy for their hematologic disease and 41% were under treatment at COVID19.

Overall 53% of the patients needed hospitalization and 9.6% required Intensive Care Unit (ICU) admission. After a median follow-up of 4 months, 12 patients died, 9 due to SARS-CoV2 infection. Mortality was similar to general population in patients managed at home (2.7%), but increases among hospitalized patients (22.5%) and those needing ICU admission (33.3%, p=0.0187, Figure 1A). Patients who died were older (66.7%≥75years) and more comorbid (78% CIRS≥6).

The median OS was not reached and after 6 months from COVID19 88% of patients were alive. In univariate analysis CIRS≥6 (HR 10.5, p=0.0001), age>75years (HR 4.4, p=0.0154) (Figure 1B-C) and hospitalization (HR 4.1, p=0.0065) were associated with a shorter OS. Conversely, gender, the type of the disease, active treatment and low IgG had no impact on OS.

Conclusions: We herein analyzed the outcome of SARS-CoV2 infection in patients with hematological malignancies. We found that elderly, comorbid patients, with a severe infection requiring hospitalization had a poor outcome.

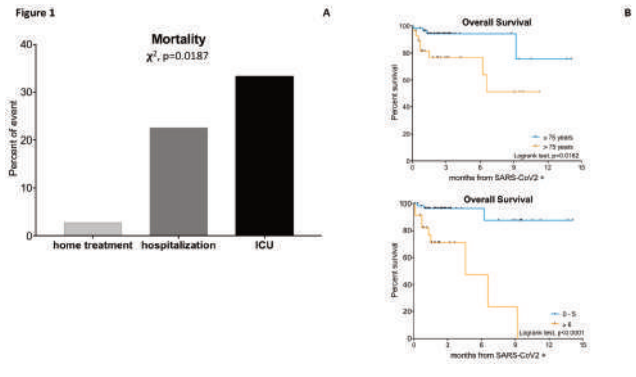

Figure 1.

D041

HEALTH CARE-ASSOCIATED INFECTIONS AND VISITING POLICIES IN A HEMATOLOGY UNIT: A RETROSPECTIVE OBSERVATIONAL STUDY TO ASSESS THE IMPACT OF RESTRICTIVE VISITING POLICY ON THE INCIDENCE OF INFECTIONS

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Most Intensive Care Units (ICUs) worldwide adopt restrictive visiting policies to avoid the risk of an increased rate of acquired infections, though there is no evidence demonstrating a correlation between partially unrestrictive visiting policies and an increased incidence of infections. This concept is particularly stressed in Hematology Units, where restrictive visiting policies are often an integral part of the non-pharmacological prophylaxis of infections in the immunocompromised patient setting. The COVID19 pandemic required us to adopt a restrictive visiting policy. No visits have been permitted since the 24th of March 2020 (Restrictive Visiting Policy: RVP). Before this time, one visitor per patient was allowed twice a day and visitors were required to wear gloves, shoe covers and a mask, and wash their hands before admission and on departure (Partially Unrestrictive Visiting Policy: UVP). We compared the incidence of fever, bacteremia and pneumonia during a 6-month period in patients admitted during UVP and to those admitted during RVP. The aim of our study was to demonstrate if the presence of visitors in the ward increases the risk of infections. We analyzed data from a group of 43 patients during the UVP, and a group of 50 patients during the RVP. Patients were admitted for acute leukemias (48.8% during UVP and 62% during RVP), pathologies other than leukemias (39.5% during UVP and 32% during RVP), diagnostic procedures or complications due to treatment (11.6% during UVP and 6% during RVP). There was no difference in incidence of fever between the two groups (39.5% in UVP group, 54% in RVP group, test Chi-square p:0.24). There was no difference in incidence of bacteremia (13.9% in UVP group, 28% in RVP group, test Chi-square p: 0.164) and there was no difference in incidence of pneumonia (11.6% in UVP group, 8% in RVP group, test di Fisher p:0.73). There was no increased detection of community-acquired microorganisms responsible for the observed infections. These data support the evidence, which is already reported in literature for ICUs but not for the Hematology Units, that the shift from a partially unrestricted visiting policy to a restricted visiting policy is not associated with a decreased incidence of acquired infections. Larger cohorts of patients are warranted to confirm these preliminary data.

D042

MICROBIOLOGICAL EFFECTS OF TAUROLIDINE CONTAINING LOCK SOLUTION IN PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) OF HEMATOLOGICAL PATIENTS: A PROSPECTIVE STUDY

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A catheter lock solution containing active antimicrobials including taurolidine (1,35%) plus citrate (4%), *i.e.*, Taurolock 3ml fl, could affect Gram-positive, Gram-negative and fungal pathogens growth, that causes catheter-related (CR) bloodstream infection (BSI) in immunocompromised patients. Tunneled lines (TLs) and, most recently, PICCs are common access devices for inpatient care of patients undergoing chemotherapy. From September 2020 to March 2021, we prospectively evaluated PICC-related BSI and/or venous thrombosis (VT) in 40 adult patients (lymphoma, n=17; multiple myeloma, n=12; acute leukemia, n=11) receiving intraluminal installation of Taurolock (3ml for single lumen; and 1,5 ml for each lumen in case of double lumen) during PICC insertion and weekly during medications (Arm A). Thirty PICCs were inserted in the right basilica vein, 9 PICCs in the left basilica vein and 1 PICC in the right brachial vein [single lumen PICCs (4 Fr), in 30 patients; double lumen PICCs (5 Fr), in 10 patients]. Arm A median follow-up was 4 months. PICC-related BSI and/or VT rates were compared with that of a historical cohort of 40 patients with similar characteristics, except for placed TLs (Port-a-cath, n=18; Broviac, n=12; Hickman, n=10) which were managed without lock solution installation (Arm B). All patients in the Arm A received catheter lock solution as scheduled, with a median of 25 mL of Taurolock (r., 3-40 mL). The CR-BSI and CR-VT rate in Arm A and in Arm B was 5% and 30%, with a difference of 25 percentage points (relative risk for CR-BSI or CR-VT 0,1667; P= 0.0064; Figure1). CR-BSI events in Arm B were 10: six oxacillin-resistant coagulase-negative *Staphylococcus* spp. (*haemolyticus*, 4; *epidermidis*, 2); three *Enterobacteriaceae* spp. (*E.coli*, 2; *K. pneumonia*, 1) and one *C. parapsilosis*. The CR-BSI incidence was zero and 3,9 per 1000 catheters daily in Arm A and Arm B. Among CR-thromboses, symptomatic VT rate was 5% in Arm A and 5% in Arm B (with two cases of septic thrombophlebitis in the latter group). Our preliminary data have confirmed that BSI and VT are the major complications affecting intravascular device-related morbidity in the hematological setting, and Taurolock infusion is effective against pathogens especially involved in biofilm formation. The use of routinely irrigated PICCs with prophylactic Taurolock, led to an approximately six-fold lower risk of CR-infection/thrombosis than that of TLs without Taurolock prophylaxis in patients undergoing chemotherapy

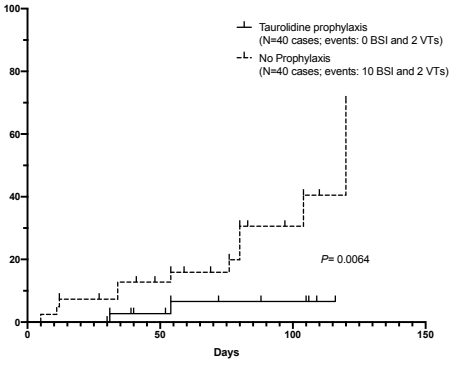

Figure 1.

D043

SEVERE SARS-COV-2 INFECTION AND AUTOIMMUNE CYTOPENIAS: A CASE SERIES

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Background: SARS-CoV-2 is associated with host's immune dysregulation and autoimmunity. Here we describe the clinical course and management of 4 consecutive cases of autoimmune cytopenias (AIC) associated to COVID, observed in Cernusco s/N Hospital from 03/2020 to 04/2021.

Case 1: 81y, M. Comorbidities: MGUS, cold agglutinin disease (CAD) in remission without therapy, COPD, diabetes, hypertension. He was admitted for COVID in 03/2020 with Hb of 6 g/dl and DAT positive for C3d. Treatment: transfusion of 1 unit of PRCs, prednisone 1 mg/kg/d p.o with antibiotic cover and glicemic control, folic acid, darbepoetin 100 mcg/w. In two weeks, Hb rose to 9.5 g/dl (without other transfusions) and COVID gradually resolved. The patient was then evaluated as an outpatient, Hb normalized and steroids were slowly tapered and stopped in 07/2020.

Case 2: 40y, F. Hematologic diseases: LGL Leukaemia in W/W with blood count values unremarkable. No other conditions. She was admitted for COVID with need of ICU support in 11/2020. Severe neutropenia was noted (PMN 400/cmm). Treatment: remdesivir, LMWH, 3 Units of hyperimmune plasma, G-CSF 30 MU/d i.v. for 5 days and piperacillin/tazobactam with neutrofil count recovery and gradual improvement of COVID disease.

Case 3: 67y, F. No relevant comorbidity. She was admitted for COVID in 03/2021 with need of ICU support. She developed severe thrombocytopenia (PLT 14,000/cmm) and renal failure during LMWH treatment. We stopped LMWH and started fondaparinux 1,5mg/d and support with 1 pool of PLTs (oral cavity bleeding). PF-4 antibodies were negative. We started high dose IVIG (400 mg/kg/d for 5 days) without steroids because of P. aeruginosa superinfection. PLT fully recovered (> 100,000/cmm). The patient is still in hospital.

Case 4:. 86y F. Comorbidities: CKD, intestinal teleangectasias with mild chronic anemia. She was admitted for severe COVID in 04/2021 with Hb levels 10.6 g/dl (nadir Hb 5.6 g/dl). Blood tests showed IAT and DAT positivity (DAT positive for C3d with anti-I auto-antibodies and C4 complement consumption). She was transfused with 1 U PRCs and treated with steroid boluses (dexamethasone 20 mg/d i.v. for 4 days then methylprednisolone 1 mg/kg/d i.v), darbepoetin 40 mcg twyce/w, folic acid with no benefit. She developed Evans‘ syndrome and died.

Conclusions: AIC can complicate SARS-CoV-2 but can be managed (if treated promptly) without hindering recovery from COVID.

Acute Leukemia

D044

EFFICACY OF VENETOCLAX IN RELAPSED TRIPLE NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA WITH JAK/STAT PATHWAY ALTERATIONS

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Introduction: Acute Lymphoblastic Leukemia (ALL) prognosis in patients (pts) relapsing after Hematopoietic Transplant (HCT) is poor and needs new drugs. Venetoclax (VEN) is a BCL2-inhibitor (i) showing a promising role in ALL preclinical models. It is mandatory to identify pts that might benefit from BCL2-i.

Methods: We describe the case of a 28 y-old-male affected by B-ALL Triple-Negative (TN) for Ph, t(4;11) and t(1;19). After entering the GIMEMA LAL1913 trial the pt underwent HCT (due to MRD at C5) from a 10/10 HLA-matched unrelated (UD) reaching complete remission (CR) lasting 6 months (m). At relapse he received chemotherapy and 2nd HCT from a 9/10 UD. A 2nd relapse, 5m later, was treated with Blinatumomab (4 cycles) and 2 DLIs achieving CR. After 3m a 3rd relapse at bone marrow (BM) and pelvic nodes (PET-scan: SUV10) was treated with Inotuzumab-Ozogamicin (6 cycles) and radiotherapy reaching MRD-neg and nodal partial response (PET-scan: SUV2.9). In December 2019 started VEN 400 mg/d. Up to April 2021 MRD and PET are neg and VEN is well tolerated. In order to characterize molecular background, we analyzed on relapse samples transcriptome profiling (TruSight RNA Pan-Cancer, Illumina-1385 genes), flow cytometry (*CRLF2*), FISH, mutational screening [*TP53* CDS (NGS), *JAK1, CRLF2, IL7R, JAK2* (by SS), *IKZF1* deletions (Δ4-7, 2-7, 4-8; SS). Gene-expression profile (GEP) analysis were performed on 16 Ph+, 10 Ph-like, 53 TN and 9 donors.

Results: We found no mutations in *TP53, JAK2, CRLF2, IL7R* genes and no rearrangements with RNAseq analysis. *CRLF2* was not expressed in blasts. GEP analysis was neg. for Ph-like CRLF2-up pattern. We detected and validated *JAK1* mutation in kinase domain (KD), while Sanger showed *IKZF1* deletion. GEP compared to normal controls, Ph+, Ph-like adult and TN pts not harboring Ph-like features showed a significant down-regulation of *BCL6, MPL* and *STAT5B*.

Conclusions: Abnormal activation in JAK/STAT pathway is thought to play an important role in malignancies and in particular in ALL pathogenesis. These alterations translate in modified transcription of genes involved in cell survival, proliferation and differentiation including *STAT3, STAT5* and *BCL2*. In our pt we found JAK/STAT pathway alterations in terms of *JAK1* KD-mutation and *MPL, STAT5B* downregulation. Inhibition of BCL2, by acting downstream of the JAK/STAT pathway, might explain the efficacy of VEN in our pt and this suggests use of BCL2-is in B-ALL showing JAK/STAT dysregulation.

D045

PRE-EXISTING CYTOPENIA HERALDING DE NOVO ACUTE MYELOID LEUKEMIA: UNUSUAL PRESENTATION OF NPM1-MUTATED AML IN A SINGLE-CENTER RETROSPECTIVE STUDY

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While NPM1 mutation is not detectable in individuals with CHIP, it is widely recognized that NPM1-mutated AML could develop from pre-existing clonal hematopoiesis, with NPM1 mutation acting as gatekeeper for AML.

Table 1. Single-center AML patient series: clinical and biological characteristics, focusing on cases with pre-existing unexplained cytopenia.

Number of patients/Sex (entire cohort)		226 (120 M/126 F)	
Age at AML diagnosis (years), median (range)		63 (21-90)	
WBC count at diagnosis (x 10^9/L), median (range)		9.5 (0.3-390)	
Hemoglobin level (g/dl), median (range)		9.2 (4-15)	
Platelet count (x 10^9/L), median (range)		62 (6-516)	
Normal karyotype, number of cases (%)		127 (56.2%)	
***NPM1*-mutated AML, number of cases (%)**		77 (34%)	
		Positive for *FLT3*-ITD 24 (31.2%)	
		Positive for *FLT3*-TKD mutation 10 (13%)	
WBC count at diagnosis (x 10^9/L), median (range)		31.9 (1.1-280) / 17 (1.1-280) when *FLT3* WT	
WBC count >10 x 10^9/L, number of cases (%)		52 (67.5%)	
WBC count <2 x 10^9/L, number of cases (%)		5 (6.5%)	
ANC (x 10^9/L), median (range)/number of cases with <1.5 x 10^9/L (%)		3.0 (0.1-110)/25 (32.5%)	
Patients with AML diagnosis following pre-existing unexplained cytopenia* (11 cases)		**Patients with *de novo* AML (171 cases)**	
Age at AML diagnosis (years), median (range)	63 (44-79)	61 (21-90)	p=0.77
Patients aged <60 years, number of cases (%)	5 (44.5%)	80 (46.8%)	p=0.93
WBC count at diagnosis (x 10^9/L), median (range)	1.8 (1.3-18.7)	12.6 (0.6-390)	p=0.001
ANC (x 10^9/L), median (range)	0.5 (0.2-3.3)	1.7 (0-110)	p= 0.02
Hemoglobin level (g/dl), median (range)	11.2 (6.6-13.2)	9 (3.5-15.9)	p=0.61
Platelet count (x 10^9/L), median (range)	99 (34-270)	62 (8-516)	p=0.01
Normal karyotype, number of cases (%)	10 (90.9%)	90 (52.6%)	p=0.01
BM cellularity (%), median (range)	72 (30-95)	90 (15-100)	p=0.055
BM blast count (%), median (range)	30 (20-80)	50 (20-100)	p=0.2
MDS morphologic features, number of cases (%)	4 (36.4%)	46 (26.9%)	p=0.49
Positivity for *NPM1* mutation, number of cases (%)	4 (36.4%)	68 (39.7%)	p=0.8
Frequency (median, range) of gene mutations by NGS analysis on BM samples at AML diagnosis (8 cases with pre-existing cytopenia)	2.5 (1-5) mutations		
Epigenetic/Chromatin regulators (n° of cases, %)			
DNMT3A	4 (50%)		
IDH1	1 (12.5%)		
IDH2	2 (25%)		
TET2	1 (12.5%)		
ASXL1	1 (12.5%)		
Spliceosome factors (n° of cases, %)			
SRSF2	2 (25%)		
ZRSR2	1 (12.5%)		
Cell signaling (n° of cases, %)			
NRAS	2 (25%)		
FLT3	1 (12.5%)		
PTPN11	1 (12.5%)		
Transcription factors (n° of cases, %)			
RUNX1	2 (25%)		

AML, acute myeloid leukemia; WBC, white blood cell; ANC, absolute neutrophil count; BM; bone marrow; MDS, myelodysplastic; NGS, next generation sequencing

* Cytopenia was defined as hemoglobin level <11 g/dl, platelet count <100 x 10^9/L or ANC <1.5 x 10^9/L, persisting for at least 4 months.

Scanty information is actually available on the frequency of NPM1-mutated AML arising in patients observed for unexplained cytopenia. We unexpectedly diagnosed NPM1-mutated AML with concurrent IDH1 mutation in a 65-year old man observed for 36-month history of isolated moderate to severe neutropenia, without evidence of NPM1 mutation or myelodysplastic features on BM examination, performed 6 months earlier. Based upon this observation, we retrospectively analyzed the frequency of pre-existing unexplained cytopenia among 226 consecutive AML patients over a 11-year period (2010-2020). Of interest, 11 (4.9%) subjects had previous, mainly mild to moderate, unexplained cytopenia (unilineage or multilineage in 5 and 6 cases, respectively), with a median duration of 12 months (range 4-48). After having excluded 44 patients with secondary AML, we compared clinical features of the 11 AML patients with a history of cytopenia to those of remaining 171 de novo AML cases, as summarized in Table. Surprisingly, in 4 of 11 cases (36.4%), NPM1 mutation was eventually documented at AML diagnosis, comparable to the frequency found in de novo AML. Overall, NPM1-mutated AML with previous unexplained cytopenia was thus observed in 4 of 226 patients from the entire cohort (1.8%), accounting for 5.2% of the 77 NPM1-mutated AML cases. Only one of these 4 patients showed leukocytosis at AML onset. Moreover, somatic mutations mainly involving either DNA methylation or spliceosome genes were retrospectively found by NGS analysis at AML diagnosis from 8 patients with previous cytopenia, including 3 NPM1-mutated AML. This observation suggests that clonal cytopenia of undetermined significance could potentially have been identified at least in some cases before AML occurrence. In conclusion, although de novo NPM1-mutated AML usually shows high WBC count, in a small subgroup of patients pre-existing unexplained cytopenia may herald NPM1-mutated AML, presenting with leukopenia. This uncommon picture could be under-recognized, therefore extensive sequential molecular analyses in patients with persistent/worsening ICUS could be suggested to investigate the presence of somatic mutations in myeloid-relevant genes, including NPM1, which could eventually drive clonal evolution to AML.

D046

GOOD RISK AML, A HETEROGENEOUS GROUP: ANALYSIS OF OUTCOME ACCORDING TO MOLECULAR SUBGROUPS

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Introduction: ELN-2017 guidelines are a critical tool for risk stratification in AML patients. NPM1-mut AML has been "historically" associated with higher rates of remission and longer survival compared to other subtypes of AML. Recent studies have shown that the prognostic role of NPM1 mutation in several subsets of patients is still controversial as it may depend on a variety of accompanying mutations other than FLT3-ITD.

Methods: In our retrospective analysis, we included all consecutive patients diagnosed with AML at Hematology Clinic, Ospedali Riuniti Ancona, between 1997 and 2020.When cryopreserved sample was available, retrospective genetic analysis, including FLT3-ITD allelic-ratio calculation, were performed. Patients were subsequently assigned to genetic risk group following ELN 2017 recommendations. For time-to-event analysis, we calculated survival estimates using the Kaplan–Meier method and compared groups by the log-rank test. Patient classified as "ELN risk unknown" (*e.g* patients without available genetic studies) were excluded from survival analysis.

Results: Outcome analysis included a total cohort of 608 patients. Four hundred-twenty-eight patients underwent intensive chemotherapy. According to the ELN-2017 recommendations, they were classified as follows: 95 patients favorable risk ; 172 patients intermediate risk ; 125 patients adverse risk. NPM1mut-FLT3-ITDwt AML and NPM1mut-FLT3-ITDlow AML had no significant difference in terms of OS (p=0.62). *NPM1-mutated AML had a significantly lower OS ($p<0.01$) compared to CBF AML.*

Conclusions: Our study demonstrated that NPM1-mut AML, independently from FLT3-ITD mutational status/allelic ratio, had a significantly worse outcome compared to CBF AML, despite sharing the same ELN 2017 risk class. This could be explained by small sample size and lack of extended evaluation of co-occurring mutations, such as DNMT3a, IDH1 and IDH2, whose prognostic influence on NPM1-mut AML has been already demonstrated. Our data suggest that NPM1-mut AML has

not an estabilished favorable outcome per se, but rather the favorable prognostic impact of NPM1 mutation is context-dependent and is particularly influenced by the presence of other genomic mutations that should be evaluated at diagnosis. A multi-center project , with the aim to confirm our speculations, is actually underway.

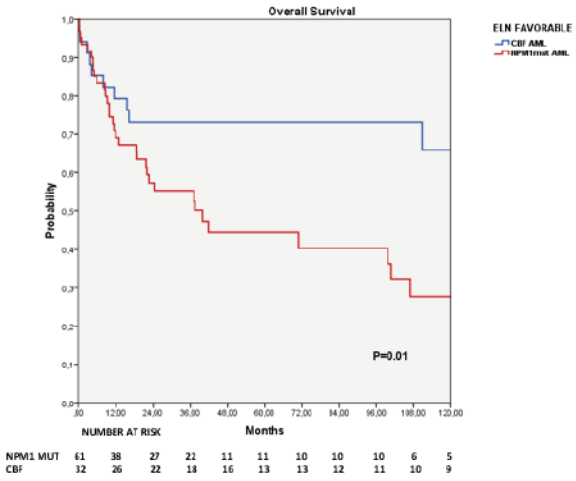

Figure 1.

D047

INVASIVE FUNGAL INFECTIONS IN FLT3-POSITIVE ACUTE MYELOID LEUKAEMIA PATIENTS TREATED WITH CHEMOTHERAPY AND MIDOSTAURIN: PRELIMINARY RESULTS OF A MULTICENTER OBSERVATIONAL SEIFEM STUDY

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The potential interactions of midostaurin (M) with cyp450 inhibitors may influence the choice of antifungal (AF) prophylaxis in FLT3-pos acute myeloid leukemia (AML) patients (pts). To evaluate the incidence of invasive fungal infections (IFI) during induction and consolidation of FLT3-pos AML pts, within the SEIFEM Group we planned a retrospective/prospective observational study enrolling all AML FLT3-pos pts treated with M+chemotherapy in 20 Italian Centers. Potential relationships between IFI and AML characteristics, phase of treatment and type of AF prophylaxis were evaluated. Forty-one pts have been enrolled, M/F ratio 14/27, median age 56 years (range 29-73). NPM1 was expressed in 22 (54%) and patients were classified according to ELN classification as low risk in 9 (22%) of cases, intermediate 18 (44%) and high in 14 (34%). A total of 109 courses have been delivered (41 induction and 65 consolidation). Twenty-eight (68%) pts achieved complete remission after the first induction. AF prophylaxis was delivered in all but one pt during induction (13 posaconazole, 10 echinocandins, 11 posaconazole for 7 days followed by either micafungin or caspofungin 50 mg/d, 6 other AF prophylaxis) and in 36 (55%) during 65 consolidation courses (15 posaconazole, 9 echinocandins, 5 posaconazole for 7 days followed by either micafungin or caspofungin 50 mg/d, 10 other AF prophylaxis). M was discontinued in 6 pts during induction and in 2 during consolidation; reasons for discontinuation were interaction with voriconazole in 2 cases, gastroenteric toxicity in 3, refractory thrombocytopenia in 1 and severe infections in 2. Overall, IFI incidence during induction was 27% (11/41); probable/proven IFIs were 4 (10%), 3 aspergillosis and 1 candidemia. IFI incidence was higher in pts older than 60 years (7/15, 47% *vs* 4/22, 18%, p=0.064), while it was lower in those receiving posaconazole containing AF prophylaxis (4/25, 16%) than other AF regimens (7/16, 44%) (p=0.074). Four IFIs (3 possible and 1 probable) have been observed during consolidation (6%), all in pts not on anti-mold prophylaxis. IFIs did not correlate with NPM expression nor with ELN risk category. IFI-related 30-day mortality was 7% (1 probable aspergillosis during induction). IFI incidence is quite high among FLT3-pos AML pts, probably also because of the different AF prophylaxis strategies adopted. Posaconazole containing regimens, including the sequential schedule, seem to be protective for IFI development.

D048

CPX-351 TREATMENT IN SECONDARY ACUTE MYELOID LEUKEMIA (SAML): THE REAL LIFE EXPERIENCE FROM THE "ITALIAN TRIVENETO REGISTRY"

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Background: CPX-351, a liposomal encapsulation of cytarabine and daunorubicin, has been approved for the treatment of patients affected by therapy-related Acute Myeloid Leukemia (t-AML) or AML with myelodisplasia-related changes (MRC-AML), improving survival probabilities in comparison with standard chemotherapy. However, outside of clinical trials or compassionate use program, no data are available regarding efficacy and safety of CPX-351 in clinical practice after his commercial approval.

Patients and Methods: We performed a preliminary analysis from cohort of 57 newly diagnosed secondary AML pts treated with CPX-351 in 8 Italian Hematological Centers (Udine, Treviso, Mestre, Padua, Verona, Bolzano, Aviano, Vicenza) from August 2019 to April 2021 (the recruitment of cases is still open). Median age was 65 yrs, 31/57 (54%) have sAML evolving from myelodysplastic syndrome, 19/57 (33%) pts have been previously treated with hypomethylating agent. The median baseline bone marrow blast percentage was 30%. Median WBC count at diagnosis was $3{,}5x10^9$/L. All pts received the induction cycle at a standard dose of 44 mg/mq repeated on day 1,3,5 and 21/57 (36,8%) pts proceeded to HSCT of which seven after only the first course.

Results: In 49/57 evaluable pts the Overall Response Rate (ORR=CR+CRi) after the induction course was 72% after a median of 36 days from first day of CPX-351 administration. After a median follow up of 7 months (range 0,3-22) relapse was observed in 10/57pts (17%). At last follow-up 42/57 pts (74%) are still alive and 15/ 57 (26%) are dead. The main cause of death was disease progression. The drug was generally well tolerated without onset of severe mucositis. The most common toxicities were myelosuppression and documented infectious complications (9 pneumonias and 13 sepsis; 3/57 pts died early from in-

fections during the aplasia post induction). Twelve-months OS from the start of CPX-351 therapy was 77%. Univariate and Multivariate analysis of factors affecting response and OS are ongoing (pts recruitment still open).

Conclusion: These data show the efficacy and the emerging role of CPX-351 in the real word management of secondary AML. Future directions include evaluating dose intensification with CPX-351, combining this agent with targeted therapies, and better understanding the mechanism of improved responses.

D049

INCIDENCE, TREATMENT AND OUTCOME OF CENTRAL NERVOUS SYSTEM RELAPSE IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED FRONT-LINE WITH PEDIATRIC-INSPIRED REGIMENS. A RETROSPECTIVE MULTICENTER STUDY OF THE CAMPUS ALL

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Despite great progress in the management of acute lymphoblastic leukemia (ALL) with modern pediatric-inspired regimens, the prognosis of patients (pts) with a central nervous system (CNS) relapse remains very poor. We aimed at analyzing the incidence, characteristics, treatment and overall survival (OS) of CNS relapse in adult ALL pts treated front-line with pediatric-inspired regimens. In the framework of Campus ALL group we retrospectively analyzed a total of 1,035 consecutive, newly diagnosed adult ALL pts (B 757, T 278) who were treated in 25 Centers between 2009 and 2020. Philadelphia (Ph)+ pts were included if treated with chemotherapy (CT) in addition to TKIs. A total of 71 pts (6.8%) experienced a CNS relapse: 41 (58%) had an isolated CNS relapse, 21 (29%) had a concomitant bone marrow (BM) relapse and 9 (13%) had a molecular BM involvement. Overall, CNS relapse was more frequent in T-ALL (28/278; 10%) than in B-ALL (43/757; 5.7%) (p=0.017). CNS relapse was observed in 9 Ph+ pts (21% of B-ALL cases). Notably, within B-ALL pts devoid of major molecular lesions (n=32), the BCR/ABL1-like status was available in 13, and 6 of them proved Ph-like (46 %). An early CNS relapses - defined as occurring <12 months from diagnosis - was observed in 41 pts. Risk factors for early CNS relapse included T-cell phenotype (p=0.006) and hyperleukocytosis >$100x10^9$/L (p<0.001). Treatments were heterogeneous, including systemic CT, radiotherapy, intrathecal therapy, TKI and novel agents. A CR was obtained in 39 pts (55%). No treatment modality was associated to a superior CR rate compared to the others. After CR achievement, 26 pts underwent an allogeneic transplant, with a significant OS benefit compared to non-transplanted pts (p=0.039). The use of TBI as part of the conditioning regimen did not affect OS compared to non-TBI-based regimens (p=0.56). After a median observation of 31.5 months (range 3-99), 23 pts (32%) are still alive. Median OS after isolated CNS relapse, CNS plus molecular BM relapse and CNS plus hematologic relapse was 14, 10 and 5 months, respectively (p=0.01). Pts with early CNS relapse had a particularly poor outcome, with a 5-year OS rate of 2.1% (p<0.05).

In the era of pediatric-inspired regimens, CNS relapse still represents a major challenge. Some biological subsets of pts, including Ph-like, might be at a higher risk and may deserve a more aggressive prophylaxis. After CNS relapse, subsequent transplant of pts achieving CR improves survival.

D050

VENETOCLAX IN COMBINATION WITH HYPOMETHYLATING AGENTS IN PREVIOUSLY UNTREATED PATIENTS WITH ACUTE MYELOID LEUKEMIA INELIGIBLE FOR INTENSIVE TREATMENT: REAL-LIFE RESULTS FROM A SINGLE CENTRE EXPERIENCE

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Introduction: The addition of Venetoclax to hypomethylating agents (HMA-V) improved the outcome of elderly patients with newly diagnosed acute myeloid leukemia (AML), in terms of response and survival. The aim of our study was to confirm, in a real-life single center experience, the efficacy and safety of HMA-V in elderly AML naïve patients.

Patients and methods: We retrospectively evaluated naïve AML patients who received HMA-V at the Hematology Department of the Maggiore Hospital-ASUGI, Trieste. We collected cytogenetic and molecular data and stratified patients by genetic risk according to the 2017 European Leukemia net (ELN) recommendations, while considering that mutational status of TP53, ASXL1 and RUNX1 was not available for all patients. Patients were treated with HMA at standard labeled dose and V was added starting from cycle 1 to 3. Dose adjustments of either V or HMA were allowed in case of toxicities. Time-to-response (TTR) was the period for achieving complete response (CR) or CR with incomplete hematological recovery (CRi). Response duration (RD) was the time between CR/CRi and relapse. Transfusion independence (TI) was defined as ≥ 8 weeks without red blood cell and/or platelet transfusion. Finally, we evaluated overall survival (OS).

Results: Between September 2018 and June 2020, 16 previously untreated AML patients started HMA-V treatment, with a median age of 76.5 years (range 63-79). Patients characteristics are summarized in Table1. The median number of cycles was 8 (range 1-24). With a median

follow-up of 12.5 months (range 2-27), CR and CRi were achieved in 9 (56%) and 4 (25%) cases, respectively, with a median TTR was 2 months (range 1-6). Seven out of 13 responders (53.8%) relapsed. The median RD was 10 months (range 2-24), with a projected 12 and 24-months RD rate of 58% and 35% respectively. TI was obtained in 9 (60%) patients. Four patients (25%) died, all for leukemia-related causes. Median OS was not reached, and the 12 and 24-months OS were 80% and 69%, respectively. All 13 responders required transient V interruption, and 4 patients needed HMA dose reduction, due to hematological toxicity. Three patients (18%) had febrile neutropenia, and 4 (25%) had serious grade 3-4 pneumonia. Diarrhea and nausea were reported in 4 (25%) and 7 (43%) patients, respectively.

Conclusions: In conclusion, our real-life single center experience confirms that HMA-V is a feasible and active front-line treatment for elderly AML patients.

Table 1. Baseline characteristics of 16 patients evaluated for the analysis. A: azacitidine; AML-MRC: acute myeloid leukemia with myelodysplasia related changes; AML-NOS: acute myeloid leukemia not otherwise specified; AR: allelic ratio; CR: complete response; CRi: complete response with incomplete hematological recovery; D: decitabine; HMA: hypomethylating agents; NE: not available; OS: overall survival; PE: provisional entity; RD: response duration; TTR: time to response; V: venetoclax.

Patients	Sex/Age	2016 WHO Classification	Cytogenetic	Identified gene abnormalities	2017 ELN risk category	BM blast (%)	HMA	Cycles of therapy	Response to V+HMA	TTR (months)	Relapse	RD (months)	OS (months)
#1	m/78	AML with mutated NPM1	46,XY	*FLT3-D835, NPM1*	favourable	69	D	22	CR	3	no	24	27
#2	f/83	AML-MRC	46,XX	*NPM1*	favourable	20	A	13	CR	2	yes	13	18/died
#3	f/77	AML with mutated *RUNX1* (pe)	46,XX,t(10;21)	*FLT3-ITD, RUNX1*	adverse	90	A	17	CRi	2	no	22	24
#4	f/73	AML-MRC	46,XX	/	intermediate	30	A	18	CR	5	yes	14	22
#5	m/73	AML-MRC	46,XY	*BAALC*	intermediate	26	A	18	CR	6	yes	12	23
#6	m/78	AML-NOS	NA	*FLT3-D835, IDH2*	intermediate	90	A	2	NR				2/died
#7	m/78	AML with *BCR-ABL1* (pe)	46,XY,t(9;22)	*FLT3-D835, BCR-ABL1*	adverse	80	A	7	CR	2	yes	5	18
#8	f/79	AML with mutated NPM1	46,XX	*FLT3-ITD^{low} AR, NPM1*	intermediate	50	A	15	CR	2	no	16	18
#9	m/73	AML with mutated NPM1	45,XO,-Y	*NPM1*	low	40	A	12	CR	4	no	10	14
#10	f/79	AML-NOS	46,XX	*BAALC*	intermediate	70	A	1	NR				9/died
#11	m/75	AML-MRC	46,XY,del(5q)	/	adverse	35	A	7	CRi	5	yes	2	11
#12	f/76	AML-NOS	46,XX	*BAALC, WT1*	intermediate	50	A	6	CR	3	no	7	10
#13	f/70	AML-MRC	complex	*WT1*	adverse	65	A	4	CRi	1	yes	5	9
#14	f/73	AML-MRC	complex	/	adverse	30	A	1	NR				2/died
#15	f/76	AML-NOS	47,XX,+8	/	intermediate	40	A	4	CR	1	yes	4	7
#16	m/77	AML-MRC	complex	*FLT3-ITD*	adverse	90	A	4	CRi	1	no	6	7

D051

EXPLORING THE ATR-CHK1 PATHWAY IN THE RESPONSE OF DOXORUBICIN-INDUCED DNA DAMAGES IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Doxorubicin (Dox) is one of the most commonly used anthracyclines for the treatment of solid and hematological tumors such as B-/T-cell acute lymphoblastic leukemia (ALL). Dox compromises topoisomerase-II enzyme functionality, thus inducing structural damages during DNA replication and causes direct damages intercalating into DNA double helix. Eukaryotic cells respond to DNA damages by activating the ATM-CHK2 and/or ATR-CHK1 pathway, whose function is to regulate cell cycle progression, to promote damage repair and to control apoptosis. We evaluated the efficacy of a new drug schedule combining Dox and specific ATR (VE-821) or CHK1 (prexasertib, PX) inhibitors in the treatment of human B-/T- cell precursor ALL cell lines and primary ALL leukemic cells. We found that ALL cell lines respond to Dox activating the G2/M cell cycle checkpoint. Exposure of Dox-pretreated ALL cell lines to VE-821 or PX enhanced Dox cytotoxic effect. This phenomenon was associated with the abrogation of the G2/M cell cycle checkpoint with changes in the expression pCDK1 and cyclin B1, and cell entry in mitosis, followed by the induction of apoptosis. Indeed, the inhibition of the G2/M checkpoint led to a significant increment of normal and aberrant mitotic cells, including those showing tripolar spindles, metaphases with lagging chromosomes and massive chromosomes fragmentation. In conclusion, we found that the ATR-CHK1 pathway is involved in the response to Dox-induced DNA damages and we demonstrated that our new *in vitro* drug schedule that combines Dox followed by ATR/CHK1 inhibitors can increase Dox cytotoxicity against ALL cells, while using lower drug doses.

D052

SAFETY AND EFFICACY OF COMBINED HMAS AND VENETOCLAX AS FIRST LINE TREATMENT IN AML PATIENTS UNFIT FOR INTENSIVE CHEMOTHERAPY

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Venetoclax (VEN) is an oral BCL-2 protein inhibitor, used, in combination with hypomethylating agents (HMA) (azacytidine – AZA- or decitabine – DEC-), for the first-line treatment of unfit adult acute myeloid leukemia (AML). From March to December 2020, we collected data about treated patients at the University Hospital of Catania, Italy, focusing on adverse drug reactions (ADRs), grouped according to the Medical Dictionary for Regulatory Activities (MedDRA®), and response, as per ELN guidelines. 24 patients were treated with VEN combined with AZA (15 patients, 63%) or DEC (9, 38%) (Table 1).

Table 1. Patients characteristics

Characteristic	N (24)	%	AZA (15)	%	DEC (9)	%	p
Age, years, median (range)	73.5 (54-86)	-	75.6 (54-86)	-	69.3 (56-76.6)	-	0.3
Male/Female	14/10	58/42	11/4	73/27	3/6	33/67	0.09
AML type							
De novo	13	54	9	69	4	31	0.7
Secondary to MDS or MDS/MPN	7	29	3	43	4	57	
Therapy-related	1	4	1	100	0	0	
Myeloid blast crisis from previous MPN	3	13	2	67	1	33	
Cytogenetic risk							
Low	0		0		0		0.9
Intermediate	16	67	10	67	6	67	
High	6	25	4	27	2	22	
Failed	2	8	1	6	1	11	
ELN risk classification*							
Low	0		0		0		0.8
Intermediate	13	54	8	54	5	56	
High	7	29	5	33	2	22	
Not available	4	17	2	13	2	22	

* based on NPM1 mutations, occurrence of FLT3-ITD and cytogenetics assessment

Median age was 73.7 years (range 54-86). 13 patients out of 24 (54%) were male. Median follow-up was 4 months (range 0.5-13.8). 2 patients did not complete the first cycle due to adverse events (intolerance to the drug) and 1 was lost-to-follow-up. Patients pursuing treatment received on average 5 cycles 10 patients out of the remaining 21 (48%) discontinued permanently treatment due to disease progression (n=5/10; 50%), drug-related death due to febrile neutropenia (FN) (n=2/10; 20%), FN in resistant patient (n=1/10; 10%) or malaise (n=2, 20%). 21 patients (88%) were assessed for treatment response. Best response was complete remission (CR) in 11 (52%) and CR with partial hematological recovery (CRh) in 3 patients (14%). Median duration of response was 3.6 months (range 0.14-13.8). 3 patients (15%) showed partial remission (PR) and 4 (19%) stable disease. Among the 14 patients showing CR/CRh, 3 (21%) relapsed and among the 3 patients with PR, 2 progressed (66%) and 1 died cause FN. Response assessment did not differ depending on used HMA (100% *vs* 66% in DEC and AZA groups respectively, p=0.1). Regarding ADRs, 19 patients (9 females and 10 males) experienced at least one ADR, 10 of them more than one. Reported ADRs were mostly serious (n = 28, 87.5%), including 3 deaths (11% of serious ADRs; 12.5% of patients) in FN, 1 in a patient with resistant disease and 2 in patients in CRh. More than half of them showed a positive outcome (n = 4, 12.5% improved, and n = 15, 47% fully recovered). Hematological toxicity and infections were the most reported ADRs (84%). Causality assessment with treatment was 'possible' in 23/28 (82%) and 'probable' in 5/28 (17%). In conclusion, we found that the combination VEN plus HMA is active in unfit AML patients with an overall response rate of 66%, although frequently complicated by FN (46% of cases).

D053

IMPACT OF COVID-19 ON DIAGNOSIS AND MANAGEMENT OF ACUTE LEUKEMIA IN THE REAL LIFE: THE EXPERIENCE OF THE GIMEMA NETWORK

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The overwhelming information on the growth of SARS-CoV-2 cases and related deaths, as well as the necessity to avoid interpersonal contacts produced significant anxiety in the general population. This phenomenon has led patients with hematological disorders to underestimate a variety of symptoms other than fever and respiratory failures, to postpone laboratory and radiological tests and to defer medical and hematological examinations. We retrospectively analyzed data from 25 Italian hematological centers, listed in Table 1, collected in a pre-pandemic (Dec 2019-Feb 2020) and in two pandemic trimesters (Mar-May 2020 and Sep-Dec 2020) investigating on the impact of SARS-CoV-2 lockdown and restriction measures on the number and delay of leukemia diagnosis and outcome, focusing on the mortality rate within 30 days. Eight centers were COVID-free, 16 COVID-mixed and 1 COVID-dedicated. During the first pandemic wave, the period with the most restricted measures and therefore with the greatest anxiety and fear of the population to go to hospitals, we observed a significant reduction of the average number of diagnosis compared with the second (35 *vs* 48 cases). In particular, the average number of AML was significantly lower in the first pandemic wave (25 *vs* 33), while no differences were noted between the pre-pandemic and the pandemic periods when ALL were considered. To assess whether the delay of leukemia diagnosis was homogeneous in the different periods, patients were stratified in three different groups: high (≥30 days), intermediate (10-30 days) and low (≤10 days) delay, respectively. A high delay was observed more frequently in the first outbreak (16%) compared with the second (7.9%) and the pre-pandemic period (0%). An intermediate delay was more frequently observed in the second pandemic wave (20%) in comparison with other trimesters (10% each). Although the most significant delay on diagnosis was in the first pandemic wave, we observed an increased rate of early mortality in the second pandemic period, as compared with both the pre-pandemic and the first pandemic wave (10.7% *vs* 4.1% *vs* 5.7%, respectively). The assessment of patients' clinical and biological characteristics at diagnosis, the development of co-morbidities, the response to induction treatment and a longer follow-up will clarify how much anxiety and restrictive measures can affect the results on acute hematological diseases and indicate the possible measures to prevent this critical aspect.

Table 1.

Center	PI	Survey AML	Survey ALL
AOU CITTÀ DELLA SALUTE E DELLA SCIENZA, OSPEDALE S. GIOVANNI BATTISTA MOLINETTE - TORINO - SC EMATOLOGIA 2	Audisio Ernesta	Yes	Yes
AULSS 3 SERENISSIMA, OSPEDALE DELL'ANGELO - MESTRE - UO EMATOLOGIA	Bassan Renato		Yes
AOU INTEGRATA DI VERONA, POLICLINICO G.B. ROSSI - UOC EMATOLOGIA	Bonifacio Massimiliano		Yes
ASST DEGLI SPEDALI CIVILI DI BRESCIA - UO EMATOLOGIA	Borlenghi Erika		Yes
ASST GRANDE OSPEDALE METROPOLITANO NIGUARDA - MILANO - SC EMATOLOGIA	Cairoli Roberto	Yes	
OSPEDALE MAURIZIANO UMBERTO I - TORINO - SCDU EMATOLOGIA	Cignetti Alessandro		Yes
AOU DI BOLOGNA - POLICLINICO S. ORSOLA-MALPIGHI - UOC EMATOLOGIA	Curti Antonio	Yes	Yes
ASL ROMA 2, OSPEDALE S. EUGENIO- OSPEDALE S.EUGENIO - UOC EMATOLOGIA	De Fabritiis Paolo	Yes	Yes
AOU POLICLINICO TOR VERGATA - ROMA - UOC TRAPIANTO CELLULE STAMINALI	Del Principe Maria Ilaria		Yes
CTC U.O DI EMATOLOGIA CON TRAPIANTO DI MIDOLLO OSSEO - CATANIA	Di Raimondo Francesco		Yes
ASL LECCE, OSPEDALE 'V. FAZZI' - UO EMATOLOGIA	Di Renzo Nicola	Yes	Yes
AOU CITTÀ DELLA SALUTE E DELLA SCIENZA, OSPEDALE S. GIOVANNI BATTISTA MOLINETTE - TORINO - SC EMATOLOGIA - UNIVERSITÀ DEGLI STUDI DI TORINO	Ferrero Dario		Yes
032 - AOU DI SASSARI - CLINICHE UNIVERSITARIE - STABILIMENTO CLINICHE DI SAN PIETRO - UOC EMATOLOGIA	Fozza Claudio	Yes	
FONDAZIONE IRCCS CA' GRANDA, OSPEDALE MAGGIORE POLICLINICO - MILANO - EMATOLOGIA - PADIGLIONE MARCORA	Fracchiolla Nicola	Yes	Yes
IRCCS ONCOLOGICO ISTITUTO TUMORI GIOVANNI PAOLO II - BARI - UO EMATOLOGIA	Guarini Attilio		Yes
AOU MAGGIORE DELLA CARITA' DI NOVARA - SCDU EMATOLOGIA	Lunghi Monia	Yes	Yes
AO DI PERUGIA, OSPEDALE S. MARIA DELLA MISERICORDIA - EMATOLOGIA E TRAPIANTO MIDOLLO OSSEO	Martelli Maria Paola	Yes	Yes
AOU POLICLINICO P. GIACCONE - PALERMO - UO EMATOLOGIA	Mitra Maria Enza		Yes
AS DELL'ALTO ADIGE, OSPEDALE CENTRALE DI BOLZANO - EMATOLOGIA E CENTRO TRAPIANTO MIDOLLO OSSEO	Mosna Federico		Yes
AO OSPEDALI RIUNITI VILLA SOFIA CERVELLO - PALERMO - UO EMATOLOGIA CON UTMO	Mulè Antonino		Yes
AOU SAN LUIGI GONZAGA - ORBASSANO - SCDU EMATOLOGIA GENERALE E ONCOEMATOLOGIA	Rege Cambrin Giovanna		Yes
AO BROTZU, PRESIDIO OSPEDALIERO A. BUSINCO - CAGLIARI - SC EMATOLOGIA E CTMO	Romani Claudio		Yes
ASL PESCARA, PRESIDIO OSPEDALIERO 'SPIRITO SANTO' - UOC EMATOLOGIA CLINICA	Salutari Prassede		Yes
AOU "SAN GIOVANNI DI DIO E RUGGI D'ARAGONA" - SALERNO - UOC EMATOLOGIA E TRAPIANTI DI CELLULE STAMINALI EMOPOIETICHE	Selleri Carmine		Yes
ASL DELLA PROVINCIA DI BARLETTA, ANDRIA, TRANI, OSPEDALE "MONS. DIMICCOLI" - BARLETTA - UO EMATOLOGIA	Tarantini Giuseppe	Yes	Yes

D054

COVID19 AND ACUTE LEUKEMIAS: A REAL-LIFE PERSPECTIVE

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Background: In 2020 COVID19 was declared a new pandemic virus. Since then, major concerns have been expressed regarding its impact on hematological patients treatment and mortality. Few data are available for sarsCOV-2 and acute leukemias; here we present a small real-life cohort of patients from a high-incidence region.

Methods and Results: From December 2020, 20 patients received diagnosis of acute leukemia and COVID19, confirmed by molecular

transnasal swab. Among infected patients, 10 were acute myeloid leukemia (AML), 6 were acute Lymphoid leukemia (ALL), and 4 acute promyelocitic leukemia (APL); male/female 11/9, median age 50 (21-69). Concomitant comorbidities were present in 12 (60%), with a median number of medication of 2 (range 1-3). In 17 patients (85%) COVID19 was diagnosed at the end of a cycle of chemotherapy; 2 (10%) patients received concurrent diagnosis of AML and COVID19; 1 patient (5%) received diagnosis of COVID19 during treatment with TKI. Interstitial pneumonia was confirmed in 9 patients (5 AML/2 APL/2 ALL) by CT scan; supportive measures included oxygen in all patients, with need of Non Invasive Positive Pressure Ventilation for 2 of them, and transfer to ICU unit and intubation for 4 of them (2 AML/2 APL). All intubated patients died of interstitial pneumonia. The patient on TKI continued treatment without interruption. 3 patients with persistent swab positivity started treatment with targeted agents (2 venetoclax; 1 gilteritinib). Intensive chemotherapy was restarted in 12 patients (10 AML/2 ALL); in ALL patients, treatment was restarted despite a low COVID19 positivity. In evaluable patients (16/20), median time to swab negativization was of 39 days (11-60), with no impact on type of diagnosis (AML *vs* ALL, 39 *vs* 38 days, p=0.44), and significant impact in type of treatment (Intensive *vs* No-intensive, 42 *vs* 14 days, p=0.0009).Only one atypical extra-hematological toxicity with pleural effusion, responsive to steroids and drainage, was observed.

Conclusion: SARS-CoV-2 infection is associated with worst outcome in patients who develop interstitial pneumonia, with an observed death rate of 20%. In our cohort, 2 of deaths occurred in APL. This suggests that treatment intensity do not necessarily correlate with pneumonia severity. However swab negativization time suggest to avoid intensive therapy when a treatment need to be started due to disease progression.

D055

T(10;11)(P13;P14-21) PICALM-MLLT10 ACUTE LEUKEMIA: CHARACTERIZATION OF TWO CASES WITH DIFFERENT PHENOTYPIC PRESENTATION

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Introduction: Recurrent chromosomal translocations identify specific leukemia subtypes. The translocation t(10;11)(p13;p14-21), results in PICALM-MLLT10 fusion gene and is described in a wide spectrum of hematologic malignancies most frequently T-Acute Lymphoblastic Leukemia (T-ALL) and Acute Myeloid Leukemia (AML), especially with immature phenotype (FAB M0-M1). This fusion is very rare in AML and accounts for <1% of ALL. Clinical presentation includes high platelet count, deep vein thrombosis and extramedullary involvement (spleen, liver, mediastinum, central nervous system). Due to the rarity and variety of clinical manifestations treatment is not well defined and the outcome remains poor in the majority of patients. Here we described two new cases with different phenotypes and mutational profiles (Table 1).

CASE 1: A 32 years old man was referred to our Center with hyperleukocytosis, mediastinal mass, and lower-limb thrombosis. He was diagnosed with AML with minimal differentiation (AML NOS) and a PICALM-MLLT10 t(10;11)(p12-13;q14?) rearrangement. He received D3A7 induction obtaining on day 31 a full hematological recovery with the persistence of disease quantified by flow cytometry in the 3.5% of the cells. FLA-Ida regimen was administered as intensification, leading to a complete remission (CR) with minimal residual disease (MRD) negativity by flow and FISH. The patient received haploidentical hematopoietic stem cell transplant (HSCT) in first CR, with PTCy, CSA, and MMF as GVHD prophylaxis. On day +80 after HSCT he maintains CR and full donor chimerism.

Table 1.

	WHO 2016	PHENOTYPE	KARYOTYPE/FISH	NGS (VAF%)	CLINICAL PRESENTATION
Pt 1	AML NOS (minimal differentiation)	CD34+, CD33+ HLA-DR+, CD38+ CD11b+, cCD68+ CD11c+, CD117+/- CD7+/-, CD15+/- cCD3-, cMPO- CD79a-	46 XY, add10(p12-13)del11(q14) PICAL-MLLT10 fusion gene	ETV6 (1%) p.(Gln289Argfs*29) RUNX1 (50.7%) p.(Leu56Ser)	Iperleukocytosis mediastinal mass, lower limb thrombosis
Pt 2	Acute Leukemia of ambigous lineage (Undifferentiated)	CD34+, CD33+, CD38+, HLADR+ CD71+, CD7 CD123dim+ cCD79a +/-, CD11+/- CD56 +/, cMPO- cCD3-, CD117-	46 XY del (14)(q21), add (10)(p12) add(17) t(10;11)(p12-13; q14-21) PICAL-MLLT10 fusion gene	FLT3 ITD (2.2%) p.(Leu610_Glu611ins20) FLT3 ITD (1.1%) p.(Leu601_Leu610dup) NRAS (50%) p.(Gly12Cys) ETV6 (46.9%) p.(Met319Thr) EZH2 (46.7%) p.(Asp664Ala) TET2 (48.8%) p.(Leu1721Trp)	Iperleukocytosis mediastinal mass splenomegaly lymphoadenopaties lower limb thrombosis

CASE 2: 36 years old man, with hyperleukocytosis, mediastinal mass, massive splenomegaly, lymphadenopathies, and lower-limb thrombosis, was diagnosed with Acute Undifferentiated leukemia (AUL) with PICALM-MLLT10. He received an ALL-like induction regimen with HyperCVAD but displayed a refractory disease. FLA-Ida was used as salvage treatment and allowed to obtain a CR with low MRD positivity (0.2% by flow). The patient underwent splenectomy and after one-month sibling HSCT, with CSA and ATG as GVHD prophylaxis. On day+80 after HSCT he maintains CR and full donor chimerism.

Conclusions: Despite different phenotypes and mutational profiles, Acute Leukemias with PICALM-MLLT10 confirm similar clinical features. FLA-Ida regimen was feasible and led to CR in two high-risk patients. This approach allowed to proceed to HSCT as consolidation treatment, and suggest a Fludarabine-based induction regimen in these patients.

D056

COAGULOPATHY IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH FIRST LINE ARSENIC TRIOXIDE IN COMBINATION WITH ALL TRANS RETINOIC ACID: A MONOCENTRIC EXPERIENCE

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Patients with acute promyelocytic leukemia (APL) often show some clinical and/or laboratory features of coagulopathy. The characteristic coagulopathy in APL is unique among the leukaemias with a frequency of thrombosis higher than all other forms of leukemia. Few data exist about the effect on hemorrhagic risk by the use of arsenic trioxide (ATO) plus all-trans retinoic acid (ATRA). The aim of our study is to evaluate coagulation-related parameters in APL patients at presentation, and explore the mechanism of APL coagulopathy by measuring changes in these parameters prior to and during ATO plus ATRA treatment through our real life monocentric experience. We censored each patient affected by APL who was treated according to ATO plus ATRA at low and intermediate risk; high-risk patients were excluded as per protocol. Twenty-two patients admitted to our Department from January 2009 were included in the study and their characteristics are shown in Table 1. The first parameter to normalize was fibrinogen, after a median time of 11 days (range 3-44 days) after the beginning of the therapy, but only 5 patients received fresh frozen plasma. The need of platelet transfusion was higher with a median of 8 units (range 3-23); the 13 patients requiring platelet transfusion normalized platelets count (> 30000/mmc without transfusion) in 25 days (range 11-31). No major hemorrhagic events were registered. D-dimer levels normalized after a median of 35 days without any clinical evidence of complications except for 2 thrombotic events (1

deep vein thrombosis and 1 superficial vein thrombosis of the leg after 34 days from ATRA introduction) properly managed with low molecular weight heparin treatment. All patients were discharged after the completion of ATO plus ATRA induction and the achievement of hematologic complete remission. All patients obtained molecular remission after a median time of 3 months (range 1-6) and all, but one patient, dead for progressive disease, are alive and in molecular response at a median follow-up of 48 months (range 8-145). Our data on coagulation pattern are in line with previously published data in terms of trombocytopenia and coagulation profile. The evidence of alterations of blood clotting tests seems not to correspond to clinically significant thrombotic or hemorrhagic complications. ATO plus ATRA regimen allows to treat patients non eligible to chemotherapy and to reduce possible complications, also in the setting of coagulopathy.

Table 1. Patients' clinical and lab characteristics at diagnosis.

		ATO plus ATRA 22 pts
Age (median years)		45 (range 18-72)
Sex M/F		12/10
WBC count (median, 109/L)		1300 (range 100-9770),
Hemoglobin (median, g/dl)		8.7 (range 6.1-12.8)
Platelets count (median, 109/L)		27 (range 6-136)
PT (median, sec)		12.5 (range 10.1-16.8)
aPTT (median, sec)		27.4 (range 21.5-31.6)
Fibrinogen levels (median, mg/dl)		258 (range 70-417)
AT levels (median, %)		108 (range 80-150)
D-dimer (median, ng/dl)		12426 (range 786-60734)
Molecular analysis	bcr1	11
	bcr3	10
	bcr2	1
Risk category	High	0
	Intermediate	17
	Low	5
ISTH-DIC score (≥5: DIC probable)	score 6	2
	score 5	12
	score 4	4
	score 3	1
	score 2	3

M=male, F=female, WBC=white blood cells, PT= prothrombin time, aPTT= Activated Partial Thromboplastin Time, AT: antithrombin, ISTH-DIC= ISTH criteria for Disseminated Intravascular Coagulation.

D057

EFFICACY AND TOLERABILITY OF THERAPY WITH SORAFENIB OR GILTERITINIB AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN 13 FLT3 POSITIVE AML PATIENTS

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Background: Acute myeloid leukemia with FLT3 mutation (FLT3+AML) still represents a therapeutic challenge due to high rate of relapses even after allogeneic hematopoietic stem cell transplantation (HSCT) particularly in patients (pts) without cytologic complete remission (CR) at HSCT or transplanted with positive Minimal Residual Disease (MRD).

Patients and results: In our center, from January 2018 to March 2021, we treated 13 FLT3+AML pts, at high risk of relapse after HSCT, with sorafenib or gilteritinib. Table 1 resumes characteristics of pts. Median age was 60,1 years (range 32-73). Nine pts received FLT3-inhibitor (midostaurin in 5/13 cases) during induction or as salvage therapy pre-HSCT. In 11/13 (85%) pts at high risk of relapse, FLT3-inhibitors were administered as prophylaxis and in 2/13 (15%) as pre-emptive therapy. Treatment was started at a median time of 3,9 months (range 3,0-10,0) post-HSCT; most pts had a complete hematological recovery after HSCT (Table 1), 11 pts were in immunosuppressive therapy and 10 in prophylaxis with azoles. Initial dose of sorafenib was 200 mg/day; 8 pts treated with gilteritinib started with 40 mg/day and 1 with 80 mg/day. In 9/13 (69%) cases the dose was rapidly increased with only one temporary treatment suspension due to transitory toxicity (grade 3 cytopenia). In 7/13 (54%) pts, drug was discontinued because of cytologic relapse (2/13), GVHD (2/13), infection (2/13) or other causes (1/13). The median follow-up was 11 months (range 4,6-41,3). At the last follow-up, 10/13 (77%) pts were alive (7 of them were in complete remission with negative MRD and 3 were in cytologic relapse). Of these pts, 7/10 (70%) were still in therapy with FLT3-inhibitors. No significant side effects were reported.

Conclusions: In our case series, FTL-3-ihibitors post-HSCT have proven to be effective and globally well tolerated. Most of our pts (7/13, 54%) were alive and in CR at last follow-up. FLT3-inhibitors didn't increase risk of severe GVHD even if administered in the early phase post-HSCT. Probably, in very high-risk AML pts (with active disease or MRD positive at HSCT), FLT3-inhibitors should be initiated as soon as possible after HSCT to reduce relapse risk. Prospective controlled studies are ongoing and may clarify efficacy, timing, best drug and dose for post-HSCT maintenance therapy in FLT3+AML pts.

Table 1. Patients characteristics.

	SORAFENIB	GILTERITINIB	TOTAL*
N° PATIENTS	6	9	13
MEDIAN AGE (years)	60,0 (44-72)	62,5 (32-73)	60,1 (32-73)
FLT3 MUTATIONAL STATUS AT DIAGNOSIS			
• ITD	6/6 (100%)	7/9 (78%)	11/13 (84%)
• D835	0	1/9 (11%)	1/13 (8%)
• ITD AND D835	0	1/9 (11%)	1/13 (8%)
DISEASE STATUS AT HSCT:			
• Complete Remission (MRD+)	4/6 (67%)	6/9 (67%)	9/13 (69%)
• Partial Remission	0	1/9 (11%)	1/13 (8%)
• Relapse/Refractory	1/6 (17%)	1/9 (11%)	2/13 (15%)
• Stable Disease	1/6 (17%)	1/9 (11%)	1/13 (8%)
DONOR:			
• MUD	5/6 (83%)	6/9 (67%)	9/13 (69%)
• Aplo	1/6 (17%)	3/9 (33%)	4/13 (31%)
FLT3-INHIBITOR PRE-HSCT	3/6 (50%)	7/9 (78%)	9/13 (69%)
• Midostaurin	1/3 (33%)	4/7 (57%)	5/9 (56%)
• Sorafenib	2/3 (67%)	2/7 (28%)	3/9 (33%)
• Gilteritinib	0	1/7 (14%)	1/9 (11%)
• Quizartinib	0	1/7 /14%	1/9 (11%)
FLT3-INHIBITOR POST-HSCT			
• Maintanence	4/6 (67%)	7/9(78%)	11/13 (84%)
• Pre-emptive	2/6 (33%)	0	2/13 (16%)
• Relapse*	0	2/9 (22%)	0
START FLT3-INHIBITOR (months)			
• Mean	4,8 (2,5-9,3)	6,0 (3,0-16,4)	4,8 (3,0-10,0)
• Median	4,1 (2,5-9,3)	4,1 (3,0-16,4)	3,9 (3,0-10,0)
BLOOD COUNT AT THE START OF FLT3-INHIBITOR			
• Hb< 10 g/dL	0	2/9 (22%)	1/13 (8%)
• Hb>10 g/dL	6/6 (100%)	7/9 (78%)	12/13 (92%)
• PLT>20.000/mmc	6/6 (100%)	9/9 (100%)	13/13 (100%)
• PLT> 50.000/mmc	6/6 (100%)	8/9 (89%)	13/13 (100%)
• PLT> 100.000/mmc	4/6 (67%)	4/9 (44%)	8/13 (61%)
• PMN>500/mmc	6/6 (100%)	8/9 (89%)	13/13 (100%)
• PMN >1000/mmc	6/6 (100%)	6/9 (67%	12/13 92%)
DRUG DISCONTINUATION	4/6 (67%)	5/9 (56%)	7/13 (54%)
• Relapse/progression	1/6 (17%)	3/9 (33%)	2/13 (15%)
• GVHD	1/6 (17%)	1/9 (11%)	2/13 (15%)
• Infection	1/6 (17%)	1/9 (11%)	2/13 (15%)
• Other	1/6 (17%	0	1/13 (8%)
RELAPSE/PROGRESSION DURING MAINTANENCE	2/6 (33%)	5/9 (55%)	5/13 (38%)
LAST FOLLOW UP STATUS:			
• Alive in complete remission	3/6 (50%)	4/9 (44%)	7/13 (54%)
• Alive in relapse	0	3/9 (33%)	3/13 (23%)
• Deaths	3/6 (50%)	2/9 (22%)	3/13 (23%)

** Two patients at the beginning were treated with sorafenib and subsequently (in consideration of non-response/relapse) with gilteritinib.*

D058

PRELIMINARY DATA OF CPX-351 TREATMENT IN A MULTICENTER REAL-LIFE EXPERIENCE IN YOUNG PATIENTS (<60 YEARS OLD) AFFECTED BY THERAPHY RELATED ACUTE MYELOID LEUKEMIA AND ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES

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Therapy-related acute myeloid leukemia (t-AML) and AML with myelodysplasia-related changes (AML-MRC) are two AML subtypes with very poor prognosis. The CLTR0310-301 study showed survival advantages in favour of CPX-351 compared to "7+3" in newly diagnosed t-AML or AML-MRC, 60-75 years old. The aim of our study is to explore the efficacy of CPX-351 in younger patients in a real-life setting. Since September 2019 we treated 13 patients with CPX-351, with a median age of 51 (range 32-59) and ECOG range 0-2. Our cohort consisted in 9 patients with MRC-AML, 3 patients with t-AML and 1 patient with t-AML after a therapy-related myelodysplastic syndrome (t-MDS). 2 patients (both with AML-MRC) harboured *FLT3-ITD* (Allelic Ratio 0.1), 1 patient *FLT3-TKD*, 1 patient *NPM1* (with AML secondary to MDS), 2 patient *IDH1* and 1 patient *TP53* mutation. 7 patients had a complex karyotype and 2 patients had received prior treatment with hypomethylating agents (HMA). All patients underwent induction therapy with intravenous administration of CPX-351 at day 1, 3 and 5 except 1 patient who received just two doses due to pneumonia outbreak. Twelve out of 13 patients were evaluable at the end of treatment. One patient died during induction by sepsis event. Seven patients reached a complete remission (CR) and 1 patient a CR with incomplete haematological recovery (CRi), with 66% overall response rate (ORR). One patient showed a partial remission (PR), while 3 patients were refractory (two showed complex karyotype and one in addition was *TP53* mutated) and were switched to other regimens. Median days of severe neutropenia (defined as neutrophils lower than 500/uL) were 30. Median days of severe thrombocytopenia (defined as platelets lower than 20.000/uL) were 24. We were able to bridge 6 out of 12 (50%) evaluable candidate patients to HSCT. In conclusion we found that CPX-351 is active in young patients with t-AML and AML-MRC with a rate of ORR higher than that reported in the pivotal study (66% *vs* 47%). Regarding the safety profile, the most frequent complication was febrile neutropenia (66%), successfully managed with supportive therapy. Finally, our results are in line with those reported by other institution real-life data in patients <60 years.

D059

VENETOCLAX PLUS AZACITIDINE AS FIRST LINE THERAPY IN PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA: A REAL-LIFE EXPERIENCE

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Older or unfit patients (pts) with acute myeloid leukemia (AML) retain a poor outcome, also after treatment with an hypomethylating single agent. Recently Venetoclax (Ven) and Azacitidine (Aza) combination has been approved for newly diagnosed AML (ND-AML) pts > or = 75 years or ineligible for standard induction chemotherapy, after publication of a Phase -3 trial. However, data on real world efficacy and safety are still limited.

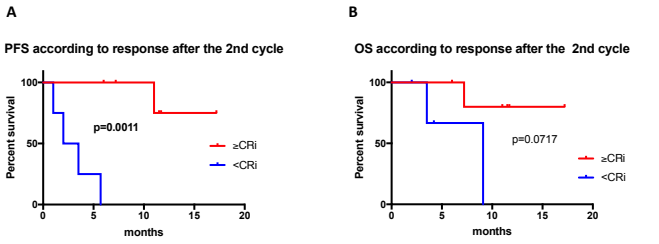

Figure 1.

In this retrospective study we have evaluated the efficacy in terms of overall response rate (ORR), progression free survival (PFS) and overall survival (OS) and the safety profile of Ven-Aza combination in a real-life cohort of ND-AML. Ten consecutive pts with ND-AML (median age 72 y, range 67-77 y), 6 (60%) unfit for standard induction chemotherapy according to Ferrara score have been evaluated. Six had de novo AML and 4 myelodysplastic related changes (MRC)-AML. According to ELN 2017, one patient presented standard, 4 intermediate and 5 high cytogenetic risk. Next generation sequencing (NGS) was available in 6 (60%) cases showing 1 to 4 mutations per patient, 3 cases being *TP53* mutated. Except for one case who progressed after one cycle, all pts received at least 2 cycles of Aza 75 mg/m^2 for 7 days every 28 days plus Ven at maximum dose 200 mg/daily in association with azole prophylaxis. Subsequently, most pts received Aza 75 mg/m^2 in 5 days and Ven 200 mg/day for 21 days every 28 due to infections and prolonged cytopenias. All pts started treatment during hospital admission. No tumor lysis syndrome was observed. Four pts (40%) developed grade 3 pneumonia of whom one (10%) complicated with septic shock and cardiac arrest while in severe neutropenia during the first two cycles. Two pts (20%) developed SARS-Cov2 infection, one asymptomatic while in severe neutropenia during the first cycle, the other died due to bilateral pneumonia despite being in CR. ORR evaluated in 9 pts after 2 cycles was 77%, five pts (55%) obtaining a complete response (CR) and 1 (10%) patient a CR with incomplete hematological recovery (CRi). With a median follow up of 8.2 months, median PFS and OS were 11 months and not reached, respectively. Responding pts (CR+Cri) within two cycles showed improved PFS (not reached *vs* 2.8 months, p=0.0011) and OS (not reached *vs* 9.1 months, p=0.0717) (Figure 1). In conclusion, Ven-Aza combination proved efficacy with an acceptable safety profile in this group of pts with ND-AML and unfavorable profile as for age, fitness and cytogenetic risk.

D060

COVID-19 IN ACUTE LEUKEMIA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Patients with hematologic malignancies appear to have a greater risk of SARS-CoV-2 infection and severe disease due to myelosuppression; delays in treatment of patients with hematologic malignancies are associated with a risk of disease progression. Data on COVID-19 in hematology are still limited.

Methods: Since February 2020 to February 2021, a total of 310 Hospitalizations, → 163 adult patients were admitted in our Center for treatment of hematologic malignancies. The indication for admission was AML in 50 (30%) patients, ALL in 14 (8%), LNH in 54 (33%), MM in 14 (8%), other 31 (19%). Diagnosis of SARS-CoV-2 infection was based on virus detection by RT-PCR in respiratory tract specimens. Standard preventive measures were applied to all patients care, accordance with National disease control and prevention GL.

Table 1.

Tab.1 Overview of each patient's characteristics

ID	Age	Sex	Diagnosis	Time of Covid-19 infection	Covid-19 complications	Change or delay in the Hematological Treatment	Days delay	Deaths owing to Covid-19	Remission status after current therapy
1	82	M	AML	No yet treated	progressive respiratory failure	No treatment	Na	died owing to progressive respiratory failure before treatment could be started	Na
2	56	F	AML	in peak cytopenia post I consolidation	None	delay	60	no	RC
3	62	M	AML	Relapsed post I induction	progressive respiratory failure	N a	Na	died owing to progressive respiratory failure	Na
4	64	M	AML	in peak cytopenia post –re-induction	Interstitial pneumonia	delay	30	no	RC
5	53	M	AML	in peak cytopenia post I induction	None	delay	10	no	In peak cytopenia after allo-BMT
6	67	F	AML	Relapsed post I induction	DVT-EP; Interstitial pneumonia	delay	30	no	RC.schedule d to allo-BMT
7	62	F	AML	in peak cytopenia post I consolidation	Interstitial pneumonia	delay	40	No	RC
8	66	M	ALL Ph-	in peak cytopenia post I consolidation	Interstitial pneumonia	delay	50	No	RC
9	21	M	ALL Ph-	RC post II consolidation	None	delay	20	No	RC
10	59	M	AML	Refractory to I induction	progressive respiratory failure	Na	Na	died owing to progressive respiratory failure	Na

AML: Acute myeloid Leukemia; ALL Ph-: acute lymphoblastic Leukemia Philadelphia negative. RC: Complete Remission, allo-BMT: allogeneic bone marrow transplant. DVT-EP: Deep vein Thrombosis-Embolism Pulmonary; Na: not applicable

Results: Ten (6%) patients tested positive for SARS-CoV-2 via PCR in a unique Covid-19 outbreak during hospitalization stay, and they were transferred to Covid Infectious Unit. All these patients, were affected by Acute Leukemia (8 pts AML, 2 pts ALL ph negative), the majority of them was in peak of cytopenia at the Covid-19 infection time. Nine patients had been treated with intensive chemotherapy before SARS-CoV-2 confirmation. At SARS-CoV-2 diagnosis, 1 patient had untreated, newly diagnosed AML; 3 patients had refractory/relapsed AML. One patient was in CR. DVT complicated by PE and interstitial pneumonia was observed in a patient despite anticoagulation and in thrombocytopenia. After SARS-CoV-2 infection, no leukemia-specific treatment was adjusted.Three patients (30%) died due to severe acute respiratory distress syndrome in deep aplasia, all of them in refractory disease. Seven patients delayed in chemotherapy for a media of 34 days; chemotherapy started until COVID-19 symptoms have completely resolved and two viral testing becomes negative. However, these patients are still alive and maintained their CR, remaining for long-time negative for SARS-CoV-2. One patient underwent to bone marrow transplantation.

*Conclusions:*We reported a high COVID-19 infection mortality of 30%, in accordance with other hematological case series. However, deaths owing to Covid-19 were observed in patients in disease leukemia progression; furthermore, our recovered COVID-19 leukemia patients remained negative for SARS-CoV-2 after delivery of chemotherapy, and underwent to their following chemotherapy and allo-BMT program without any other complications

D061

CPX 351: A THERAPEUTIC CHALLANGE IN SECONDARY ACUTE MYELOID LEUKEMIA: A SINGLE INSTITUTION EXPERIENCE

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Secondary acute myeloid leukemia(sAML) accounts for about 25% of AML. Previous hematological disorders,age,cytogenetics and biomolecolar featurs affect the poor prognosis.The standard induction therapy 3+7 (daunorubicina plus cytarabine)has been associated to lower overall response rate,relapse-free survival and overall survival in sAML when compared with de novo AML.CPX351 is a dual-drug liposomal encapsulation of cytarabine (C)and daunorubicin (D) that has improved the outcome in sAML. From december 2019 to december 2020 we observed six consecutive patients (pts)with sAML eligible for intensive chemotherapy as bridge to Allogenic Hematopoietic Stem Cell Transplant (alloHSCT). Patient's characteristics are listed inTable1. All pts receveid CPX351: three pts one induction cycle (ind1):D44mg/m^2+ C100mg/m^2 on days 1,3,5; three pts second induction cycle (ind2):D44mg/m^2+C100mg/m^2 on days 1,3,5.

Five pts receveid one consolidation cycle D29mg/m^2+C65mg/m^2 on days 1,3.Response to first induction was evaluated in six pts after a median of 45 days.CR was observed in three pts (50%),PR in two pts (33.3%).One pt (16.6%) had a resistant disease. At the end of treatment (EOT) five pts achieved a CR (83.3%).The median time to neutrophil recovery>0.5x10^9/L was 45,44 and 66 days at the ind1,ind2 and consolidation respectively. The median time to platelet recovery>25x10^9/L was 40,35 and 50 days at ind1,ind2 and consolidation respectively. AEs-Grade>1CTCAE.5 were febrile neutropenia in all pts, skin rash in two pts (33.3%)and pneumonia in four pts (66.7%). One pt (16.6 %) died in CRat day 60 for sepsis; the other two pts died in refractory/relapsed disease seven and eight month later since EOT respectively. Two latter pts were carriers of complex karyotype and TP53 mutation. Four pts(66.6%) were alive in CR after a median of seven months from the end of therapy. One of them underwent alloHSCT. Our data confirm that CPX351 is an effective therapy for sAML.The slow molecular release could facilitate the overcoming chemoresistence by neoplastic cells with better therapy efficacy. The delayed blood recovery did not impact on safety. The allo HSCT could be offered to larger number of selected patients fit to intensive chemotherapy with an advantage for disease-related mortality and risk of relapse. Even if on small size population sample, our data overlap with the literature data, describing the use of CPX351 in a single center so as in the "real life experience".

Table 1.

BASELINE DEMOGRAPHIC AND CLINICAL PATIENT CHARACTERISTICS	
Age (y)	N (%)
50-59	1 (16.6)
60-69	4 (66.7)
70-75	1 (16.6)
Sex	
F/M	4/2 (66.7/33.3)
ECOG PS	
0	4 (66.7)
1	2 (33.3)
s-AML subtype	
AML-MRC	4 (66.7)
t-AML	2 (33.3)
Bone marrow blast (%)	
< 50	5 (83.4)
>50	1 (16.6)
White blood cell count (x10 ^{3}/µl)	
<10	5 (83.4)
>10	1 (16.6)
Karyotype	
Favorable	1 (16.6)
Intermediate	1 (16.6)
Adverse	4 (66.7)
TP53	
Wild type/Mutated	4/2 (66.7/33.3)
IDH1	
Wild type/Mutated	5/1 (83.4/16.6)

Lymphomas

D062

PRESENTATION, EFFECTS ON TUMOR TREATMENT AND OUTCOME OF SARS-COV-2 INFECTION IN 50 PATIENTS WITH PRIMARY CNS LYMPHOMA: A STUDY OF THE INTERNATIONAL PCNSL COLLABORATIVE GROUP

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Introduction: COVID-19 is associated with high mortality in cancer patients (pts); its course varies greatly among pt subgroups and tumor status. Herein, we report an study on pts with primary CNS lymphoma (PCNSL), an aggressive tumor where dose intensity is crucial, and concurrent SARS-CoV-2 infection diagnosed in 12 centers of 5 countries.

Methods: Presentation, management and outcome of pts with PCNSL and SARS-CoV-2 infection were analyzed to define effects of infection on timing of PCNSL treatment and outcome. Pts were grouped in 1st and 2nd pandemic waves (cut-off: July 31, 2020).

Table 1.

Patient's characteristics	N= 50
n. of participating centers/countries	12/5
median age (range; years)	68 (22-87)
Males/females	28 / 22
Race: Caucasian/Asiatic/Hispanic	44 / 4 / 2
Disease site	
Brain parenchyma	42 (84%)
Brain + Meninges	6 (12%)
Brain + eyes	1 (2%)
Brain + spinal cord	1 (2%)
IELSG risk	
Low	4 (8%)
Intermediate	18 (36%)
High	16 (32%)
Undefined	12 (24%)
Comorbidity	
High blood pressure	22 (44%)
Hypercholesterolemia	17 (34%)
Vasculopathy/coronaropathy/cardiac arrhythmia	12 (24%)
Obesity/overweight	12 (24%)
Type-II diabetes	10 (20%)
Chronic respiratory disease	5 (10%)
Renal failure	3 (6%)
Hepatitis virus infection/HIV	3 (6%)
Prior solid or hematological tumor	3 (6%)
None	9 (18%)
SARS-CoV-2 detected	
before first-line treatment for PCNSL	8 (16%)
during first-line treatment for PCNSL	27 (54%)
during follow-up	11 (22%)
during salvage treatment (relapsed PCNSL)	4 (8%)
COVID-19 symptoms	
Fever	28 (56%)
Cough	19 (38%)
Dyspnea	17 (34%)
Fatigue	10 (20%)
Pain	1 (2%)
Anosmia	2 (4%)
Ageusia	1 (2%)
Diarrhea	1 (2%)
Treatment of COVID-19	
Hydroxychloroquine	11 (22%)
Antiviral agents (include hyper-immune plasma)	6 (12%)
Dexamethasone	19 (38%)
Anticoagulant therapy or prophylaxis	22 (44%)
Ongoing or prior first-line treatment for PCNSL	
MATRIX or similar regimens	16 (32%)
High-dose methotrexate monotherapy	3 (6%)
High-dose methotrexate plus oral alkylating agent and rituximab	28 (56%)
None	3 (6%)

Results. 50 pts were registered (Table 1): 30 at 1st and 20 at 2nd wave. SARS-CoV-2 was diagnosed before/during 1st line PCNSL therapy in 35 (70%) pts, with a median time between PCNSL diagnosis and virus detection of 45 days (d) (range -27-179); 26 (75%) of them were hospitalized (median 22 d) for pneumonia, 9 admitted to ICU (median 14 d); 13/26 (50%) cleared the virus (median 31 d), resumed PCNSL treatment (median delay 27 d) and are alive. The 13 pts with pneumonia who did not clear virus died of COVID-19 or related infections within 25 d from symptoms onset. 8/9 pts without pneumonia cleared virus and resumed/initiated PCNSL treatment (median delay 16 d); none died of COVID-19. Virus clearance and pneumonia were significantly associated with resumption of PCNSL therapy. 5/11 pts affected by SARS-CoV-2 during follow-up required hospitalization for pneumonia (median 25 d); all 11 pts cleared virus and are alive. Conversely, the 4 pts infected during salvage PCNSL therapy interrupted treatment, did not clear virus and died of lymphoma or COVID-19. At a median follow-up since virus detection of 214 d, 30 (60%) pts are alive, 15 without evidence of lymphoma and 28 cleared virus. 12 (24%) pts died of COVID-19, 4 of other infections, 4 of lymphoma. The 6-month OS was 63%; virus persistence was independently associated with poor outcome. Mortality among pts in 1st line treatment was higher during the 2nd wave (4-month OS 75% *vs* 37%; p=0.03), and associated with lower viral eradication rate (75% *vs* 40%; p=0.03).

Conclusions: COVID-19 was a strong outcome-defining event, especially in pts receiving PCNSL therapy and diagnosed during the 2nd wave. Virus eradication and completion of planned therapy, with acceptable timing and short-term OS, were achieved in half of pts with pneumonia. For pts in follow up, SARS-CoV-2 infection was not associated with worse OS.

D063

ABSTRACT WITHDRAWN

D064

THE BASELINE METABOLIC TUMOR VOLUME PREDICTS THE TIME TO TREATMENT START IN PATIENTS WITH FOLLICULAR LYMPHOMA ON WATCHFUL WAITING

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Introduction: Patients with low tumor burden follicular lymphoma (FL) are often managed with a watchful waiting (WW) strategy. Clinical prognostic models as Follicular Lymphoma International Prognostic Index (FLIPI) are used to predict progression-free survival (PFS) for patients treated with systemic therapy. Measuring baseline tumor burden by PET-CT is a promising parameter to predict PFS following therapy. No prognostic parameters have been established for patients with FL candidates to WW.

Aim: Our study aims to evaluate baseline PET-TC parameters, total metabolic tumor volume (tMTV) and total lesion glycolysis (TLG) as predictors for time to treatment (TTT) in patients with FL and low disease activity according to GELF criteria.

Methods: We included 54 patients with FL diagnosed between 2010-2019 who performed initial FDG PET/CT at our center and were followed by WW approach. TMTV and tTLG were calculated using automatic whole-body segmentation (LesionID, MIM Software Inc). TTT was calculated from the date of diagnosis until start of treatment. Using Receiver operating characteristic (ROC) analysis we identified optimal cutoff of PET parameters for prediction of TTT within 24 months. Survival functions were calculated by Kaplan-Meier estimates. Cox regression model was applied to evaluate PET prognostic power and Wilcoxon-Mann Whitney test for multivariable analysis.

Results: With a median follow-up of 33 months, 22 (41%) patients started therapy due to progression reaching GELF criteria and 32 (59%) patients were on observational strategy at a median of 43 months. The optimal cut-points identified for TTT within 24 months were 14 cm^3 for tMTV (AUC 0.70, 95% CI 0.51-0.88) and 64 for tTLG (AUC 0.71, 95% CI 0.52-088) ($p<0.005$). The probability of not starting treatment at 24 months after diagnosis was 87% (95% CI, 69-95) in patients with tMTV<14cm^3 and 53% (95%, CI, 28-74) in patients with tMTV>14cm^3 ($p<0.005$). The median TTT was 28 months for tMTV>14cm^3; it was not reached for tMTV<14cm^3. We obtained similar results for tTLG. Combining FLIPI and tMTV, we identified a subgroup of patients with both intermediate-high FLIPI and tMTV>14cm^3 who had only 18% probability of not starting therapy at 36 months.

Conclusion: Results suggest that PET-CT functional parameters at diagnosis may help to predict time to treatment in patients with low tumor burden FL managed by WW strategy. This might help to stratify these patients for interventional studies.

D065

RISK-ADAPTED PREEMPTIVE TOCILIZUMAB TO PREVENT SEVERE CYTOKINE RELEASE SYNDROME AFTER CD19 CAR T CELLS: THE HUMANITAS CANCER CENTER EXPERIENCE

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CAR T-cell therapy has revolutionized treatment for patients with relapsed/refractory (r/r) Diffuse Large B Cell Lymphoma (DLBCL) or Primary Mediastinal B Cell Lymphoma (PMBCL). Although impressive durable responses can be achieved, this is weighted by adverse events. Cytokine release syndrome (CRS) immune effector cell–associated neurotoxicity syndrome (ICANS) are the most notable toxicities of CAR T-cell therapy. We report about 20 patients with r/r DLBCL (n=15) and PMBCL (n=5) treated with CD19 CAR T-cells at Humanitas Cancer Center from November 2019 to April 2021, according to AIFA restrictions. The aim of the study is to evaluate the effectiveness of risk-adapted preemptive Tocilizumab administration in preventing severe (grade 3-4) CRS after CAR T-cell. Patients received a dose of Tocilizumab (8 mg/kg) at the time of developing persistent grade 1 CRS, defined as fever (TC ≥ 38°C) for a 24-hour period. ASCT grading system was used to grade CRS and ICANS. Patients characteristics are listed in Table 1. Seventeen (85%) patients developed CRS: CRS was graded as G1 in 8 patients, G2 in 8 patients and G3 in 1 patient. No patients developed grade 4 CRS. Median time to CRS onset was 3 days (range, 0-8). Tocilizumab was administered in 15 cases and only 3 patients received steroids. ICANS was observed in 3 patients (15%). Median time to ICANS onset was 8 days (range, 7-10). ICANS grading was G2 in 1 patient, G3 in 1 patient and G4 in 1 patient. All patients with ICANS were treated with steroids with resolution of symptoms. Intensive care unit (ICU) admission was required for only 3 patients, 2 of them with severe ICANS and one with severe pleural effusion due to uncontrolled progressive disease. Two patents developed infections: one probable pulmonary aspergillosis and one Pneumocystis Carinii pneumonia. Non relapse mortality was 0%. The best overall and complete response rates were 70% and 60%, respectively. The progression free survival and overall survival at 12 months were 48% and 62%, respectively. In conclusion, although the small number of patients analyzes, we found that preemptive administration of Tocilizumab decreased the expected incidence of severe CRS with no impact on neurotoxicity or infections and no death from CAR-T toxicity. Moreover, early intervention with Tocilizumab reduced the ICU admission without adversely impacting on the antitumor efficacy of CD19 CAR T cells, compared with previously reported real world experiences.

Table 1.

Patients characteristics	N=20	%
Age, median (range)	54 years (26-68)	
Disease		
DLBCL	15	75
PMBCL	5	25
ECOG performace status @apheresis		
0	18	90
1	2	10
Stage @apheresis		
I-II	6	30
III	1	5
IV	13	65
Prior ASCT		
no	15	75
si	5	25
LDH @ infusion, median (range); normal range	231 UI/L (121-1528); <248	
CRP @ infusion, median (range); normal range	1.55 mg/dl (0.08-13.9); <0.5	
Ferritin @ infusion, median (range); normal range	207 ng/ml (40-336); 24-336	
Disease status post bridging therapy		
CR/PR	6	30
SD/PD	14	70
CART type		
Tisagenlecleucel	15	75
Axicabtagene ciloleucel	5	25

DLBCL, Diffuse Large B Cell Lymphoma; PMBCL, Primary Mediastinal B Cell Lymphoma; ECOG, Eastern Cooperative Oncology Group; ASCT, autologous stem cell transplantation; LDH, lactate dehydrogenase; CRP, C-reactive protein; CR, complete response; PR, partial response; SD, stable disease; PD progressive disease; CART, chimeric antigen receptor T-cell.

D066

ORBIT IRRADIATION AS SALVAGE TREATMENT FOR PATIENTS WITH OCULAR ADNEXAL MALT LYMPHOMA (OAML) RELAPSED AFTER OR REFRACTORY TO CHLAMYDIA PSITTACI-ERADICATING ANTIBIOTIC THERAPY

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Background: OAML is related to *Chlamydia psittaci (Cp)* in ~75% of Italian patients (pts). *Cp* eradication with doxycycline has been followed by lymphoma regression in two-thirds of pts enrolled in prospective trials. Several therapies, in particular radiation therapy (RT), are available for pts with unresponsive or relapsed disease; however, postponing RT while waiting for the tumor response to antibiotic could be a cause of concern. Herein, we report safety and efficacy of salvage RT in pts with OAML relapsed after or refractory to *Cp* eradication.

Methods: Pts with stage-IEA OAML diagnosed at our institution between 2005 and 2019 were reviewed. Selection criteria were: 1) first-line *Cp*-eradicating therapy with doxycycline; 2) lymphoma relapsed or progressed locally after doxycycline; 3) orbital irradiation as salvage treatment. Data of diagnosis, treatment and outcome of selected pts were analyzed to establish safety and efficacy of RT as salvage treatment after doxycycline.

Results. 25 pts (median age 66; range 37-92; 14 males) were assessable. Eleven pts had conjunctival lesions, 6 had viral hepatitis, 9 had gastric *H. pylori* infection, one had Sjögren syndrome. All considered pts but one (partial response) experienced progressive disease during doxycycline (n=10) or after a period of disease stability (n=14), with a median time to progression from doxycycline of 14 months (IQR 4-40). RT dose was 30/30.6 Gy delivered in 15/17 fractions; ocular function was maintained in all pts with mild side effects (only 2 cases of grade-1 blepharitis). RT was followed by objective response in all pts (ORR=100%), with a complete response in 23 (92%; 95%CI= 82-100). At a median follow-up from RT of 42 months (range: 5-168), 8 pts experienced relapse: within the irradiated volume in 2 (8%), at the contralateral orbit in 1 (4%) and at distant organs in 5 (20%), with a 4-year PFS of 68±10%. The 8 relapsed pts received 10 further lines of treatment: antibiotics (n=5), RT (n=2; distant), tumor resection (2; distant), and lenalidomide (1). All pts are alive; 20 (80%) pts are disease-free.

Conclusions: To postpone RT until relapse after *Cp*-eradicating antibiotic therapy is a safe and effective strategy in pts with limited-stage OAML. In-field, contralateral and distant relapse rates after this strategy are similar to those reported in large OAML series treated with upfront RT. Treatment without chemotherapeutic agents and delaying RT until relapse does not affect survival of OAML pts.

D067

SAFETY AND EFFICACY OF THE "CARMEN" REGIMEN, A NEW DOSE-DENSE SHORT-TERM THERAPY, IN PATIENTS WITH AGGRESSIVE B-CELL LYMPHOMA AND MYC REARRANGEMENT. A MULTICENTER ITALIAN EXPERIENCE

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Introduction: Pts with aggressive B-cell lymphoma and MYC rearrangement exhibit poor outcome after R-CHOP. In the last decade, pts with Burkitt lymphoma (BL) or high-grade B-cell lymphoma with MYC rearrangement (HGBCL) were treated with a new dose-dense, short-term therapy termed "CARMEN regimen", at seven Italian Centers. Herein, we report efficacy and tolerability of CARMEN in a multicenter series of 66 pts with BL or HGBCL.

Methods: Adults (18-80 years) with BL or HGBCL and MYC rearrangement at FISH were treated with CARMEN: a single 36-day course of sequential doses of cyclophosphamide, vincristine, rituximab, methotrexate, etoposide, and doxorubicin (induction) plus intrathecal chemotherapy, followed by high-dose-cytarabine-based consolidation (plus cisplatin in HIV-negative pts). Pts who did not achieve CR after induction received BEAM/ASCT after consolidation.

Results: 25 pts with HGBCL and 41 with BL were treated (Table 1). Treatment was well tolerated: 21 (84%) HGBCL and 38 (93%) BL pts completed induction, 20 (80%) and 38 (93%), respectively, completed consolidation. Per protocol, 8 HGBCL and 9 BL pts received ASCT. G4 hematological toxicity during induction was: neutropenia in 50 (76%) pts, thrombocytopenia in 24 (36%) and anemia in 7 (11%), which were recorded after consolidation in 34 (59%), 38 (66%) and 1 (2%) pt, respectively. G4 non-hematological toxicity was uncommon: mucositis in 4 (6%) pts and TLS in 1 (2%) during induction; heart failure and bleeding in 1 (2%) pt each after consolidation. G4 infections were recorded in 4 (6%) pts during induction and in 2 (3%) after consolidation. 4 HGBCL and 2 BL pts died of toxicity (sepsis in 4; respiratory failure; COVID-19), with a TRM of 9%. After induction, 21 (84%) HGBCL and 37 (90%) BL pts achieved a response, which was CR in 11 (44%) and 26 (63%) pts, respectively. After the whole treatment, CRR was 68% for HGBCL pts and 78% for BL pts. At a median follow-up of 54 (2-131) months, 17 (68%) HGBCL and 29 (71%) BL pts remain relapse-free, with a 5-yr PFS of 67% and 70%, respectively. 17 HGBCL and 32 BL pts are alive, with a 5-yr OS of 66% and 77%, respectively. HIV seropositivity did not modify outcome. Age and LDH serum level were independently associated with OS.

Conclusions: With the limitations of a retrospective series, this study shows that CARMEN regimen is a safe and active treatment in HGBCL with MYC rearrangement and BL pts, independently from HIV positivity.

Table 1.

	HGBCL (n=25)	BL (n=41)
Median age (range)	56 (26 – 77)	43 (21 – 69)
Gender - males	18 (72%)	33 (80%)
ECOG-PS >1	4 (16%)	15 (37%)
Stage (Ann Arbor) III-IV	23 (92%)	35 (85%)
CNS involvement	2 (8%)	5 (12%)
Bone marrow infiltration	3 (12%)	9 (21%)
Bulky disease	12 (48%)	7 (17%)
High LDH serum level	21 (84%)	33 (80%)
IPI >2	15 (60%)	33 (80%)
HIV seropositivity	11 (44%)	32 (78%)
HBV or HCV seropositivity	5 (20%)	14 (34%)
Single hit (FISH; *MYC*)	16 (64%)	40 (98%)
Double Hit (*MYC + BCL2 or BCL6*)	8 (32%)	0 (0%)
Triple hit (*MYC + BCL2 + BCL6*)	1 (4%)	1 (2%)

D068

PROGRESSION-FREE SURVIVAL IN ADVANCED CLASSICAL HODGKIN LYMPHOMA PATIENTS WITH BULKY DISEASE AFTER ABVD WITHOUT RADIATION THERAPY IN ERA PET

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Introduction: The role of radiotherapy (RT) in patients (pts) with classical Hodgkin lymphoma (cHL) in advanced stage and bulky disease who present a complete metabolic responseafter chemotherapy is currently debated. We report here our experience in a cohort of pts with advanced cHL with bulky disease who did not receive RT.

Methods: We retrospectively collected data of pts with cHL stage IIB-IV with bulky disease, defined as >5 cm, treated at our Institution from 2010 to 2020. We excluded pts with positive interim PET-CT (iPET-CT) or positive end-of-treatmentPET-CT (EOT-PET-CT), as well asthose who

received RT as part of first-line treatment. PET-CT scans were evaluatedinitially with the Juweid criteria, then with the Lugano criteria based on the Deauville score. We performed a descriptive analysis of the principal pts' characteristics and Kaplan-Meier analysis of progression-free survival (PFS) and overall survival (OS).

Results: We report data from 49 consecutive advanced stage cHL pts with bulky disease and negative iPET-CT and EOT-PET-CT. Median age was 36.7 years (range, 16.5–61.07),63.3% pts were male, nodular sclerosis was the most frequent histologic subtype (55.1%), B-symptoms were present in 69.4% of pts. 14 pts were in stage IIB, 17 in stage III, 18 in stage IV. In 37 pts bulky lesions were localized in mediastinum, in 7 pts in the abdomen, while a laterocervical and inguinal bulky was present in 2 and 3 pts, respectively. Dimension of the bulky lesion was 5-7 cm in 24 pts, 8-9 cm in 14 pts, > 10 cm in 9 pts; in 2cases dimension was not available. All pts were treated with six cycles of ABVD. After a median follow-up of 43.5 months (range, 8-136) all pts were alive, andtwo pts relapsed at 6 and 18 months after restaging, both outside of the bulky area. 5-years PFS was 95.0%; according to bulky dimension 5-years PFS was 95% in pts with 5-7 cm bulky, 92.3% in pts with 8-9 cm bulky, 100% in pts with a bulky larger than 10 cm.

*Conclusions:*With the limitations of a monocentric and retrospective studyour results showed a high PFS in a selected cohort of pts withadvanced stage, bulky cHL pts with negative iPET-CT and EOT-PET-CTdespite the omission of RT, as previously assessed by Gallamini et al (J Clin Oncol 2020; 38:3905-13) in a large randomized, phase III trial. Results of other randomized trials are awaited to appropriatelyevaluate the role of RT as consolidation in advanced cHL in the PET era.

D069

IMMUNE-RELATED ADVERSE EVENTS IN THE TREATMENT OF NON-HODGKIN LYMPHOMA WITH IMMUNE CHECKPOINT INHIBITORS: A RETRO-PROSPECTIVE CASE SERIES

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Immune checkpoint inhibitors (ICIs) show efficacy in the treatment of non-Hodgkin lymphomas (NHL). However, the improved immune response induced by this class of agents is related with a peculiar group of adverse events, known as immune-related AEs (irAEs). Currently, no real-world prospective data were already published on these peculiar AEs, and recommendations for their management are based on information coming from the ongoing clinical trials. An observational retrospective/prospective study was conducted on patients with relapsed/refractory NHL treated with ICI to determine the incidence of irAEs assessing the type, severity, and timing of onset, management, outcome and relationship with ICIs of these events. Secondary objectives were activity and disease control of ICIs along with their relationship with irAEs onset. Thirty-two patients underwent ICI as single agent (N=20) or in combination (N=12). Ten patients (31.3%) developed at least one irAE for a total of 17 irAEs. Median time to presentation of irAEs was 69 days (range, 0-407) with a median resolution time of 16 days (ranges 0-98). No statistically significant difference in irAEs frequency resulted between different ICIs, histologies and outcomes. Progression free survival at 24 months for patients who developed an irAE was 40% and 31.8% for who did not. Overall survival for the two groups did not differ (at 24 months 40.0% and 62.5% for patients without and with irAE, respectively), but the median for patients who developed an irAE was not reached. No dose reduction for ICIs has been necessary and only 2 patients had an early drug discontinuation due to AEs. The incidence of irAEs was associated with better long-term survival in NHL treated with ICI but patients' disease conditions need to be carefully evaluated to decide the optimal actions to be adopted. Lymphomas-adapted guidelines for irAEs diagnosis and management are needed.

D070

UPDATED RESULTS OF THE FIL (FONDAZIONE ITALIANA LINFOMI) "MIRO" STUDY, A MULTICENTER PHASE II TRIAL COMBINING LOCAL RADIOTHERAPY AND MRD-DRIVEN IMMUNOTHERAPY IN EARLY-STAGE FOLLICULAR LYMPHOMA

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Early-stage follicular lymphoma (FL) is managed with involved-field radiotherapy (IFRT), allowing eradication only in 40-50% of patients (pts). The aim of this multicenter phase II prospective study was to evaluate the role of MRD in identifying pts unlikely to be cured by IFRT, for whom an immunotherapy (IT) could improve outcome. 110 pts with stage I/II FL were treated with 24 Gy IFRT. Peripheral blood (PB) and

bone marrow (BM) samples were centralized to the FIL (Fondazione Italiani Linfomi) MRD Network. In BCL2/IGH+ pts at baseline by both nested PCR (NEST) and RQ-PCR (RQ) in BM a/o PB, MRD was analyzed after IFRT and every 6 months over 3 years. Pts with MRD+ by both NEST and RQ in BM a/o PB after IFRT or who became MRD+ during FU were treated with 8 weekly doses of the anti-CD20 MoAb ofatumumab (OFA). Primary objective: to define the efficacy of IT in obtaining a negative MRD. Of 106 evaluable pts at baseline, 32 (30%) were BCL2/IGH+ in BM a/o PB. All but one obtained a clinical response after IFRT; one additional pt died soon after IFRT for unrelated causes. MRD evaluation after IFRT revealed the persistence of BCL2/IGH+ cells in PB a/o BM in 60% of pts. MRD+ pts, either after IFRT (n=18) or in case of conversion to MRD+ during FU (n=8), received OFA, obtaining a conversion to MRD- in 22/24 pts (91.7% - CI 73.0-99.0), significantly superior to the expected 50% (Figure 1). After a median FU of 38 months, 17 pts who achieved a MRD- with OFA are still negative; 5 converted to MRD+. Of the latter, 2 received OFA retreatment, achieving a second MRD-; 2 pts were not retreated due to Sars-Cov2 pandemic. Clinical relapse or progression was observed in 23 pts: 18 (24.6%) among the 73 "no marker" pts and 5 (15.6%) among the 32 BCL2/IGH+ at baseline (p=0.3), with no significant difference in PFS (p=0.25). Two early relapses were observed among the 12 pts who became MRD- after IFRT and 3 among the 24 treated at least once with OFA (1 MRD+, 1 MRD-, 1 converted from MRD- to MRD+). Only 1 Pt relapsed while MRD- after OFA. MRD data indicate that IFRT alone is often insufficient to eradicate the disease, inducing a MRD- only in 40% of pts, long-lasting in half of them. The primary objective, MRD conversion after IT, was largely achieved. The strategy of an IT consolidation after IFRT in MRD+ pts allowed to increase molecular responses, although applicable only to 30% of pts. A clinical advantage of the MRD-driven treatment strategy is suggested although not significant.

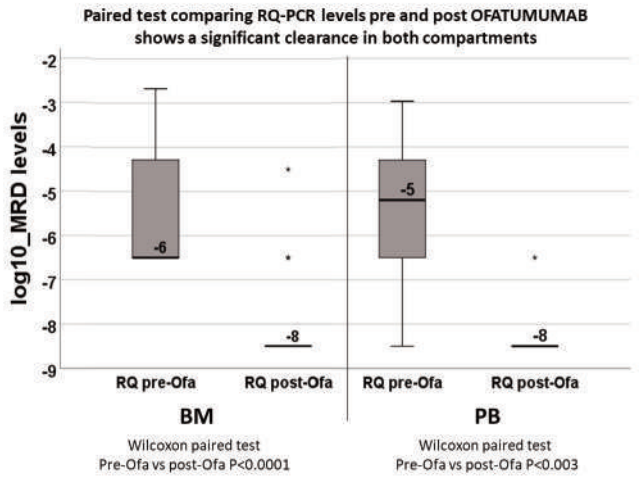

Figure 1.

D071

HIGH GRADE B-CELL LYMPHOMA WITH *MYC, BCL2* AND/OR *BCL6* REARRANGEMENTS: UNRAVELING THE GENETIC LANDSCAPE OF A RARE AGGRESSIVE SUBTYPE OF NHL

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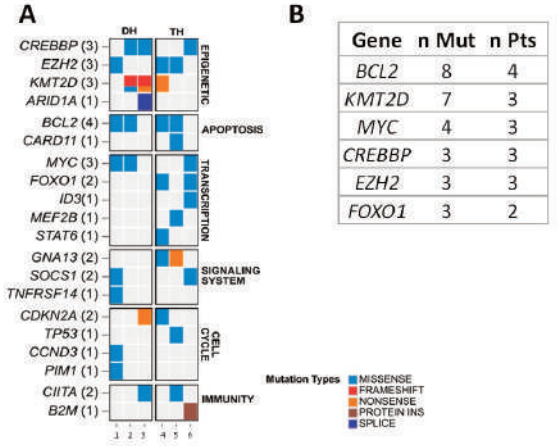
Background: High-grade B-cell lymphomas, with *MYC* and *BCL2* and/or *BCL6* translocations, i.e, double-hit or triple-hit lymphoma (HGBL-DH/TH) are a distinct subgroup among high-grade lymphomas *(WHO 2017)* characterized by an increased risk of central nervous system involvement and low sensitivity to standard chemotherapy. Although, they are well defined by cytogenetic markers, concurrent genetic events contributing to their genomic landscape are still largely unknown.

Aim: Elucidating the genomic background of HGBL-DH/TH by identifying concomitant molecular cytogenetic events. Patients. Eighty patients with a diagnosis of diffuse large B-cell lymphoma (DLBCL) were recruited. DNA was extracted from Formalin-Fixed Paraffin-Embedded samples or bone marrow (QIAamp DNA FFPE and AllPrep DNA/RNA, Qiagen).

Materials and methods: FISH with break-apart assays (Vysis-Abbott) was done for *MYC, BCL2*, and *BCL6*. CytoscanHD/OncoscanCN-VPlusAssay (=5 cases) was performed according to manufacturer's protocols (Affymetrix). Filters were set at 1,5Mb for CNA and at 10 Mb for cnLOH with at least 50 markers. On 6 cases, targeted NGS (Lymphoma Solution) was performed with Sophia Genetics tools (SOPHiA DDM software).

Results: HGBL-DH/TH accounted for 13/80 cases (16%). There were 5 TH and 8 DH. Overall, 43 CNA were detected (range: 4-14; median: 8), *i.e.* 21 losses, 16 gains, and 6 cnLOH. There were no common events. One case of HGBL-DH showed chromothripsis of chromosome 3. Mutational analysis identified 49 variants (range: 7-11; median: 8) in 20 genes. Mutations mainly involved epigenetic modulators and transcriptional factors (Figure 1A). *BCL2* (n =8), *KMT2D* (n =7), and MYC (n =4) (Figure 1B). *FOXO1* was mutated in 2/3 cases of TH (Figure 1A). All six patients presented potentially druggable marker(s).

Conclusions. Our FISH screening on unselected DLBCL reclassified 15% of cases as HGBL-DT/H, confirming FISH testing as the preferential diagnostic approach (Friedberg JW, Blood 2017). Mutational screening revealed that: a) *BCL2* and *MYC* are frequently mutated, suggesting that limiting their evaluation to the rearrangement probably underestimates their pathologic impact and b) the SNVs median number per patient, the kind of genes affected by mutations, and the specific inhibitor availability (HDAC, EZH2, BCL2, AKT, MALT1 inhibitors) suggest the need for deeper diagnostic evaluation to design new target treatments in this poor risk lymphoma subgroup. Supported by GILEAD Fellowship program 2018/2019



B

Gene	n Mut	n Pts
BCL2	8	4
KMT2D	7	3
MYC	4	3
CREBBP	3	3
EZH2	3	3
FOXO1	3	2

Fig.1: **A)** Heatmap of 6 patient detected mutations across Double Hit (DH) and Triple Hit (TH) subtypes and considering gene biological functions; **B)** List of the most mutated genes.

Figure 1.

D072

NAÏVE T CELLS ARE REDUCED FOR PROLONGED PERIODS AFTER BENDAMUSTINE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Treatment of follicular lymphoma (FL) can induce severe and prolonged lymphocytopenia, more common after Rituximab-Bendamustine (RB) regimen. Limited data exist on the composition of the peripheral T cell pool after RB. The single-platform Lyotube Immunomonitoring was used to determine the percentage and absolute count of lymphocyte subsets in peripheral blood. T cell maturation was studied as follow: CD45RA-FITC, CCR7-PE, CD95-PerCP-Cy5.5, CD4-PE-Cy7, CD27-APC, CD8-APC-H7, CD3-BV450, CD31-BV500. Data were acquired on FACSCantoII and analyzed with Diva Software (BD Biosciences). CD4 and CD8 maturational subsets were defined as Naïve (CD45RA+CCR7+), Central Memory (CD45RA-CCR7+), and Effector Memory (EM, CD45RA-CCR7-). Wilcoxon-Mann-Whitney test was used for statistical analysis. We prospectively studied patients (pts) with FL (8 F, median age 55 and 6 M, median age 62) in complete remission after RB induction therapy. We analyzed 22 samples, 14 in female and 8 in men, between 18 and 24 months during Rituximab maintenance. We compared these data to a group of 26 age-matched healthy controls (HC). We observed a lower CD4+ count in pts compared to HC (median value $248x10^6$/L *vs* $780x10^6$/L, p<0.00001). CD8+ count did not differ between pts and HC. The CD4/CD8 ratio was significantly lower in pts (median 0.7 *vs* 1.6, p<0.00001). Regarding the maturational subset the percentage and absolute count of CD4+ Naïve cells were significantly lower in pts compared to HC (median 23.5% *vs* 44.4% p=0.001 and median $72x10^6$/L *vs* $275x10^6$/L p<0.00001, respectively), while CD4+ EM cells were higher in pts (median 31% *vs* 22% p=0.001). The percentage and absolute count of CD4+ and CD8+ naïve cells were significantly lower in males pts than in females (11.7% *vs* 29% p=0.001 and $18x10^6$/L *vs* $89x10^6$/L p=0.001 for CD4+; 8.4% *vs* 23% p=0.007 and $44x10^6$/L *vs* $88x10^6$/L p=0.009 for CD8+, respectively). Four pts developed infections: pneumonia in 2 male pts (Haemophilus Influenzae, Klebsiella), recurrent cystitis in 1 female pt, recurrent bronchitis and Pseudomonas cellulitis in 1 male pt. CD4+ counts are significantly reduced for a prolonged period up to two years in FL pts after RB. Most strikingly, we observed a severe long-term reduction of CD4+ naive T cells in pts compared to HC, most evident in male pts. This might have clinical implication for the risk of viral and opportunistic infections. Further studies are warranted to corroborate the clinical significance of our finding.

D073

IMMUNOLOGICAL AND KINETIC HETEROGENEITY IN CAR-T CELLS TREATED LYMPHOMA PATIENTS

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Background: Chimeric antigen receptor (CAR)-T cell therapy is associated with relevant, life-threatening side-effects such as cytokines release syndrome (CRS), macrophage activation syndrome (MAS), ipo/agammaglobulinemia, immune effector cell-associated neurotoxicity syndrome (ICANS) and infections. Here, we show the preliminary results of a prospective biological study conducted on a cohort of patients affected by Relapsed/Refractory B-cell non-Hodgkin Lymphoma subtypes receiving commercial/AIFA approved anti-CD19 CAR-T cell products at IRCCS-Azienda Ospedaliera-Universitaria of Bologna.

Methods: We report on the kinetics of CAR-expressing and CAR-non-expressing cell subpopulations in 3 consecutive patients who developed ICANS by multiparametric flow cytometry at various time points (pre-apheresis, pre-lympho-depletion, after 1 hours and 3, 7, 13, 21, 30, 90 days post infusion). Surface markers of maturation (CD45RA, CD95, CD62L), differentiation/senescence (CD28, CD57) and exhaustion (PD-1, BTLA) status were analyzed within the CD45+CD3+CAR-expressing and CD45+CD3+CAR-non-expressing-cell subsets by incubation with CD19 CAR Detection Reagent and anti-Biotin (Miltenyi Biotec).

Results: ICANS occurred on day 6, and first-line therapy with dexamethason (10 mg/kg per 4) was administered. CD45+CD3+CAR-expressing cells peaked at day 7 (ranging from 1% to 30%). A dramatic decrease in the percentage of the CAR-cell population was observed after steroid administration, and was paralleled by the CAR-non expressing cell population shrinkage. CAR-non-expressing cell population reappraisal was observed after steroid withdrawal. Notably, the CD3+CD8+ T cells showed a more differentiated phenotype (CD45RA+CD28-CD57high) within the CAR-expressing cell compartment at day 7, whereas most CD3+CD4+ T cells (CD45RA-CD28+CD57-) acquired a central and effector memory phenotype. Interestingly, one patient showed an increase in the PD-1 expression within the CD3+CD8+CAR-expressing cells. Patients showed short-term response to therapy, in spite of the important corticosteroid-induced lympho-depletion.

Conclusion: The treatment of clinical complications related to CAR T-cell therapy can significantly modify CAR-expressing and CAR-non-expressing cell population kinetics. A larger series of patients is warranted to correlate the lymphocyte populations expansion kinetic to clinical complications and disease response to therapy.

D074

LONG LASTING COMPLETE RESPONSES TO BRENTUXIMAB VEDOTIN AS LAST THERAPY IN HODGKIN LYMPHOMA PATIENTS FAILING AUTOLOGOUS TRANSPLANTATION: A SINGLE-CENTER EXPERIENCE

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Background: Follow-up of the pivotal trial and large case series report that a proportion of patients between 5% and 9% with relapsed or refractory Hodgkin lymphoma (HL) failing autologous stem cell transplantation (ASCT) and treated with brentuximab vedotin (BV) achieve and maintain long lasting complete responses (CR) with no further treatment. Very long-term data on the outcomes of such patients are indeed underreported.

Methods: Our institutional experience with patients failing ASCT and receiving BV as their last treatment line was reviewed. Records of patients in CR for more than 5 years after BV discontinuation have been analyzed in more details. Five patients who received BV at a median time since HL diagnosis of 50 (range 14-71) months met these characteristics.

Results: The median number of previous treatment lines was 3 (range 3-4) including ASCT. ASCT was the last therapy in all patients but one, who received an ifosfamide-containing regimen immediately before BV. Three patients relapsed at a median time of 9 (range 7-9) months after ASCT; one was true refractory to ASCT; the one who relapsed after ifosfamide did so after 29 months. All but one patients received 16 cycles of BV. One patients interrupted the treatment after 12 courses because of grade 3 peripheral sensory neuropathy. All patients obtained an initial response after 4 courses, which was a CR in 3 out of 5 cases. Four achieved a CR at the end of their treatment. In one patient, a partial re-

sponse converted into a CR 6 months after the 16th course of BV. Patients have a median duration of CR of 7.5 (range 6.9-8.9) years, and none of them encountered disease relapse nor received any subsequent consolidation, including allogeneic transplantation at the latest available follow-up.

Conclusions: BV confirms its efficacy in inducing CR in HL patients failing ASCT. A proportion of them reach a long-lasting CR with no need of further treatment and are therefore considered cured. The role of allogeneic transplantation in patients in CR after BV remains matter of debate.

D075

OBINUTUZUMAB DOES NOT AFFECT MOBILIZATION OR ENGRAFTMENT OF PERIPHERAL STEM CELLS IN DIFFUSE LARGE B CELL LYMPHOMA: FINAL RESULTS FROM THE PHASE II FIL GIOTTO STUDY

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Second line chemotherapy do not permit to obtain satisfactory results particularly in patients previously treated with Rituximab. Patients with first R/R DLBCL were prospectively treated with a combination of the new anti-CD20 antibody Obinutuzumab in association with DHAP (G-DHAP). The primary end point was to demonstrate an increase in the complete metabolic response (CMR) with this regimen. Secondary end points were stem cell mobilization and stem cell engraftment. In this prospective, phase-2, single-arm trial (EudraCT 2014-004014-17), R/R DLBCL patients received the standard three doses (1000 mg) of Obinutuzumab in the first cycle ad then one dose for the remaining three cycles. The stem cells apheresis was programmed after the third or the fourth cycle. At the end of therapy a restaging was performed and patients with CMR were consolidated with BEAM/FEAM conditioning regimen and autologous stem cell transplantation (ASCT). The protocol provided an interim analysis after the first 29 patients enrolled to confirm the null hypothesis of obtaining at least 10 CMR. At first interim analysis 29 patients were evaluated. The median age was 56 years, 17 patients (59%) had a primary refractory lymphoma and 12 were relapsed. Fifteen patients completed therapy and were evaluated for CMR. In an intention to treat analysis six patients obtained a CMR (6/29 patients: 21%). According to the results of the interim analysis study enrolment was stopped. We have evaluated the peripheral blood progenitors harvest. Nineteen patients started stem cell mobilization, one failed and 18 patients mobilized. Sixteen patients (89%) mobilized after 1 or 2 apheresis and the other two patients after 3 or 4. The median number of CD34+ cells was 5.5 (IQR: 5 – 6.75). Nine out 18 patients reinfused and 9 did not. The mean number of reinfused CD34+ cells was 4.1 (IQR: 3.5 – 5).

Seven out 9 (78%), after a median follow up of 41 months, are alive and without evidence of disease. Overall after a median follow-up period of 46 months survival and progression free survival were 45% and 41% respectively. The GIOTTO study did not pass the interim analysis because a lower rate of CMR reported in comparison with the hypothesis. Obinutuzumab associated with a standard mobilizing chemotherapy did not compromise stem cell mobilization and engraftment after ASCT in DLBCL patients. Globally 13 patients are alive and seven patients are alive and free from disease after a median follow-up of 41 months.

D076

INCREASE OF BONE EVENTS (FRACTURES) IN PATIENTS WITH AGGRESSIVE NON-HODGKIN LYMPHOMA: NEGATIVE SYNERGISM BETWEEN STEROIDS AND LOW MOLECULAR WEIGHT HEPARIN (LMWH)? OUR EXPERIENCE

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Low molecular weight heparins (LMWH) are widely used in thrombosis prophylaxis in lymphoma patients with central peripheral venous device (PVD). The negative effect of heparin on osteogenesis is known, it is not clear how anticoagulants vit K inhibitors act while the role of LMWH is controversial. The negative effect of steroids on osteogenesis is also documented but there are no data on negative synergy related to the simultaneous intake of steroids and LMWH. The aim of our study is to evaluate the bone events (bone fractures) observed retrospectively in a consecutive cohort of lymphoma patients treated with chemotherapy (with or without steroids) with at least 6 months of follow-up. From January 2014 to January 2021 we observed 197 patients with a median follow-up of 38 months (range 6-85 months); 90 with NHL treated with CHOP or similar 34 female and 56 male with a median age of 59 years (range 23-77 years); 53 with NHL treated with bendamustine based therapy; 23 female and 30 male with median age of 65 years (range 42-81 years) and 54 with HD treated with ABVD or similar; 26 female and 28 male with a median age of 34 years (range 16-75 years). All patients with PVD and all treated in prophylaxis with LMWH (enoxaparin or nadroparin) 4000 U / day. In the NHL group treated with steroids and LMWH the observed bone events (fractures) were 13/90 patients (14.5%), 6 M and 7 F with a mean age of 63 years (range 39-73 years) all patients had vertebral involvement, while in 2 patients, in addition to the vertebral, with the femur involvement were documented. In the NHL group treated with LMWH but non-steroids, the observed bone events were 3/53 patients (5.5%) all female with a mean age of 66 years, while in the HD group it was 2/54 patients (3.8%) all females with a mean age of 72 years, all with vertebral involvement only. These data show a higher incidence of bone events in patients receiving steroid and LMWH therapy. This evidence suggests a negative synergism between the association of steroids and LMWH on bone metabolism and also probably confirms that vitamin D metabolism in patients with aggressive NHL may be implicated in the prognosis of these lymphomas. This evidence suggests the need to integrate vit D with or without calcifying into the therapy of patients with aggressive NHL and to evaluate the possibility of proposing prophylaxis for thrombosis not with LMWH but with the new oral anticoagulants. A prospective study is needed which also includes the study of calcium metabolism and bone mineralization both at diagnosis and over time in the various subtypes of NHL and which supportive treatments they have received.

D077

IBRUTINIB IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: A REAL-LIFE, RETROSPECTIVE, MULTICENTER TRIAL ON BEHALF OF THE "RTL" (REGIONAL TUSCAN LYMPHOMA NETWORK)

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Background: Relapsed or refractory (R/R) mantle-cell lymphoma (MCL) patients have a poor prognosis and their management is challenging, in absence of a golden standard as salvage treatment. Bruton's tyrosine kinase inhibitor ibrutinib represents an effective treatment for R/R MCL patients.

Aim: We investigated ibrutinib efficacy and safety in daily clinical practice in Tuscany, together with factors that could predict disease outcome.

Patients and Methods: In this multicentre, single-arm, observational study we retrospectively analyzed a cohort of 69 consecutive, R/R MCL patients managed at 10 onco-hematological centers in Tuscany from 2005 to 2019. We identified PFS as primary endpoint, while OS, DOR, ORR and CR rate were secondary endpoints; we also investigated the potential predictive factors associated with disease response and survival. In addition, we analyzed overall toxicities and therapeutic strategies used in patients who relapsed during treatment.The treatment regimen consisted of oral, continuous, single-agent ibrutinib, maximum dosage of 560 mg once per day, until disease progression.

Results: Median duration of treatment was 9 cycles (range 1-45); 66/69 patients were evaluable for response (95.7%), the remaining 3 patients died within 3 months from the beginning of treatment due to infections. Overall response rate was 62.3%, with a CR rate of 39.1%. Reasons for treatment discontinuation included PD (30/69 cases, 43.5%), second malignancies (2 cases), acute renal insufficiency (1 case, considered as unrelated to ibrutinib), treatment toxicity (8/69 cases). After a median follow-up of 15.6 months, 40/69 patients (58%) were alive, the main cause of death was progressive disease (PD, 22/69 cases, 31.9%). Median PFS was 17 months, median DOR was not reached (estimated 2-y DoR 68%), median OS was 34.8 months. Inferior PFS was associated with >1 prior line of therapy and B symptoms. Ibrutinib refractoriness was associated with inferior OS, median OS after ibrutinib failure was only 5 months. The majority of the adverse events (AE) were mild to moderate (grade 1-2). Grade 3-4 neutropenia occurred in 7/69 cases (10.1%).

Discussion and Conclusion: In this real-life setting ibrutinib treatment prolonged survival in R/R MCL patients, with PFS and OS comparable to clinical trials, without unexpected adverse events. Patients receiving ibrutinib as 2nd line regimen had the most favorable outcome, while survival was dismal after ibrutinib failure.

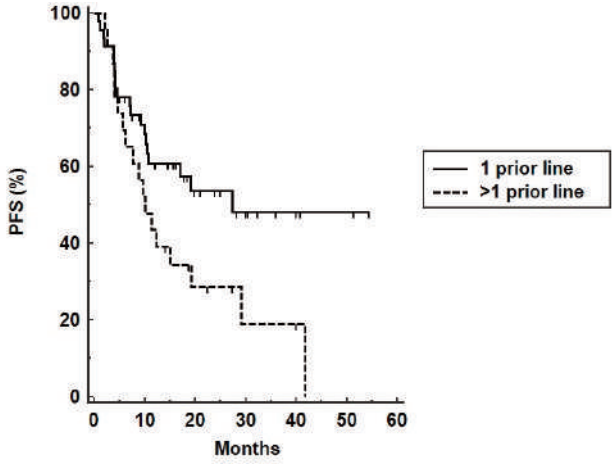

Figure 1.

D078

ABSTRACT WITHDRAWN

D079

R-VEMP FOR ELDERLY DLBCL PATIENTS: DATA FROM A MULTICENTER RETROSPECTIVE STUDY

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Background: About 30% of diffuse large B-cell lymphoma (DLBCL) occur in patients older than 70 years. The outcome of these patients with standard first line regimens remains poor, compared with that of younger patients, with an excess of early toxicity and discontinuation. The association of etoposide, cyclophosphamide, mitoxantrone and prednisolone (VEMP) demonstrated a good efficacy in a previous study (Santini G. et al. Haematologica. 1991).

Aims: We retrospectively evaluated the outcome and the tolerability of the association Rituximab-VEMP (R-VEMP), in 115 newly diagnosed elderly DLBCL not eligible for standard R-CHOP or R-CHOP like, treated between 2004 and 2020 in two hematological centers from Veneto region.

Methods: Median age was 77 years (range 60-91, only one patient <65 years). The majority were female (63%). Baseline characteristics were: 62% Ann Arbor Stage III/IV, 22% bulky disease (>10 cm), 43% high-int/high aaIPI and 30% high CNS-IPI. Median number of cycles was 6 (1-8). Seventy-one (62%) patients received from 70 to 100% of the dose and 44 (38%) had a dose reduction (20 to 69% of the full dose). Four patients received Lenalidomide maintenance. Response was assessed using the revised Lugano criteria.

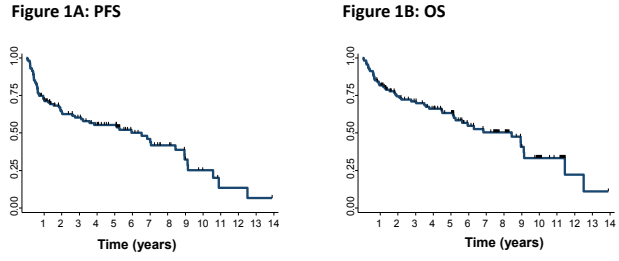

Figure 1.

Results: ORR was 77%, with 74 patients achieving a CR (64%); 26 patients (23%) were in SD or PD at the end of the treatment. With a median follow-up of 51 (9-166) months, 2 years PFS and OS were 64% (95%CI 54-72) and 74% (95%CI 65-81), respectively (Figure 1a-1b); among the 40 patients >80 years, 2 years PFS and OS of 59% (95% CI 42-72) and 74% (CI 56-85). In univariate analysis advanced stage, poor aaIPI, bulky disease, dose reduction and achieving a CR at the end of treatment were significatively associated to a better PFS ($p<0.05$). In multivariate analysis only response at the end of the treatment ($p<0.05$, HR 10, 95% CI 3.9-30-2) retained significance. Eighty (70%) patients developed hematological toxicity (≥ grade 2) while the most frequent non-hematological toxicity were infectious events (27%). Only 7 (6%) patients suffered from cardiovascular complications. The main cause of death was disease progression in 26 patients (51%).

Conclusion: With the limits of a retrospective analysis, R-VEMP is a feasible and well-tolerated treatment in elderly patients with de novo DLBCL, and could represent a valid alternative choice with curative potential for those patients not eligible for more toxic first-line regimens.

D080

COMPARISON BETWEEN LUGANO AND RECIL CRITERIA FOR TREATMENT RESPONSE ASSESSMENT TO FIRST-LINE THERAPY IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Diffuse large cell B-lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphomas (NHL). The Lugano 2014 criteria are currently used for response assessment. In order to make radiological evaluation less time-consuming, in 2017 the RECIL criteria were created, in which only the longest diameter of target lesions is considered and the number of target lesions is reduced (max 3 instead of 6). 18F-FDG PET scan was evaluated according to Deauville score (DS) in both Lugano and RECIL criteria. Despite a good agreement demonstrated by analyzing imaging from patients enrolled on clinical trials, there is a paucity of real-life data.

Aims: The primary objective was to verify the level of concordance of RECIL and Lugano response criteria in a cohort of newly diagnosed and homogeneously treated DLBCL patients.

Methods: In this single-center study, 33 patients with available clinical and radiological data were retrospectively analyzed. All cases received R-CHOP or R-COMP. Response evaluation was performed as early assessment by CT and at final re-staging by CT and 18F-FDG PET (considered negative if DS 1-3). Radiological images were retrospectively evaluated according to RECIL criteria and compared with Lugano criteria. Agreement between the 2 criteria was assessed by Cohen's k index. At the end of treatment only patients achieving a CR were considered as responsive.

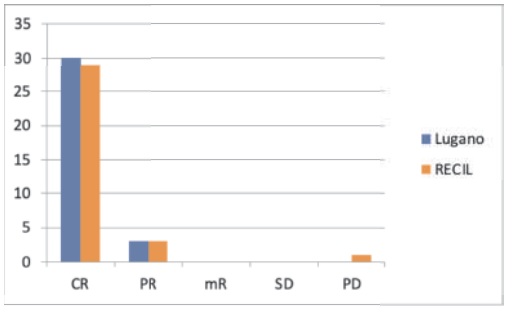

Figure 1. Response evaluation at final restaging.

Results: At the early assessment, response was comparable in 31/32 cases; the only discordant case showed a SD according to Lugano and a PR according to RECIL (k index 0.652, good agreement). At final restaging, 30/33 patients had a negative PET result and achieved a CR according to Lugano criteria. According to RECIL criteria, 29 patients obtained a CR, 3 a PR and 1 a PD because of the appearance of a new nodal lesion defined as target, even if PET-negative (thus considered in CR for Lugano criteria). The concordance index was 0.841 (excellent). It should be noted that the patient given as PD according to RECIL and CR according to Lugano criteria had never relapsed.

Summary/Conclusion: Response assessment according to Lugano and RECIL criteria showed a good agreement in our cohort of DLBCL patients. The main problem is represented by PET-negative patients with a RECIL morphological response <30%, who would have been considered as poorly responsive, with the risk of overtreatment. We suggest the possibility of re-evaluating PET role in RECIL criteria in future studies.

D081

RITUXIMAB AND NONPEGYLATED LIPOSOMIAL DOXORUBICIN (R-NPLD) TREATMENT IN PATIENTS 80 YEARS OF AGE OR OLDER AFFECTED BY DIFFUSE LARGE B CELL LYMPHOMA (DLBCL): A 2020 UPDATE AND IMPLICATIONS OF CLINICAL AND PATHOLOGICAL FACTORS

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Background: In 2018 we report a rituximab plus nonpegylated liposomial doxorubicin (R-NPLD) combination for patients 80 years or older with diffuse B cell lymphoma (DLBCL) or grade 3 b follicular lymphoma. The overall 3-year survival, cause-specific survival and progression-free survival rates were 46%, 55%, and 44%, respectively. According to these results, R-NPLD has become the new standard treatment in patients > 80 years old with aggressive B lymphoma, in our institution. To better investigate the prognostic role of clinical and pathological factors, we analyzed the same immunochemotherapy combination in a larger cohort of patient 80 years or older with DLBCL.

Methods: We retrospectively and prospectively analyzed data of patients 80 years or older with untreated histologically-proven CD20-positive DLBCL, Ann Arbor stage I to IV from our institution. Patients received a combination treatment with rituximab plus nonpegylated liposomial doxorubicin. The regimen consisted of R 375 mg/sqm and NPLD 50 mg/sqm administered intravenously on cycle day 1, plus prednisone 50 mg orally on days 1 to 5, every 21 days for 6 courses.

Results: Between May 2010 and April 2019, we enrolled 50 patients (median age 84, range 80-96, M/F:27/23). The median follow-up time was 28 months (range 10-104). The overall 3-years survival, cause-specific survival, and disease free survival rates were 49.9+7.6%, 55.5+7.9%, and 48.5+7.8%, respectively. Treatment was well tolerated with only mild toxicities, without treatment related hospitalization or toxic deaths. Patients achieving EFS12 and EFS18 had an overall3-years survival of 66+13.0% and 67.9+7.0%, respectively.

Conclusion: Our results confirm that, in patients 80 years or older with DLBCL, R-NPDL is very effective and safe combination. Among prognostic factors, only the elevated LDH (> 1.25 upper limit) strongly correlates with overall survival and risk of relapse, in univariate (p=0.001, p=0.003) and multivariate (p=0.002, p=0.005) analysis, respectively. In patients who achieved EFS18 the probability to survive 24 and 36 months is of 90.5 and 67.9%, respectively. This analysis suggests that EFS18 will be useful in patient counseling and should be considered as a robust end point for future studies of newly diagnosed very elderly DLBCL patients.

D082

IN VITRO 3D CO-CULTURE MODEL FOR DRUGS SCREENING IN DIFFUSE LARGE B CELL LYMPHOMA

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Purpose: Diffuse large B cell lymphoma represents the most common

type of non-Hodgkin lymphoma. Although the curability rate is high, around 40% of patients will relapse or exhibit refractory disease (r/r DLBCL). About 15% of DLBCL patients have bone marrow (BM) involvement and this represents a poor prognostic factor. The close interaction of lymphoma cells with stromal and immune cells within the BM directly influence lymphoma survival and drug resistance. Thus, we want to develop a three-dimensional (3D) in-vitro model to reproduce the tumour microenvironment and study MSC/DLBCL interaction with the aim to establish a tool for evaluating patients-specific therapies.

Methods: Human decellularized femoral bone fragments were used as a scaffold and recellularized with MSC. 3D spatial configuration was analyzed with two photon microscopy. DLBCL cells were allowed to flow into the model by a microfluidic system and spatial interaction was studied. Viability of DLBCL cells after drugs treatments was also evaluated by *in vitro* co-culture and analyzed with cytofluorimetric assays.

Results: We optimized a two-step recellularization protocol providing direct MSC seeding on the scaffold surface and MSC flowing through it by an in-house made device. We digitally recreated the 3D structure of the model identifying that MSC autonomously adhered and grew on scaffold (Figure 1). MSC formed a 3D web creating niches in which DLBCL cells stably adhere. Preliminary data suggest that this physical interaction reduces spontaneous and Dexamethasone-induced apoptosis upon treatments. Interestingly, Ibrutinib treatments inhibited the protection given by MSC in the 3D model.

Discussion: We found that DLBCL cells are able to migrate, adapt and grow in a 3D scaffold generated from human decellularized femoral bone fragments. In this setting, we could confirm the previously described ability of human MSC to promote neoplastic cell growth. We also observed that sensitivity of DLBCL to dexamethasone-induced apoptosis was significantly blunted by physical interaction with MSC in the 3D model. Interestingly, MSC lost their protective activity when DLBCL in the 3D scaffold were exposed to ibrutinib.

Conclusions: A 3D scaffold reproducing a bone marrow microenvironment is a promising tool for exploring MSC/DLBCL cells interaction and may be exploited to develop a patient-specific platform for drug screening and personalized therapy in r/r DLBCL.

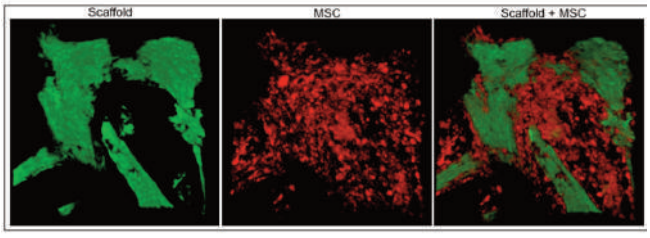

Figure1. Two photon microscopy 3D reconstruction.
a.) Green scaffold autofluorescence. b.) MSC HS-5 mCherry+ cell line adhered on the scaffold. c.) Merge. Spatial disposition of the cells in the scaffold can be appreciated.

Figure 1.

D083

HIGH BASAL MAXIMAL STANDARDIZED UPTAKE VALUE IN FOLLICULAR LYMPHOMA IDENTIFIES PATIENTS WITH LOW RISK OF LONG-TERM RELAPSE

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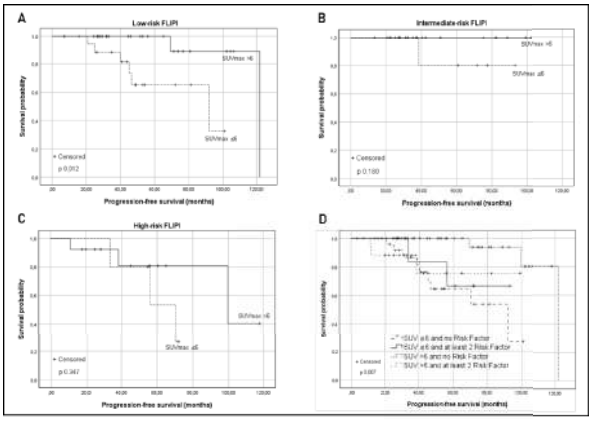
Background: Follicular Lymphoma (FL) is an indolent disease: despite the efficacy of treatment strategies in inducing remission, high risk patients relapse within two years and others relapse on long term. Despite encouraging studies on the prognostic role of Total Metabolic Tumor Volume (TMTV) the role of SUVmax at baseline PET/CT needs to be better defined.

Patients and Methods: Retrospective observational monocentric cohort study performed at Sapienza University of Rome. Patients affected by FL who underwent baseline PET/CT were included. According to the SUVmax value assessed at baseline two subgroups were identified and compared in terms of PFS and OS: A) Basal SUVmax ≤ 6; B) Basal SUVmax > 6.

Results: Ninety-four patients were included, 34 in group A (36,2%) and 60 in group B (63,8%). Five-year PFS in the whole cohort was 87,5%, 74,5% for group A and 95% for group B (p 0.005). Nevertheless, PFS at two years was comparable in the two groups (97%). A correlation between PFS and baseline SUVmax was observed in patients in different FLIPI risk categories. In low-risk FLIPI patients (48.3%) a significantly superior long term PFS was observed in group B compared to A, PFS at follow-up was 92% and 66,7% respectively (p 0.012) (Figure 1A-B). No significant association between PFS and baseline SUVmax was observed in the subgroup of intermediate (p 0.180) and high-risk FLIPI (p 0.317). Multivariate analysis of all the parameters assessed, confirmed the presence of a basal SUVmax >6 as an independent favorable prognostic factor for PSF (OR 0.234; 95% IC 0.58-0.934; p 0.04) as well as a correlation between a BM involvement at diagnosis and unfavorable PFS (odds ratio [OR] 5.98; 95% IC 1.5-23.3; p 0.011). Was finally explored the PFS of groups A and B based on the presence of at least two baseline features considered as an indicator of aggressive disease (bone marrow involvement, elevated LDH, elevated β2-microglobulin, extra-nodal disease, bulky disease, presence of B-symptoms) (Figure 1D).

Conclusion: Our data demonstrate the independent prognostic role of baseline SUVmax as a PFS predictor, especially in patients with low-risk follicular lymphoma. Even without other risk factors, patients with low tumor metabolic activity exhibit a higher long-term relapse probability. Baseline SUVmax evaluation, with its simple assessment, could help identify patients at risk for late relapse, requiring strict follow-up and, potentially, MRD monitoring.



A, B, C) PFS according to baseline SUVmax in relationship to FLIPI risk categories.

D) PFS according to baseline SUVmax and presence of at least 2 risk factors (bone marrow involvement, elevated LDH, elevated b2-microglobulin, extra-nodal disease, bulky disease and B-symptoms).

Figure 1.

D084

ENDOSCOPIC ULTRASOUND (EUS)-GUIDED BIOPSY IN THE DIAGNOSTIC WORK OF DEEP LYMPHADENOPATIES AND SPLEEN LESIONS

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Introduction: Endoscopic Ultrasound (EUS) has emerged as a safe and effective diagnostic procedure to histologic characterization of deep lesions reached through the gastrointestinal tract. EUS-guided biopsy represents a less invasive approach respect to surgical biopsy, and may allow an easier access to deep masses than external CT or ultrasound-guided biopsy.

Aim: To establish the accuracy of EUS as diagnostic tool for diagnosis of deep lymphadenopaties and spleen lesions.

Methods: We retrospectively collected data about 160 EUS-guided biopsies performed in our Center from June 2017 until March 2021. Three to four core biopsy samplings were performed using 22G needles in 156 cases, and 19G needles in 4 cases.

Results: Among 160 procedures revised, 76 were performed as outpatient, 84 as inpatient. 43 patients had already a previous diagnosis of cancer and EUS-guided biopsy was performed for suspected relapse or for disease staging. In 10 patients, the procedure was not completed for poor compliance of patients or inability to visualize pathologic lesions, identified by CT or PET imaging. In 8 patients multiple sites were biopsied. Sites of EUS-guided biopsy were: 61 supradiaphragmatic nodes including 27 subcarenal nodes,18 posterior mediastinal nodes,16 other mediastinal nodes; 67 subdiaphragmatic nodes including 6 at hepatic hilum,4 at splenic hilum,5 close to duodenum/jejunum,2 pelvic,7 perigastric, 5 peripancreatic,42 subdiaphragmatic not further specified;13 splenic focal lesions;14 extranodal lesions (7 stomach, 2 liver, 2 duodenum/jejunum, 1 peritoneum). Histopathologic reports were consistent with lymphoma in 51 patients, granulocytic sarcoma in 1 patient, solid neoplasm in 40 patients, chronic granulomatous nodal inflammation in 20 patients, with an Overall Diagnostic Rate of 74.6%. 38 biopsies were not diagnostic due to the insufficient material, no abnormality, reactive tissue or extensive necrosis. Lymphoma histotypes were:31 DLBCL, 3 HG-BCL, 5 FL, 4 HL, 2 ALCL, 1 MCL, 1 MZL, 1 PBL, 3 lymphoma not specified. 13 of 38 patients whose first biopsy was not diagnostic, performed a second biopsy, among them 2 EUS-guided biopsy, identifying 5 more cases of lymphomas (3 DLBCL, 1 FL, 1 ALCL).

Conclusion: EUS-guided biopsy is an effective procedure for histological definition of deep lymphoadenopaties and parenchimal lesions and allows an accurate diagnosis in about 75% of cases providing sufficient material for histology, flow-cytometry and molecular studies.

D085

IDENTIFICATION OF DIFFERENT GENE EXPRESSION RELATED TO CHEMORESISTANCE IN PRIMARY MEDIASTINAL B CELL LYMPHOMA: CLUES FROM RNA SEQUENCING ANALYSIS

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Primary mediastinal large B cell lymphoma (PMLBCL) is a rare subtype of non-Hodgkin lymphoma mostly diagnosed in young women and is currently recognized as a distinct clinical and biological entity. First line therapy with R-CHOP allows to achieve good remission rates even if chemoresistant cases remain highly challenging from the therapeutic standpoint. As such, we aimed to compare the transcriptome of R-CHOP resistant PMBCL patients to those of chemosensitive patients. We extracted RNA from embedded paraffin samples and then we performed whole RNA sequencing on 7 patients. Four of them were selected as chemoresistant (Group A), while three of them were classified as responder to R-CHOP (Group B). A First bioinformatics analysis selected a panel of 200 genes significantly differentially expressed in chemoresistant (A) samples versus chemosensitive (B). An unbiased analysis based on different ontology of the genes led to easily identify common signatures that may better profile the two groups of patients. Finally, on a bias analysis, genes were divided into categories in order to identify potential new targets and/or mechanisms of chemoresistance. We identified three genes which may be frankly related to chemoresistance in PMLBCLs due to an overexpression in the group A or a suppression of expression in group B. We selected NFKBIA, the gene which encodes for the IκBα protein, mutated in numerous Hodgkin's lymphoma cells, which cause NFkB to be chronically active in the lymphoma tumor cells. For this reason, we imagine that it could have a major role in the modulation of PMLBCLs sensitivity to chemotherapy. EPHB1 was selected for its very strong expression in the poor prognosis group, since it is one of the most expressed genes and for his association with numerous cancers. The kinase STK 33 appeared overexpressed in group A. A more thorough investigation of this gene might lead to new, significant findings. Our in silico analyses allowed to identify in PMBCL an unique profile that may modulate sensitivity to chemotherapy. Further analyses may address whether this unique phenotype has clinical implications. We may expect to: i) develop specific therapeutic strategies to target NFkB, EPHB1 and STK33 pathways in PMBCL with a chemoresistant behaviour; ii) correlate the expression profile of resistant PMBCL to other transcriptomes, including those of Hodgkin Lymphomas, DLBCL, in order to better profile those clinical and biological overlapping features.

D086

B-CELL RECEPTOR SIGNALING PROFILES IN MANTLE CELL LYMPHOMA: A BARCODING AND PHOSPHO-SPECIFIC FLOW CYTOMETRY APPROACH

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Mantle cell lymphoma (MCL) is an aggressive and incurable disease. B-cell receptor (BCR) signaling, constitutively activated in MCL, promotes tumor growth and is target of BTK inhibitors (BTKi). BTKi showed high response rates in relapsed/refractory (R/R) MCL but resistance inevitably emerges for reasons largely unknown. Recent evidence supports that resistance mechanisms can involve BCR signaling. With the aim to characterize BCR signaling profiles related to lymphoma drug responses in MCL, we used phospho-specific flow cytometry, which measures the phosphorylation status of intracellular signaling proteins at the single-cell level. To improve throughput capacity of this analysis, we combined phospho-specific flow cytometry with fluorescent cell barcoding (FCB), a multiplexing technique. In FCB each sample is labeled with different fluorescent-dye concentrations obtaining a different signature, or barcode, and then mixed with other samples before antibody staining and analysis by flow cytometry. We analyzed BCR phosphoproteins (pERK1/2, pp38, pPLCγ2, and pNF-kB p65) in both MCL cell lines and peripheral blood cells from R/R MCL patients, in the basal conditions or following BCR or CXCR4 stimulation with anti-IgM or CXCL12, respectively. H_2O_2, which inhibits phosphatases, or phorbol myristate acetate (PMA) were used as control stimuli. First, we set the FCB: based on their barcode fluorescence we deconvoluted mixed samples back to each individual condition. Then, we observed that in both basal and stimulated conditions phosphoproteins levels were heterogeneous among MCL samples, with each sample having distinct responsiveness profiles. Although further studies are needed to associate BCR

signaling profiles to drug responsiveness, this study shows that phospho-specific flow cytometry combined with FCB is a robust and reproducible approach to characterize signaling profiles in complex cells populations such as those found in MCL. Importantly, FCB reduces antibody consumption and cells need, eliminating staining variability between samples. These results form the basis for future studies aimed at generating maps of BCR signaling profiles related to lymphoma drug responses. The long-term goal is to define a predictive model based on BCR signaling features in MCL within MANTLE-FIRST BIO Project. We thank Fondazione Italiana Linfomi (PGR Ed. 2019) and the Platform of Flow Cytometry and Cellular Analysis (Verona University).

D087

ABSTRACT WITHDRAWN

D088

CLINICO-PATHOLOGICAL FEATURES OF RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: THE IMPACT OF THE TIME TO RELAPSE AS A STRATIFYING FACTOR

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Introduction: Diffuse Large B Cell Lymphoma (DLBCL) is the most common type of non-Hodgkin Lymphoma. Approximately 2/3 of cases reach remission after 1st line therapy, while refractory and relapsed (R/R) patients are characterized by a dismal prognosis. Despite the recent advances in the comprehension of lymphomagenesis, early recognition of high-risk disease is still unpredictable. Besides, just few studies have considered the stratification by time to relapse as a parameter to identify distinct groups of patients.

Aim: The aim of this study was to describe the clinicopathological features of patients affected by R/R DLBCL stratified according to the time to relapse and to identify potential prognostic and predictive factors.

Figure 1. Impact of the time of progression on OS from relapse.

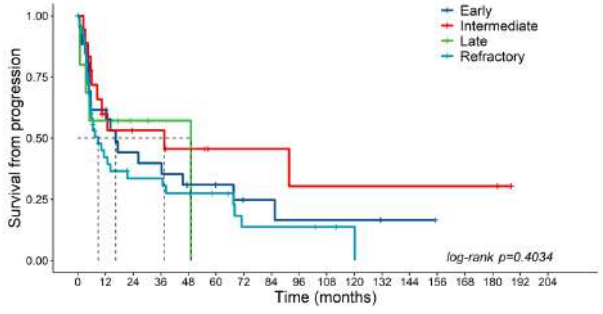

Figure 1.

Methods: After retrospective revision of medical charts of patients followed at the Hematology Unit of Padua University Hospital between 2001 and 2020, 100 R/R DLBCL cases were selected. The immunohistochemical analysis was performed on slices of formalin-fixed paraffin-embedded tissue biopsies obtained with Tissue Arrayer Minicore3. Variables were compared with Kruskall-Wallis, Fisher's exact or Chi-square test when appropriate. Survival curves were calculated according to Kaplan-Meier method and compared with Log-rank test. Hazard ratio and confidence interval were calculated with univariate Cox proportional hazards models.

Results: The R/R patients (pts) were divided into 4 groups: primary refractory, PR (no response to 1st line therapy, 43pts), early relapsed, ER (within 12 months from diagnosis, 29pts), intermediate relapsed, IR (between 12-60 months,18pts), and late relapsed, LR (after 60 months, 10pts). Male predominance, increased LDH, bulky disease and neutrophil/lymphocyte ratio ≥3.5 characterized PR-ER versus LR patients, with significance ($p<0.05$). In the whole R/R cohort, male gender, ECOG 2-4, stage IV and poor secondary R-IPI associated with worse overall survival (OS) both from diagnosis and after relapse, while poor primary R-IPI and intermediate-high CNS-IPI affected only OS from diagnosis. Moreover, bone marrow involvement, increased LDH, bulky/extranodal disease, cell of origin, expression of BLC2 or MYC and time to progression did not impact OS from relapse (Figure1).

Conclusion: Our data suggest the use of the time to progression as a mean to distinguish groups of R/R DLBCL with different features; however, no differences were found in terms of OS from progression among the four groups.

D089

BRIDGING RADIOTHERAPY TO CAR-T CELL THERAPY IN REFRACTORY NON-HODGKIN B LYMPHOMA: SINGLE-CENTER EXPERIENCE

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Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy is an effective option for the treatment of relapsed/refractory diffuse large B-cell lymphoma. In order to control lymphoma progression during the manufacturing period, a bridge therapy regimen is required for most patients. Radiotherapy (RT) may be used for patients with localized chemorefractory disease as a bridge therapy. Here we report a case series of 6 patients (1 primary mediastinal, PMBCL, and 5 diffuse large B-cell lymphoma, DLBCL) treated with radiotherapy as bridge to CAR-T. Figure 1 summarizes the treatment history. Four patients received tisagenlecleucel (tisa-cel), and one axicabtagene-ciloleucel (axi-cel); one patient died before reinfusion of CAR-T. The dose of RT was 30 Gy in 15 fractions, the site was mediastinum for pt 001, abdominal adenopathy for pt 002, 003 and 005, inguinal adenopathy for pt 004, laterocervical adenopathy for pt 007).

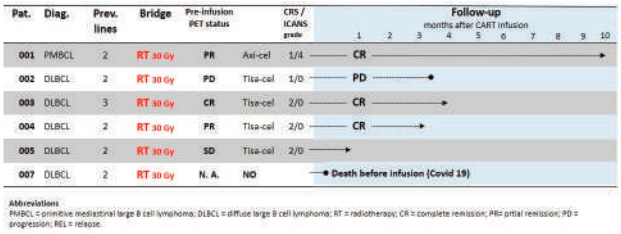

Figure 1.

Median volume of irradiation was 210 ml (avg. 270 ml, min 79,6 ml, max 635 ml). Response to bridging RT was achieved in 3/6 patients (2 PR, 1 CR), one patient had stable disease, and one patient ha disease progression at the time of CAR-T infusion. One patient died for severe Covid19 pneumonia before receiving the planned CART infusion. The outcome is favorable at the time of writing for all infused patient but one, who died for progression 3 months after infusion (the one with progressive disease at the time of infusion). Toxicity was manageable, with no grade 3-4 CRS; maximum CRS grade was 2 in 3 cases. Only one pa-

tient receiving axi-cel needed admission at ICU for grade 4 ICANS, with complete resolution after treatment with high dose steroids. We showed in this report that RT is feasible and effective as bridging therapy for patients with localized disease before CAR-T therapy.

D090

EFFECTS OF CAR-T TREATMENT ON THE NK CELL POPULATION IN DLBCL PATIENTS

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Natural killer (NK) cells are a component of the innate immune system and are important both as effector cells and as efficient producers of soluble factors, important for regulating both innate and adaptive immune responses. With the aim to gather immune phenomena after autologous, anti-CD19 CAR-T cell (tisagenlecleucel, Kymriah®) infusion for Diffuse Large B-Cell Lymphoma, we investigated their behaviour together with the peripheral blood NK cell repertoire. In particular, the effect of the CAR-T treatment on the phenotype of NK cells developing in patients after lymphodepletion has been evaluated at different time points. We evaluated patients treated at our Unit since 2020. Median number of previous lines is 2 (IC:2-4). 37% received a previous autologous transplant. 64% had a post-germinal center phenotype, 12% a double or triple hit lymphoma, 75% were refractory to the last line. CAR-T cell levels are determined through cytofluorimetric analysis: a median of $2.7x10^8$ CAR-T cells were infused; median time to maximum concentration t(max) was 10 days, with a median of 13 CAR-T/mcl. At t(max), CD8+ CAR-T levels were not significantly different compared to CD4+ (8 *vs* 4; p=n.s.). By comparing responders to non-responders, no differences were identified among maximum IL6 (993 *vs* 2520, p=n.s.) and CAR-T levels (32 *vs* 56, p=n.s.), respectively. NK cells are detectable and evaluable by cytofluorimetric analysis, even very early after CAR-T infusion (3 days). A large array of both inhibitory and activating NK receptors (including KIRs, NKG2A, NKG2C, NCRs, DNAM-1, immune checkpoints) and chemokine receptors have been investigated by multiparametric flow cytometric analyses. Preliminary results indicate that the NK cell population is enriched in less differentiated cell subsets, *i.e.*$CD56^{bright}$ NK cells and $CD56^{dim}$ NK cells characterized by a NKG2A+ KIR- phenotype. The natural cytotoxicity receptors, NKp30 and NKp46, are well expressed after treatment at any time points, while the expression of activation markers on $CD56^{dim}$ NK cells decreases along the period time considered. These analyses could be useful in defining whether CAR-T treatment may affect the phenotype and function of NK cells that develop in the patient after lymphodepletion and whether the development of particular NK cell subsets can correlate with better patient follow-up, suggesting a possible synergy between NK and CAR-T in the fight against tumor cells.

D091

ABSTRACT WITHDRAWN

D092

VENETOCLAX IN MULTI-RELAPSED MANTLE CELL LYMPHOMA PATIENTS: A REAL-LIFE MULTICENTRIC EXPERIENCE

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Background: Mantle cell lymphoma (MCL) is a very heterogeneous disease, ranging from indolent forms - candidates to the "watch & wait" strategy – to the aggressive ones - that need an intensive treatment. Recently, ibrutinib and venetoclax, alone or in combination, have been reported to be effective in relapsed patients, with overall response rate (ORR) of 40-50%, and median progression-free-survival (PFS) ranging from 3.2 to 8 months.

Aim: to describe the clinical outcomes of 12 multiply relapsed MCL patients (10 in fourth-fifth line of therapy) who received venetoclax (10 cases) or venetoclax in combination with ibrutinib (2 cases) in the venetoclax compassionate use in 8 Italian centers. Ten patients already received ibrutinib, 25% had blastoid histology and 80% presented with a poor performance status.

Results: Median follow-up from diagnosis was 7 years; at the time of venetoclax treatment all patients failed the previous therapy. Median WBC value was $8.8x10^9$/L, median Hb 11.3 and median PLT count $164.7x10^9$/L. Seven patients, after ramp-up, received a full dose of 800 mg; the median duration of treatment was 5 months (range, 1-15). Significant adverse events were observed in 7/12 cases, but only 2 patients discontinued therapy for hematological toxicity: comparison of WBC, Hb and PLT values before and after venetoclax showed a significant decrease of PLT only, with stable WBC and Hb levels. The extra-hematological adverse events included gastro-intestinal toxicity and one case of tumor lysis syndrome. Two patients are still receiving venetoclax after more than 30 months; 4 patients underwent allogeneic transplantation and other 4 received a further treatment after venetoclax discontinuation. ORR was 50%; 9 patients had progressed and 5 died; median overall and progression-free post-venetoclax survivals were 8 and 6 months, respectively, and they were not conditioned by sex, age or blastoid histology.

Conclusions: our real-life multicenter experience, even if still limited, is perfectly in line with data from literature, both for ORR and duration of response (50% *vs* 42-50%; 5 *vs* 4 months). Notwithstanding our patients were heavily pre-treated and ibrutinib resistant, survival was satisfying and venetoclax functioned as bridge to transplant in 4 cases. Toxicity also had a low impact, with thrombocytopenia being the most frequent adverse event, making venetoclax an attracting therapy for relapsed MCL patients.

D093

ABSTRACT WITHDRAWN

D094

LUNG INVOLVEMENT IN HODGKIN'S LYMPHOMA

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Hodgkin's lymphoma (HL) is a malignant disease mostly affecting lymphatic system and it is characterized by high cure and survival rates, exceeding 80% after first-line therapy. Extranodal lymphomas would account for 25–50% of all non-Hodgkin lymphomas and only 2–5% of HL. Although lungs are the extralymphatic site most commonly involved, data regarding the behavior and the consequences concerning this localization are poor. To further assess the presenting features and the prognostic significance of HL with pulmonary disease, we performed a retrospective single institution study of 25 patients affected by HL and treated at the University Hospital of Bari (Italy) between 2000 and 2021.

Fig.1: Overall survival and progression free survival in 25patients with HL and lung presentation.

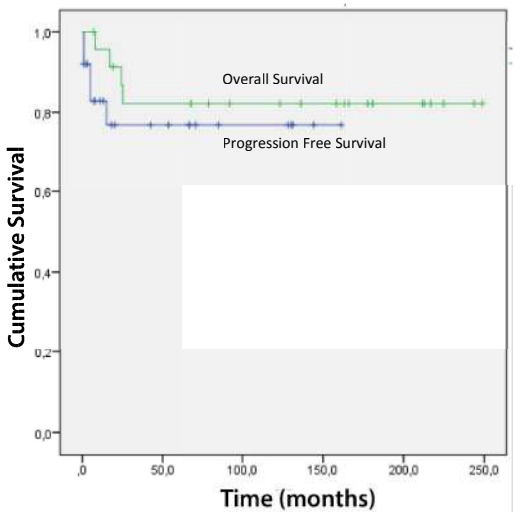

Figure 1.

The average age at time of diagnosis was 35 years (range: 16-83). Among these patients 17 (68%) were female and 8 (32%) male; 21 (84%) had stage 4, 2 (8%) had stage 2 and 2 (8%) stage 1. In 17 cases (68%) pulmonary localization was the single extranodal site, while in 8 cases (32%) it was associated with other extranodal sites (only liver in 2, only bone in 3, both in 2 cases and only breast in 1 case). All 25 patients had ABVD protocol as first line chemotherapy, 5 had second line chemotherapy and 4 had undergone to Autologous stem cell transplant (ASCT). We found that the most frequent localization at diagnosis was a nodular lesion (72%) involving both lungs (48%). In 7 patients (28%), we found a consolidated mass with or without cavitation and these patients had a worse outcome. At a median follow-up of 120 months 17 patients (68%) were in complete remission and 5 patients (20%) had refractory/relapsed disease, among them, after first line chemotherapy, 2 had a resolution of pulmonary localization and 3 had persistence of lung lesions with signs of progression. The progression free survival (PFS) and the overall survival (OS) at 3 years were respectively 76% and 81%. In our study we found that pulmonary HL has a wide range of clinical and radiologic presentations, often associated with other extranodal sites, and that only a high burden disease seems to be associated with a poor prognosis. Further studies on a wider series will be able to evaluate the prognostic value of the different presentation modalities.

D095

ABSTRACT WITHDRAWN

D096

INTENSIFICATION AND MAINTENANCE WITH RITUXIMAB IN PATIENTS WITH DLBCL IN UNCERTAIN CR OR PR (PET-ORIENTED) AFTER INDUCTION THERAPY WITH R-CHOP OR SIMILAR THERAPIES; OUR EXPERIENCE

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The addition of rituximab to induction chemotherapy improved the outcome of patients with diffuse large B-cell lymphoma (DLBCL). However the maintenance with immunotherapy not improved outcome.We have studied, in patients who achieved an uncertain or unsatisfactory response (CRu or PR) oriented by final PET (after R-CHOP or similar therapy) with a deauville score of 2-3, a intensification therapy with rituximab (R) (R 375 mg/m^2 per week for 4 weeks) followed by maintenance (R 375 mg/m^2 every 2 months for a total of 12 administrations or until any progression or unacceptable toxicity) to assess whether we observe an improvement outcome in this cohort of patients. From January 2014 to April 2021 we studied 75 consecutive patients with DLBCL (45 M and 30 F, mean age: 65 years (range 28-82); 37 ABC, 25 GC; 5 NOS; 5 T-rich and 3 immunoblastic, treated with R-CHOP or similar therapy. Of the 75 patients (with a median follow-up of 30 months) the ORR was 77% (58/75 patients: CR 39; CRu 16 and PR 3); 7 NR (9%); 7 (9%) died before completion of therapy and 3 (5%) has still on R-CHOP treatment. The total OS, PFS and EFS of this group projected at 54 months was of 75%; 78% and 75% respectively. We considered the patients in CRu or PR to evaluate responses to intensification and maintenance treatment with R oriented by PET. We treated 18/58 patients 31%, 16 in CRu and 2 in PR, with intensification and maintenance with R, 13 M and 5 F with median age 66 (range 44-77) 8 ABC; 7 GC; 2 immunoblastic and 1 rich in T. No significant side effects were observed in this patient group.After a median follow-up of 40 months (range 8-88 months) 17/18 (94%) patients are in CR (only one patient relapsed after 18 months). While after a median follow-up of 30 months of 40/58 patients in CR after induction of R-CHOP, 33 (83%)are in CR while 7 (17%) had a relapse.Observations of these 2 groups projected at 85 months were similar for OS (94% and 93% respectively); while PFS and EFS were 94% and 82% in the maintenance or non-maintenance groups, respectively.These our retrospective data, demonstrate an improvement in PFS and EFS in patients with RP or CRu with DLBCL compared to the literature. In particular an overall advantage was noted for the DLBCL ABC subgroup performing this treatment (total patients DLBCL ABC and GC with OS at 70 months 82% and 76% respectively). This improvement may be due to intensification and maintenance with R. A larger cohort of patients and a randomized trial are needed to confirm these preliminary data

D097

EFFECTIVENESS AND SAFETY OF PIXANTRONE THERAPY IN AGGRESSIVE AND REFRACTORY LYMPHOMAS: EXPERIENCE OF A SINGLE CENTER

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Introduction: Pixantrone (Pixuvri®) is an aza-anthracenedione with a new mechanism of action, recently approved in Europe for using as monotherapy in adult patients (pts) affected by aggressive B-cell non-Hodgkin's lymphoma (NHL) relapsed after at least 2 previous line of chemotherapy or with refractory disease. Diffuse large B-cell lymphoma (DLBCL) and transformed follicular lymphoma (tFCL) are clinically and molecularly heterogeneous aggressive diseases with different outcomes. One third of patients has a more aggressive clinical course showing refractory disease or an early relapse to standard first line regimen such as R-CHOP. We experienced the use of Pixantrone as salvage therapy in this setting of patients.

Patients and Methods: Between 2019-2021, 9 consecutive elderly pts affected by relapsed or refractory DLBCL or tFCL were treated with Pixantrone. Median age was 74 years (r 65-82), and the median number of previous treatment was 2.5 (r 1-4); 2 pts had refractory disease. Treatment schedule included pixantrone 50 mg/m2 day 1,8,15 q28 for a maximum of six cycles.

Results: Eight out of 9 pts (89%) achieved at least a stable disease, with 1 CR and 4 PR. Median PFS was 3.7 months (r 2.8-21.9) with 2 patient 91 CR and 1 PR) still alive after more than 1 year. Only one patient experienced a progressive disease and dead after the first cycle of therapy. Toxicity was mostly hematological with G3/G4 neutropenia, thrombocytopenia and anemia (75, 86 and 70%, respectively. One patient had treatment-related mortality.

Conclusions: Our data overlap, both for response rate and PFS, with previous reported series of Pixantrone treatment in elderly and heavily

pretreated pts. Treatment appears feasible and safe also in frail patients. More data are needed to confirm efficacy as single agent and could be interesting to test Pixantrone in combo therapy in refractory and/or relapsed pts.

D098

DA-EPOCH-R PLUS HIGH DOSE METHOTREXATE AS FIRST LINE THERAPY IN ADVANCED STAGE AGGRESSIVE LYMPHOMAS: A FEASIBILITY STUDY

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DA-EPOCH is an infusional regimen designed to improve the response rate in highly proliferative tumors by prolonging cell exposure to low concentrations of chemotherapeutic agents. It was demonstrated as effective regimen in both T and B cell high grade lymphomas with an elevated proliferative index. Moreover, the addition of Rituximab is currently the standard of care in any B cell lymphoma treatment strategy. Previous studies seem to suggest that high dose methotrexate (HD-MTX) therapy is better than intrathecal MTX administration to prevent CNS relapse in high-risk patients. Few reports are available exploring the feasibility of a combination therapy including HDMTX into the DA-EPOCH-R scheme. Our study reported the result of the above combination therapy in a series of high-risk advanced stage lymphoma patients (pts).

Patients and Method: 12 pts affected by aggressive lymphoma were enrolled: 4 double expressor DLBCL, 4 DLBCL with very high proliferation index, 1 PMDLBCL, 1 nodal ALK+ ALCL, 1 Richter and 1 Gray zone lymphoma with MYC overexpression. All out of one pts showed a stage IV disease with extranodal sites involvement, high LDH level, elevated Ki67 (median 90%, range 60-100%) and intermediate/high IPI score. Combination therapy included classical DA-EPOCH-R scheme, with exception of Rituximab in ALCL, with the addition of high dose (3.5 g/m2) Methotrexate at day 9 after urine alkalinization and hydration. All patients were scheduled to receive a total of 6 courses, two of which including HDMTX. G-CSF prophylaxis was administered to all patients starting on day +9 and day +12 in the case of DA-EPOCH or combination therapy, respectively.

Results: A total of 24 combination cycles (DA-EPOCH-R+HDMTX) were administered without any delay of the q21 regimen. 9 patients are evaluable for response: 7 got a CR and 2 a PD. At 15.3 months of median time of observation, 6 pts are in CCR and only 1 experienced a relapse. No CNS relapse were observed. All pts showed a favorable toxicity profile without severe extra-hematological AE. G3/G4 neutropenia, anemia and thrombocytopenia were observed without severe infectious complications.

Conclusion: Our preliminary results showed the feasibility of the combination therapy DA-EPOCH-R/HD-MTX with satisfactory results on controlling very aggressive disease. However, a prospective trial is needed to confirm the possibility to use this scheme as standard therapy in very aggressive lymphomas with high risk of CNS relapse.

D099

LATE CARDIAC COMPLICATIONS HODGKIN AND NON HODGKIN LYMPHOMA PATIENTS UNDERGOING CHEMOTHERAPY AND RADIOTHERAPY

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Aims: The primary endpoint of this multicenter prospective observational study was to evaluate the prevalence of late (five years from the end of therapy) cardiovascular toxicity in patients with Hodgkin (HD) and non-Hodgkin lymphoma (LNH) treated with antracycline-based chemotherapy (CHT) and 3D conformal radiation (3D-CRT) on the mediastinum. Secondarily, we correlate clinical and/or subclinical cardiac damage with cardiac substructures RT dosimetric data.

Materials and *Methods* Patients underwent cardiovascular screening based on cardiological examination, complete blood chemistry tests, blood thyroid function, blood troponin and NT proBNP o BNP, electrocardiogram, echocardiogram, cardio-pulmonary exercise test and supraortic trunk echocolor-doppler. The assessment of toxicity was obtained through retrospectively contouring and evaluating 3D-CRT dosimetric data of the heart chambers and cardiac structures, lungs, thyroid and carotids and the dose volume histogram (DVH) evaluation. Based on the expected prevalence of the primary endpoint of 16%, after 5 years, a sample of 207 patients was estimated assuming a margin of error of 5% and a confidence interval of 95% (CI 95%). For time to event endpoints the survival curve will be estimated using the Kaplan Meier method and the comparisons will be based on the log-rank test. RESULTS Since November 2019 to date we enrolled 10 patients affected by mediastinal HD and LNH, respectively one and nine Patient ages ranged from 31 to 73 years (median 42 years). All patients were treated since July 2006 to April 2016 with CHT and 30 Gy/15 fractions 3D-CRT on the mediastinum. Median FUP was 89 months (range 50-1470 months). Five patients underwent complete blood chemistry tests, blood thyroid function, blood troponin and NT proBNP o BNP: median BNP serum level 13.4 (range 10 – 108.5) and median Troponin I serum level 1.2 (range 0.9 – 12.4). Eight patients underwent electrocardiogram and echocardiogram: only one patient showed grade I left ventricular dysfunction and high BNP serum level (108.5 pg/ml). Eight patients underwent supraortic trunk echocolor-doppler without significant alterations. The expected duration of the study is 24 months so the study itself and its results are still ongoing. CONCLUSIONS The results of this study could have an impact on daily clinical practice, proposing a specific cardiological screening program reserved for selected category of patients considered at risk of developing late cardiotoxicity

D100

CASTLEMAN DISEASE OF MESENTERY TEN YEARS FOLLOW-UP

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Castleman disease (CD) is a rare disorder of unknown etiology defined by characteristic lymph node histopathology hyaline vascular, plasmacell and mixed ; unicentric UCD,benign,usually asymptomatic or at multiple sites MCDwith systemic inflammation and cytochine driven multiorgan dysfunction,involving more than one node and type B symptoms. (Dispenzieri BloodApr2020) MCD often presents with infection of immunodeficiency virusHIVand/or Human Herpes virus 8 HHV8 that plays a critical etiopathogenetic role. (Rhee OncClin.feb2018) Other viral drivers as Ebstein Barr virus (EBV)are under investigation.(Nabel PlosOne jun2019) The most common UCD presentation is mediastinal, exceptionally in the mesentery as only55 cases in leterature and the standard treatment is a complete an bloc surgical resection.(Bracale B.M.C.Surg apr2017).

Case presentation: We report on a case of UCD of angiofollicular/hyalin-vascular type with mesenteryc mass treated by laparoscopic assisted procedure with active EBVinfection at 10 years of follow-up.A 55 year's old woman was admitted on 28/10/2011to Oncology for widespread arthralgia ,mild anemia Hb11,4,ESR117;CPR61,lc adenopathy 1cm. Pet Scan revealed diffuse captation in left mesogastric area at

high metabolic activity confermed by Ct andUs scan of 3c.m.Aspirate needle diagnosed lymphoproliferative disorder and on9/12 the mass was excised by laparoscopic surgery. Bone marrow biopsy and PET total body performed on19/1/2012in the norm. At histologic examination mass of fibrotic lymphnode 4x3x3 u.c.m : B-cellCD20+ and follicular dendritic cells CD21+, hypervascular zone,smallCD3+Tcells: YA-LINEVASCULAR/ANGIOFOLLICULAR TYPE CASTLEMAN. Evidenced past CMVinfection and persistent active EBV infection EBV-VCA IgM32,7; EBV-VCA IgG 700 ;EBV-NA IgG 600. US scan in the norm ten years after surgery.

Discussion: UCD in the abdominal cavity is very rare, most commonly presents as a mediastinal nodal mass(55 only mesentery cases described) Our female had an uneventful curable postoperative course and a in norm PET 3 months after surgery. Although UCD is often asynthomatic our patient presents autoimmune artralgia. Persistent active EBV infection and past CMV infection is of difficult interpretation as the primary driver in UCD is not known and seems to highlight the heterogeneous nature of the disease:autoimmune? autoinflammatory?paraneoplastic?autoimmune reaction initiated by viral infection?

Chronic Myeloproliferative Diseases

D101

COVID-19 IN ESSENTIAL THROMBOCYTHEMIA: COAGULOPATHY AND THROMBOSIS

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Essential thrombocytemia (ET) is characterized by platelet and coagulation activation and thrombosis such as COVID-19 is disease. Therefore, COVID-19 may be an ad ditive and synergistic thrombotic factor in ET. We studied Interleukin-1α (IL-1α) and IL-8, and Tissue Factor Pathway Inhibitor (TFPI) as inflammation and endotheliopathy markers, thrombin antithrombin complex (TAT) and β-Thromboglobulin (β-TG), as coagulation and platelet activation markers, and thromboelastometric parameters. This study included 100 WHO-defined ET patients (60 men, 40 women; mean age 50 years, range 30-50). Of 100 patients, 50 had positive RT-PCR on nasopharyngeal swab COVID-19 and thrombosis (25 men, 25 women) and 50 were without COVID-19 and without thrombosis. Of 50 with thrombosis, 20 developed nonfatal myocardial infarction (15 men, 5 women) on electrocardiography, 15 had nonfatal stroke (10 men, 5 women) on magnetic resonance imaging, and 15 had deep venous thrombosis (8 men, 7 women) on ultrasonography. The mean duration of disease was 10 years. All patients were on ASA 100 mg once daily and treatments standard (hydroxyurea, interferon, anagrelide). None had comorbidities or thrombophilia. Interleukin-1α (IL-1α) and IL-8, and TFPI were measured using ELISA kits (R&D Systems) on Luminex machine and ELISA kit (American Diagnostica Inc., Greenwich, CT), respectively.TAT and β-TG were measured by ELISA (Diagnostic Stago, Boehringer Mannheim, Mannheim, Germany and R&D Systems, respectively). The viscoelastic properties of blood were analyzed using Thromboelastometry ROTEM (Pentapharm GmbH, Germany). The patients with thrombosis had high IL-1α and IL-8 (2.9±1 pg/ml *vs* 0.20±0.5 pg/ml and 60±10 pg/ml vs 21±3 pg/ml, respectively) as well as TFPI (166±69 ng/mlvs81±12 ng/ml),and increased TAT and β-TG (70±10 μg/lvs (3±1 μg/l and 245±15 IU/mlvs10±5 IU/ml). A positive correlation was found between inflammatory, endothelial and coagulation markers. A p-value of <.05 was considered statistically significant. Shortened CT (CT, unit: s. n.v. 100-240 s) (50±30 s),shortened CFT (CFT, unit: s, n.v. 30-160 s (12±10 s), increased MCF (MCF, unit: mm, n.v. 50-72 mm) (130±10 mm) and lower LY-30 (LY-30, %: v.n. 15%) (0.7%) there were in ET with thrombosiscompared with ET without thrombosis (CT 100±50 s and CFT50±5 s and MCF 0±10 mm and LY-30 15%). These findings suggest that COVID-19-associated ET has a higher thrombotic risk and needs more appropriate antithrombotic therapy.

D102

ABSTRACT WITHDRAWN

D103

HOW DID COVID-19 PANDEMIC CHANGE CLL TREATMENT APPROACH WITH VENETOCLAX?

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According to ASH suggestions for CLL management during COVID19 pandemic, "venetoclax (V) initiation, that requires multiple and extended clinic visits with lab testing, should be avoided if possible unless considered the most appropriate treatment for a particular patient". It is also recommended to skip/avoid antiCD20 monoclonal MoAb. In this analysis we evaluated V management during pandemic in 21 centre-north Italian centers. From February 2020 to March 2021, 130 pts received V+/- MoAb; 37% were treated in Lombardia, the most impacted region by COVID19. In table pts' characteristics. 33 pts received V monotherapy, 97 combined with MoAb. In 61 BTKi pretreated pts, V was considered the only available salvage option and was administered as: time-fixed combined with MoAb (36 pts); continuously as monotherapy (25 pts). The remaining 69 BTKi-naïve pts (16 TN) received V+MoAb; the choice was driven by: fixed-duration schedule (69%); BTKi conflicting comorbidities (28%); biologic profile (3%).

Table 1. Patients' characteristics

Characteristic	Value N° (%)
Median Age (range)	68 (41-91)
Sex: Male/Female	86 (66)/44 (34)
Prior Tx median (range) 0 1-2 ≥3	1 (0-8) 16 (12.3) 85 (65.4) 29 (22.3)
Prior BTKi	61 (47)
Neutropenia	12 (9.2)
Renal Impairment CrCl < 50 CrCl < 30	14 (10.7) 13 (10) 1 (0.7)
Major Comorbidities	72 (55.4)
TLS risk Low Intermediate High	 25 (19.2) 71 (54.6) 34 (26.2)
IGVH unmutated	98 (75.4)
Del(17p) and/or TP53mut	55 (42.3)
Del(11q)	23 (17.7)
Venetoclax single agent Venetoclax + MoAb	33 (25.4) 97 (74.6)

Except 2 pts, screening procedures were performed regularly as per local standards. COVID19 swab was tested in 28% of pts before tx. No changes were applied to ramp-up and standard lab monitoring. In only 22 pts (17%) tx schedule was modified: V dose reduced in 1 pt, MoAb initiation postponed in 18, monthly time-interval extended in 3. Overall, 16 pts (12%), 4 TN, 12 R/R, developed COVID19 infection while on V; all but 2 were in clinical response. Planned schedule was monotherapy in 6; MoAb combination in 10. 13 infections occurred while on V full dose, 3 during ramp-up. In 6/16 infected pts, 6 MoAb cycles had been completed before COVID19 diagnosis, in 4 the planned MoAb initiation was postponed due to the epidemiological situation. 12 pts (75%) were hospitalized; 2 had mild symptoms; 1 was pauci-symptomatic; 1 asymptomatic. V was discontinued in 14 pts with mild/severe symptoms. In the remaining 2 less severe cases, V dosage was lowered. Infection resolved with no sequelae in 10 pts, 8 of them restarted V. 6 pts (37.5%) died due to COVID19, 4 of them having received prior MoAb. Up to now 44 pts have been COVID19 vaccinated without V modifications. With a median follow up of 8 m, 120 pts (92%) are alive and 108 (83%) are still on V, after a median time of 7.8 m. COVID19 pandemic did not impact on V-based regimen choice or tx schedule. Infection rate in MoAb-pretreated pts was 6.5%. COVID19 related hospitalizations and deaths are in line with those reported in literature.

D104

IMPACT OF ADMINISTERED DOSE ON EFFICACY AND SAFETY OF PONATINIB IN RESISTANT OR INTOLERANT CML PATIENTS

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Ponatinib is currently indicated for the treatment of chronic myeloid leukemia (CML) patients resistant and/or intolerant to II gen TKIs, for Ph+ acute lymphoblastic leukemia (ALL) and for patients carrying the T315I mutation. The recommended starting dose is 45 mg once daily. The data from PACE suggested a relationship between dose and safety events, including arterial occlusive events, indicated that AOEs are dose related. Interim analysis of OPTIC trial shows a trend toward dose-dependent efficacy and safety. The aim of our study was to assess the impact of administered dose on efficacy and safety of ponatinib in a retrospective analysis of a real-life cohort of 68 patients with resistant/refractory CML from 17 Italian Hematological Institution. 60 patients were in CP, 5 in AP and 3 in BP, all failing at least one line of therapy with a first or second generation TKI. The monitoring plan, definition for response to the treatment and molecular responses were defined according to the ELN 2013. Regardless of age, 33 patients received 45 mg/day of ponatinib as starting dose, while the lower doses of 30 mg/day and 15 mg/day were selected in 24 and 11 patients, respectively. Overall, 48 of the 60 CP patients achieved at least CCyR at any time (80%). Most of these patients had a deep and sustained response to the therapy (21 patients achieved MR3 and 17 to MR4 or better). Adverse events were reported in 41 pts, all grade 1/2. the most common were dermatological, thrombocytopenia and pancytopenia. Cardiovascular events, no severe, were observed only in 12 patients, 8 of whom continued the treatment. 43 of the 68 patients reduced Ponatinib daily dose. In the statistical analysis using SPSS package the median of daily administered dose, treat-

ment duration and total administered dose were 30 mg/day, 773 days and 22000 mg, respectively. In both univariate and multivariate analysis, response to treatment was significantly influenced only from daily administered dose with a cutoff of 30 mg (best response with a dose lower than the median of 30 mg/day, with about 40% of patients obtaining at least an MR3). On the other hand, both duration of treatment and total dose administered do not seem to influence the response rate. Our real-world results suggest that the effective dose of Ponatinib could be 30 mg/day, even in resistant patients, with a reduction in exposure to side effects. Once response is achieved, the dosage could be reduced to the safety dose of 15 mg/day.

D105

ABSTRACT WITHDRAWN

D106

MANAGEMENT OF BLAST PHASE OF PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASE: A REAL LIFE SINGLE INSTITUTION EXPERIENCE

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Background: The evolution into blast phase (BP) of a myeloproliferative disease (MPN), including polycythemia vera (PV), essential thrombocythemia (ET), or myelofibrosis (MF) is a sign of bad prognosis. Currently, there is no standard of care for managing this event.

Methods: We have retrospectively analyzed 25 consecutive patients (pts) with BP-MPN, diagnosed in the last decade according to WHO 2016 criteria. We collected data regarding clinical and biological features of chronic phase (CP)-MPN and BP-MPN and treatments options, in order to evaluate differences in response rate (*Mascarenhas consensus criteria, 2012*) and survival from evolution in BP.

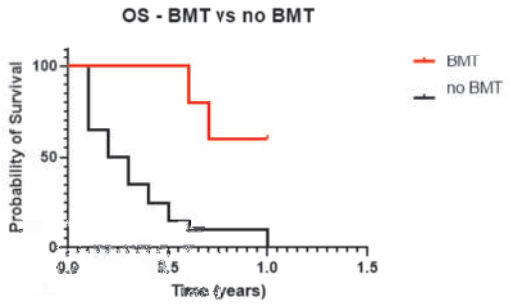

Figure 1.

Results: Median age of pts was 74 years (range 53-88) with prevalence of females (56%); the BP occurred in 12 (48%), 4 (16%) and 9 (36%) pts belonging to the MF, PV and ET groups respectively. BP occurred on average 9.4 years after MPN diagnosis (range 0-29). A total of 21 (84%) pts had JAK2V617F mutation. At the time of BP, 8 pts (47%) presented a complex karyotype; according to mutational profile, 5/22 pts had a FLT3 mutation (23%), one a NPM1 mutation (5%). The treatment choice was at the discretion of the physician, according to age and fitness status of pts. A total of 12 (48%) pts received hypomethylating therapy (HM), 5 (20%) induction with intensive chemotherapy (CH), the remaining 8 pts (32%) received supportive care (SC) (transfusions and/or oral cytoreduction). Overall response rate (ORR) was 25% and included complete remission in 3 pts (12%), 1 after HM and 2 after conventional CH, and partial remission in 5 (20%), all after HM treatment. No response was seen in SC group. Five pts (20%) received allogeneic transplantation (ASCT) as post-remission therapy: 3 after CH, 2 after HM. In univariate analysis, older age (>70 years, p=0.018) and higher blast count on peripheral blood (>20%, p=0.01) at BP onset, were related with poor outcome. The obtainment of ORR was associated with better outcome (p=0.007). After a median time of 3.1 months (range 0.2-24.8) from BP, 13 pts (52%) already died. According to type of remission treatment, no difference in survival was showed between CH *vs* HM (p=0.59). Only ASCT was associated to better survival (12 *vs* 2.1 months, p<0.001, Figure 1). In pts unfit to ASCT, no difference in survival was seen in SC *vs* HM group (1.86 *vs* 3 months, p=0.35).

Conclusion: Outcome of BP-MPN pts in the last decade remains dismal. Only ASCT can be an effective cure for these pts.

D107

LONG-TERM EFFICACY AND SAFETY OF LOW-DOSE PEGYLATED INTERFERON ALPHA2B IN CHRONIC MYELOPROLIFERATIVE MALIGNANCIES: A MONOCENTRIC REAL-LIFE EXPERIENCE

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Background: A remarkable issue in the use of Interferon for cytoreduction in chronic myeloproliferative malignancies (MPN) is the scarce tolerability and the high rate of discontinuation. Nevertheless, many improvements have been made since the introduction of pegylated formulation.

Aim: We present a series of 41 patients (17 M and 24 F) with Philadelphia-negative MPNs treated with pegylated INF alfa2B (Peg-INF) since 2003 as off-label use and observed for a median time of 153 months.

Patients: Median age was 36.8 years (14.8 – 5.9); 68.3% of patients were affected by Essential Thrombocythemia (ET), 17.1% by Policythemia Vera (PV) and 14.6% by Hypereosinophilia (HE). At diagnosis, MPNs-related systemic symptoms were reported by 41.5% of patients and 34.1% presented splenomegaly. JAK2V617F and CALR mutations were found in 71.4% and 17.2% of cases, respectively, while 11.4% were triple negative. Before Peg-INF, 63.4% of patients had been treated with at least one line of cytoreductive therapy (HU or INF), while 36.6% was untreated. First-line treatment discontinuation cause was intolerance in 13 cases, resistance in 2 and for patient's request in 8 cases. The administered dose was 50 mcg weekly (68.3%) or 25 mcg weekly (31.7%).

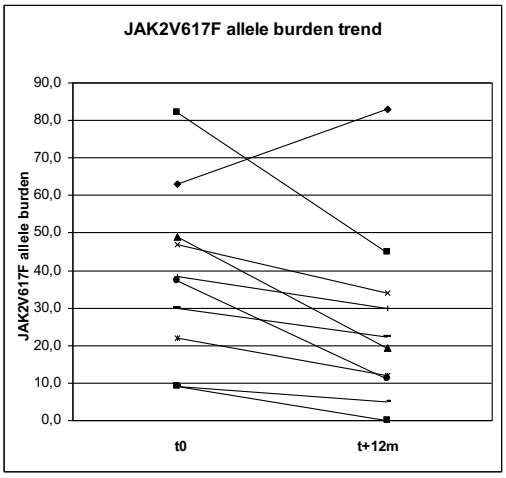

Figure 1. JAK2V617F allele burden at baseline and after 1 year of therapy with Peg-INF.

Results: According to ELN criteria ORR was 94.6% (CR 48.7% and PR 45.9%) at 1 year, 75% 100% at 5 years (CR 75.0%) and 100% at 10 years (CR 63.6%). ORR was independent from age, sex, drug dosage, MPN10 score or driver-mutations. After a median time of 17 months in 39.0% of cases adverse events occurred, including flu-like syndrome (43.8%), thyroid dysfunction (18.8%), hematological toxicities (12.5%) and tachycardia (12.5%). Discontinuation rate was 7.5% at 1 year, 40% at 5-years and 60% at 10-years follow-up, mainly due to toxicity. Moreover, JAK2V617F allele burden was measured at baseline and after 1 year of therapy in 10 cases: interestingly, in 90% a mean reduction of 12.5% was observed (Figure1).

Conclusions: Our results remark 1) the long-term efficacy of Peg INF in MPNs, even at low dose, also proven by the progressive increase of CR rate 2) a good safety profile with 60% of patients still in treatment after 5 years. These data are comparable to those already reported in literature.

D108

CLINICAL FEATURES AT ONSET AND DURING FOLLOW-UP IN PATIENTS WITH POLYCYTHEMIA VERA AND JAK2-V617F ALLELE BURDEN < 25%

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Polycythemia Vera (PV) is generally characterized at diagnosis by a high allele burden (≥ 25%) of JAK2-V617F mutation, even if some patients may have at onset lower levels. The aim of our study is to evaluate the rate and clinical features of PV patients with low allele burden (<25%) at diagnosis and to correlate it with major events in the follow-up compared to PV patients with higher allele burden. A whole cohort of 212 patients with PV according WHO 2016 criteria and with an available allele burden measurement behind 2 years from diagnosis in 2 different hematologic Centers was analysed. Allele burden was assessed in granulocyte DNA by quantitative polymerase chain reaction–based allelic discrimination assay. Patients were divided in low-allele burden (LAB) and high allele burden (HAB) groups, based on a 25% threshold. According to allele burden, 52 patients (24.5%) were allocated in the LAB group and 160 (75.5%) in the HAB group.

Table 1. Clinical features at diagnosis of the whole cohort and according to allele burden.

	All patients N° 212	Allele burden < 25% N° 52	Allele burden ≥ 25% N° 160	p
Gender, M/F (%)	106/106 (50.0/50.0)	26/26 (50.0/50.0)	80/80 (50.0/50.0)	1.00
Median age (years) (IQR)	69.3 (60.8 - 76.0)	68.8 (60.4 - 75.3)	69.5 (61.0 - 76.5)	0.873
Ht, % (IQR)	55.2 (52.1 - 59.7)	53.3 (50.7 – 56.6)	56.3 (52.8 - 60.4)	**0.020**
Hb, g/dl (IQR)	18.1 (16.9 - 19.3)	17.4 (16.7 - 18.4)	18.3 (17.1 - 19.5)	**0.007**
WBC, x 10^9/l (IQR)	10.6 (8.6 - 13.2)	9.5 (7.9 - 12.2)	10.8 (8.9 - 13.8)	**0.028**
PLTS, x 10^9/l (IQR)	464 (349 - 663)	582 (425 - 724)	442 (324 - 622)	**0.015**
Spleen, n° (%):				
Not palpable	156 (73.5)	45 (86.5)	111 (69.4)	**0.013**
< 5 cm below costal margin	48 (22.6)	7 (13.5)	41 (25.6)	
≥ 5 cm below costal margin	8 (3.9)	/	8 (5.0)	
Previous thrombotic events, n° (%):				
NO	164 (77.4)	43 (82.7)	121 (75.6)	0.281
YES	48 (22.6)	9 (17.3)	39 (24.4)	

The main clinical features at diagnosis of the whole cohort and according to allele burden are reported in the table: patients in the LAB group had lower Hb, Ht and WBC levels with a low incidence of palpable spleen size, but presented a significantly higher PLTS median count. After a median follow-up observation of 63.9 months (IQR 37.7 – 107.1), 26 thrombotic events (12.3%) occurred in the whole cohort [5/52 (9.6%) in the LAB group *vs* 21/160 (13.1%) in the HAB group, p=0.494]. Evolution in a myelofibrotic phase was observed in 15 patients (7.1%) of the whole cohort, with a trend for an higher incidence in HAB group [14/160 (8.8%) compared to 1/52 (1.9%) in the LAB group, p=0.09]: in the whole cohort, 8 patients (3.8%) developed a blastic phase, without differences according to allele burden [2/52 (3.8%) in the LAB group *vs* 6/160 (3.9%) in the HAB group, p=0.975]. At the last follow-up, 24 patients died in the whole cohort, with a 5-year and a 10-year overall survival (OS) of 94.8% (95%CI 91.3 – 98.3) and 84.4% (95%CI 77.6 – 91.2), respectively; 5-year and 10-year OS in the LAB group were 96.9% (95%CI 90.8 – 100) and 86.1% (95%CI 71.0 – 100) compared to 94.2% (95%CI 90.1 – 98.3) and 84.1% (95%CI 76.5 – 91.7) in the HAB group, respectively (p=0.550). Low allele burden at diagnosis in PV patients seems to be related to a less "policytemic" phenotype and probably to a lower incidence of myelofibrotic transformation, but did not have an impact on the occurrence of thrombotic complications and the long-term OS.

D109

PROSPECTIVE CROSS-SECTIONAL STUDY ON USEFULNESS OF ULTRASOUND ASSESSMENT IN CLL PATIENTS COMPARED TO PALPATION

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Introduction: The 2018 IWCLL and the 2020 ESMO guidelines suggested ultrasonography (US) as imaging technique to evaluate visceral involvement and palpation to evaluate superficial lymph nodes (SupLN) outside the context of clinical trials. US features of normal and diseased SupLN, and normal value of spleen dimensions have been published. In our study we tested the hypotheses that US can be a reliable tool to assess SupLN and spleen dimensions in clinical practice and verify the degree of concordance with palpation.

Methods: We enrolled N=55 consecutive CLL patients. Each patient was assessed by two independent physicians (P1 and P2) with palpation of SupLN and spleen. A third physician, blinded to P1 and P2 assessed splenic dimensions and SupLN with US using two different US machines. SupLN regions evaluated: inguinal, axillary, supra/subclavicular, and cervical. Parameters assessed by both palpation and US of SupLNs: presence/absence, dimensions, pathological *vs* normal.

Results: We found poor concordance in SupLNs assessment between P1 and P2 and good concordance between the two US machines in SupLN and spleen dimensions assessment. We found no concordance between palpation and US in: (i) in splenic assessment (Choen k=0.063), (ii) in SupLN: inguinal right and left (k=0.031 and k=0.001, respectively), (iii) axillary right and left (k=0.001 and k=0.00, respectively), (iv) cervical right and left (k=0.005 and k=0.001, respectively). In subclavicular regions inaccessible by palpation, US found N=19 right and N=22 left pathological lymphnodes. In the supraclavicular regions, hardly accessible with palpation, US found N=10 right and N=6 left pathological LN. Age and BMI did not have a statistical impact on concordance/discordance between US and palpation of spleen (p=0.925 and p= 0.529, respectively) and SupLN (p=0.322 and p=0.607, respectively).

Discussion. This is the first cross-sectional study to evaluate comparison between palpation and US assessment in CLL patients. We found a low concordance between 2 independent physicians using palpation and good concordance between 2 different sonographers. We found no concordance between palpation of SupLN and spleen size and US neither in the number of SupLN detected for each anatomical region, nor in their dimensions and pathological assessment. US is a non-invasive, radiation free tool to assess SupLN and spleen in CLL patients and allows a more precise staging of both SupLN and spleen dimensions.

D110

NANOPORE SEQUENCING APPROACH FOR IMMUNOGLOBULIN GENE ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The evaluation of the somatic hypermutation (SHM) of the clonotypic immunoglobulin heavy variable (IGHV) gene has become essential in the management of chronic lymphocytic leukemia (CLL) patients. The gold standard method is performed in two steps: a) clonality detection by PCR and capillary electrophoresis (CE); b) sanger sequencing (SS) of the clonotypic IGHV gene. The sequencing result is then evaluated for its deviation compared with the closest matched germline IGH gene, and the 2% threshold is used to discriminate unmutated from mutated status. Next-generation sequencing (NGS) for SHM analysis has widely tested, showing comparable accuracy but distinct advantages. However, the adoption of NGS requires a high sample number (run batching) to be economically convenient, which could lead to a longer turnaround time. Here we present data from nanopore sequencing (NS) for the SHM evaluation compared to the standard method. Thirty-six CLL patients were included in this study. According to the European BIOMED-2 collaborative study and the ERIC recommendations, the IGHV region was amplified for clonality and SHM assessment. To the aim of NS data analysis, we developed a pipeline starting from nanopore basecalled, and demultiplexed reads to perform sequence assembly, correction, clonality assessment, and mutational status analysis in 12h. The amplification results were assigned to SS and NS in a blinded manner.

The analysis produced the following results*:* 27 single VDJ (12 unmutated, 15 mutated), and 9 double (7 productive/unproductive with concordant status, 2 double productive with concordant status). Based on the final mutational status, data from both methods are then compared: 28 (78%) on 36 showed concordance, whereas 8 (22%) were discordant. In detail, in two cases, NS analysis was not able to produce the rearrangement consensus but reported correct clonality, and in one case, the rearrangement was not detected. On the other hand, NS analysis for the remaining five discordant cases showed additional VDJ recombinations not detected by SS methodology. Moreover, in four of these latter cases, the additional VDJs were validated and confirmed by specific VH-PCR.

In conclusion, our results show that NS is suitable for IGHV mutational analysis in terms of sensitivity, accuracy, simplicity of analysis and is less time-consuming. Moreover, our work showed that the development of an appropriate data analysis pipeline could lower the NS error rate attitude.

D111

HIDE AND SEEK OF CIRCULATING CD34+CD38-CD26+ LEUKEMIC STEM CELLS IN *DE NOVO* CML BLASTIC PHASE

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Recent investigations in peripheral blood (PB) samples of Chronic Myeloid Leukemia (CML) patients (pts) demonstrated that CD34+CD38-CD26+ cell population represent a "CML specific" leukemia stem cell (LSC) circulating compartment. We earlier confirmed that CD26 expression discriminates CML leukemic stem cells (LSCs) from normal HSCs or from LSCs of other myeloid neoplasms and we demonstrated that CD26+LSCs are measurable by flow cytometry in 100% of CML pts at diagnosis. In a prospective study, we documented that circulating CD26+LSCs persist, at lower level, in most pts during treatment with tyrosine kinase inhibitors (TKIs) and even after successful TKI discontinuation. Up to date, CD26+LSCs in CML Blastic Phase (BP) were not fully investigated yet. We reported here the behavior of circulating LSCs at diagnosis and after induction treatment in a 34-years-old female in which we diagnosed a de novo lymphoid BP CML (*i.e.* not preceded by a recognized CP CML). Using flow cytometry technique we assessed the antigenic expression of PB CD34+CD38-CD26+ LSCs; qualitative and quantitative BCR-ABL transcript detection were performed by RT-PCR. Cell blood count showed leukocytosis, anemia and thrombocytopenia, morphological examination of blood smear showed a consistent quote of myeloid immature cells and 23% of blasts with increased nuclear-cytoplasmatic ratio and prominent nucleoli. PB flow cytometry test showed the presence of lymphoid blasts CD34+CD19+CD10+TdT+ and RT-PCR detected the presence of p210 BCR-ABL transcript (b3a2). Bone marrow aspirate confirmed a B-Lymphoid BP of CML. As expected, circulating CD26+LSCs have not been documented. The patient underwent induction treatment with HyperCvad and at recovery, concomitantly with the response to treatment and PB blasts disappearance, we documented a slight, but evident population of CD34+/CD38-/CD26+ LSCs. Our results indicated that CD26+LSCs, yet possibly present, are not detectable during BP-CML. Indeed, a quote of CD26+LSCs resulted detectable after induction chemotherapy, suggesting the reappearance of a Chronic Phase clone that anteceded the Blastic Phase. Considering that blast cells are predominant during the BP, we hypothesized that they could surmount LSCs and mask their detection. Based on this evidence we suggest to explore the compartment CD34+CD38-CD26+ in de novo BP CML both at diagnosis and during treatment to confirm the presence of a previous, yet undetected, CP phase.

D112

IBRUTINIB TREATMENT IS FEASIBLE IN VERY ELDERLY PATIENTS INDEPENDENTLY FROM FITNESS AND POLYPHARMACY

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Ibrutinib changed CLL treatment paradigm. Pts>80y often struggle with comorbidities, polypharmacy, functional dependence and are therefore excluded from trials. The aim of this analysis is to evaluate whether fitness may play a role on treatment management. Overall, 81 pts≥80y who started ibrutinib outside clinical trials in 15 Italian centres between March 2014 and March 2020 were included. We analyzed the impact of baseline CIRS (≤6 *vs* >6), CIRS3+ (at least one organ with a CIRS≥3), ECOG (0-2 *vs* >2), cardio/renal comorbidities, polypharmacy (>3), use of antiPLT/anticoagulant and CYP3A4i on definitive treatment discon-

tinuation due to toxicity (tox-DTD), permanent dose reduction (PDR), EFS (event: tox-DTD, PDR and tox-related death) and OS. CLL complications and CLL diagnosis itself, were not included in CIRS calculation. In table pts characteristics. Median mo on ibrutinib were 17.7 (range 0.9- 72.3). Overall 41 pts (51%) discontinued treatment ≥7 days with a median of 20 days/pts interruption. A total of 34 pts (41.9%) permanently discontinued ibrutinib due to: toxicity, 21(26%); PD/Richter Transformation, 10 (12%); other reasons, 3 (4%). Most frequent adverse events (AEs) leading to tox-DTD were: cytopenia (19%), cardiological (19%), hemorrage (10%) and gastrointestinal (10%). Definitive discontinuation due to AEs was observed within the first 6 mo in 25% of pts and reached a plateau after 32 mo. At least one dose reduction occurred in 27 pts (33%) while in 21 (26%) dosage was permanently lowered. Hematologic and cardiological toxicities were the main reasons for PDR in 9 and 3 pts respectively. At univariate analysis none of fitness' variables (ECOG, CIRS>6, CIRS3+) nor cardiological/renal impairment had an impact on tox-DTD, PDR or EFS. Similarly number and type of concomitant medications influenced treatment management. We could only observe a trend (p .0710) of increased PDR in patients taking >3 drugs. Overall survival instead, was significantly affected by ECOG-PS>2 (p .0208), CIRS>6 (p .0461) and CIRS3+ (p .0280). In conclusion, our data show that ibrutinib treatment is feasible in the elderly population even when presenting high comorbidity burden or polipharmacy. Rate of tox-DTD in this series is in line with that reported in literature in younger pts. We could also confirm in this setting the occurrence of AEs leading to permanent ibrutinib discontinuation as an early event.

Table 1. Patients and disease characteristics at ibrutinib initiation.

Median Age y (range)	82 (80-95)
Sex: Male/Female	46/35 (56.8/43.2)
Tempo da diagnosi a ibrutinib (range)	63 (0-425)
Prior Tx median (range) 0 1-2 ≥ 3	3 (0-10) 25 (30.9) 45 (55,6) 11 (13.9)
IGHV unmutated	56 (69,1)
del(17p) and/or *TP53^{mut}*	32 (39,5)
High Risk^ del(17p) and/or *TP53^{mut}* and/or unmutated IGHV and/or del(11q)	33 (40,7)
ECOG-PS 0-1/>1	55/26 (67.9/32.1)
CIRSMedian (range) CIRS ≤6/CIRS*>6	7 (1-24) 39/42 (48.1/51.9)
CIRS3+	21 (25.9)
CIRS*>6 and CIRS3+	16 (19.8)
CrCl ml/min ≥50/30-49/<30	42/32/7 (51.9/39.5/8.6)
Pts with Cardio-Comorbidity Hypertension	24 (29.6) 49 (60.5)
Median N° concomitant medications (range) Pts with >3 concomitant medications	3 (1-13) 52 (64.2)
Pts treated with anticoagulant/antiplatelet	6/17 (7.4/21.0) (both 1)
Pts treated with CYP3A4 inhibitors	12 (14.8)
Steroids	12 (14.8)

D113

ATYPICAL CHRONIC MYELOID LEUKEMIA: NGS CHARACTERIZATION AND THERAPEUTIC APPROACH

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Atypical chronic myeloid leukemia is a rare clonal hematopoietic stem cell disorder with absence of a detectable BCRABL1 fusion that WHO includes in the group of MDS/MPN neoplasms. It displays both proliferative (neutrophil leukocytosis) and dysplastic (prominent granulocyte dysplasia) features. Clinical presentation includes anemia, splenomegaly, and thrombocytopenia, preceded by a myeloproliferative step with leukocytosis and/or thrombocytosis. The normal disease course typically ends in complications from cytopenias, or transformation to acute myeloid leukemia (AML) in 30–40% of cases. The cytogenetic analysis reveals recurrent abnormalities including -7, -5, del(20q), i(17)(q10). Advancements in next generation sequencing have shown an array of recurrently mutated genes involved in epigenetic regulation, RNA splicing, transcription, and cell signaling. Each entity displays a unique spectrum of somatic mutations supporting their unique pathobiology and clinical features. In aCML, the most frequent mutations (to varying degrees) have been found in SETBP1, ASXL1, (N/K) RAS, SRSF2, TET2, CBL, CSF3R, and ETNK1. Of these, mutations in SETBP1 and ETNK1 appear to be the most recurrent and are seen in up to a third of patients, while co-mutated SETBP1 and ASXL1 occurred in 48% of aCML patients. We describe here five cases of aCML, seen in the last 5 years, 4 male and 1 female showing similar clinical characteristics and evolution; in particular they show the same NGS mutation profiles with a similar percentage of VAF. All exhibited myeloproliferative features at diagnosis followed by high-grade myelodysplastic features in a median of 13.9 months in four patients, 44 months in one patient. The NGS analysis performed at diagnosis showed the prevalent presence of mutation on SETBP1, ASLX1 and SRSF2 genes in all of them with a median VAF of 42 %. Patients were treated in the myeloproliferative phase with hydroxyurea and with hypomethylating agents in the myelodysplastic phase. 2 patients died for progression, while 3 younger patients are waiting for SCT which could represent the only treatment with favorable outcomes. It will be discussed the NGS pattern in detail of all 5 patients, a review of literature and new therapeutic approach. Importantly, a detailed molecular profile with improved molecular characterization will give hope for a targeted therapy.

D114

REAL LIFE OF SARS-COV-2 (COV 2) INFECTION IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASM PH NEGATIVE: EXEPERIENCE OF THE LAZIO GROUP

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Background: The CoV 2 infection started to spread in Italy in February 2020. This infection is highly contagious. The clinical spectrum of this infection can vary from asymptomatic to symptomatic infection, with interstitial pneumonia and respiratory failure and can cause an increased risk of arterial and venous thromboembolism. Little information is available on the course of this infection in patients with Chronic Myeloproliferative Neoplasms Ph Negative (MPN), Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Myelofibrosis (MF) which are characterized by an increased thrombotic risk.

Method: This observational study involved 60 patients recruited from 10 haematological Centers (2 academic and 8 non-academic hospitals) in the region of Lazio, from 03/2020 to 03/2021. Results during the period of study, a cohort of 60 patients, 27 males and 33 females (median

age73 years, range 28-81) infected by CoV2 were analysed for the clinical outcome. Out of 60 patients, 22 had ET, 15 PV, 22 MF and 1 was unclassifiable. Regarding treatment, 10(16.6%) were managed by phlebotomies, 31(51.6%) received Hydroxyurea, 8 (13.3%) received Ruxolitinib, 1 (1.6%) received both drugs, 4 (6.6%) received other drugs and 6 (10%) patients did not receive any treatment. 37 patients with antithrombotic prophylaxis were treated with aspirin, 6 with oral anticoagulants, 2 with clopidogrel, and 2 with combinations (5 patients did not receive any prophylaxis, in 8 cases it was not known). From March to June 2020, 9 patients were infected, from July to September, 3 patients, from October to December, 31 patients, and from January to March 2021, 17 patients. Diagnosis was made by molecular swab in 42, antigenic swab in 5, antibody test 6, not known in 7 cases. The infection was asymptomatic in 26 (43.6%) patients and symptomatic in 34 (56.6%); 3 (8.8%) of the symptomatic patients had thromboembolic complications (2 MFI and 1 PV). Only 9 symptomatic patients died (5 male and 4 female), 6 were MFI, 2 PV and 1 ET; 5/9 patients were treated with Ruxolitinib. No patients in oral anticoagulants therapy died.

Conclusions: data from this study indicate a mortality rate of 15% (10% MFI, 1.6% ET, and 3.3% PV). No differences between males and females (for both infection and deaths). No deaths among patients with prior oral anticoagulant therapy treatment. There is an increased incidence of infected patients during the period October to December 2020 (during the second wave of the pandemic).

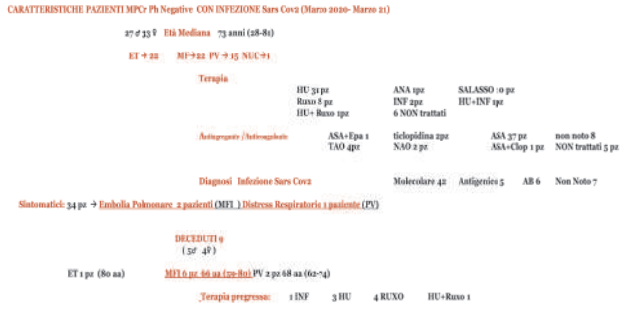

Figure 1.

D115

SAFETY AND ECONOMIC IMPACT OF CONTINUOUS TREATMENT WITH IBRUTINIB COMPARED TO FIXED-DURARATION OBINUTUZUMAB-CHLORAMBUCIL THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS. A SUBANALYSIS OF A CLL CAMPUS STUDY

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Introduction: The BTK inhibitor ibrutinib (IB) and obinutuzumab plus chlorambucil (G-CHL) are approved as first line therapies in chronic lymphocytic leukemia (CLL) unfit for fludarabine-based treatment. While IB has proven to be superior to chemoimmunotherapy in clinical trials, a relevant number of patients discontinue IB due to adverse events (AE).

Aim: The aim of this real-world project was to compare the economic impact of the fixed duration G-CHL *vs* continuous IB treatment.

Method: Patients received IB 420mg daily until progression or unacceptable toxicity, while G-CHL at standard dose up to 6 cycles (Goede V, NEJM2011). Economic outcomes (available for 4 centers: Alessandria, Roma Cattolica, Padova and Perugia) included ex-factory drug costs and administration, hospitalizations, visits and AEs management. Regional current tariffs (DRG and outpatient specialist care) were used to estimate the economic value of visits and hospitalizations. Generalized linear regression models were used to estimate differences in outcomes. The project was approved by the Ethic Committees.

Results: We recruited 183 CLL patients without TP53 disruption from 16 hematologic centers, 103 were treated with G-CHL and 80 with IB as first-line treatment. G and CHL doses were decreased in 12% and 35%, respectively. Eighty-two % of the 103 patients received all the 6 G-CHL cycles. After a median follow-up of 30 months, 44% of patients decreased the dose of ibrutinib and 79% were still under treatment. The 2-year progression free survival and time to next treatment was 76% *vs* 92% (p=0.0061) and 93% *vs* 97% (p=0.0043, Figure1A) for G-CHL and IB. Economic data were available for about 50% of the total cohort (69 G-CHL and 23 IB). Patients treated with G-CHL seemed to experience comparable AEs of any grade than those taking IB (2.98 *vs* 1.68 AE/month/person, rate ratio [RR] 1.13, 95%CI 0.6-2.37), but less clinical visits (RR 0.17, 95%CI 0.15-0.20) and hospitalizations (RR 0.42, 95%CI 0.17-1.10). Mean total monthly cost per patient was €1,545 with G-CHL and €5,587 with IB, resulting in a mean savings of €4,074 (95%CI 3,267-4,881) due mostly to the savings in first line drug cost (€1,029 *vs* €5,297) and slightly to reduction in hospitalization and outpatient visits (€95 *vs* 290€) (Figure1B).

Conclusions: The costs of continuous treatment with IB for treatment naive CLL patients is significantly higher than that of fixed duration of G-CHL, that should be carefully considered in health policy planning.

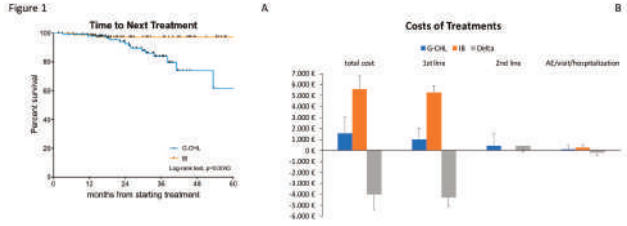

Figure 1.

D116

HBV REACTIVATION IN CLL PATIENTS WITH OCCULT HBV INFECTION TREATED WITH IBRUTINIB WITH OR WITHOUT VIRAL PROPHYLAXIS. A RETROSPECTIVE MULTICENTRIC GIMEMA STUDY

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Background: Chemo-immunotherapy (CIT) is associated to an increased risk of HBV reactivation in patients (pts) affected by lymphoproliferative disorders. Occult hepatitis B infection (OBI) is defined by the presence of anti-HBc antibodies, HBsAg negativity with or without anti-HBs antibodies and HBV-DNA serum negativity. Guidelines suggest lamivudine prophylaxis in OBI/CLL pts treated with CIT. No data are available about the need for prophylaxis in OBI/CLL pts treated with BTK inhibitors.

Aims: The objective of this study is to evaluate if OBI/CLL pts need lamivudine or HBV-DNA monitoring.

Table 1.

Table 1: OBI/CLL patients characteristics and results.

GIMEMA Study LLC1010	22 Italian GIMEMA centres			Prophylactic antiviral therapy with lamivudine and HBV-DNA monitoring		
Characteristics	Overall 111 pts			No =38	Yes =73	p- value
	Sex: M/F, n		76/35	26/12	50/23	>0.99
	Median age (range)		64 (39-86)	63 (48-81)	65 (39-83)	0.43
	Binet stage, n (%) 106/111	A B C	10 (9) 51 (48) 45 (42)	2 (5) 21 (55) 15 (39)	8 (12) 30 (44) 30 (44)	0.44
	IGHV, n (%) 84/111	unMut Mut	60 (71) 24 (29)	25 (83) 5 (17)	35 (65) 1 (35)	0.083
	FISH, n (%) 106/111	NK Del 13q Tris 12 Del 11q Del 17p	33 (31) 20 (19) 12 (11) 13 (12) 28 (26)	13 (34) 8 (21) 3 (8) 4 (11) 10 (26)	20 (29) 12 (18) 9 (13) 18 (26) 20 (29)	0.92
Response of CLL after IBR at 12 months, n (%) 98/111	CR/CRi, n (%)		10 (10)	3 (9)	7 (11)	0.56
	PR/PR-L, n (%)		74 (75)	23 (72)	53 (77)	
Time to IBR, n (%) 107/111	IBR 1 line		26 (24)	9 (24)	17 (25)	0.66
	After less than 12 months from last treatment		31 (29)	13 (34)	18 (26)	
	After more than 12 months from last treatment		50 (47)	16 (42)	34 (49)	
	Unknown		4	0	4	
Reactivation overall by therapy, n (%)	Reactivation overall, n (%)		5 (4.5)	4 (11)	1 (1.4)	**0.046**
	Reactivation (serological) overall, n (%)		2 (1.8)	2 (5.3)	0	0.12
	Reactivation (clinical) overall, n (%)		3 (2.7)	2 (5.3)	1 (1.4)	0.27
Details for Pts with reactivation, n	IBR 1 line		1	1 occult	0	
	IBR > 2 lines		4	1 occult	0	
	After more than 12 months from last treatment			2 clinical	1 clinical	

Methods: We analyzed 111 OBI/CLL pts (14%), among 781 CLL pts treated with IBR in 22 Italian GIMEMA centres until January 2019. Median age was 64 years. At IBR start, 9%, 48%, 42% pts were on Binet stage A, B, C respectively; 71% pts had unmutated IGHV, 26% pts had 17p deletion. Twenty-six (23%) OBI/CLL pts were treatment naïve at IBR start; 44 (40%) pts, 18 (16%) and 23 (20%) had been previously treated with 1, 2 or >2 lines of CIT respectively. Seventy-three OBI/CLL pts on IBR underwent prophylaxis with lamivudine, while 38 pts were only subjected to HBV-DNA monitoring every 3 months. Table1.

Results: Viral reactivation was observed in 5 pts. Four of them (2 with clinical reactivation and 2 with serological one) belonged to the HBV-DNA monitoring group; one patient experienced clinical reactivation on the lamivudine prophylaxis group (p=0.046). Both kinds of reactivation occurred in the first 3-6 months of IBR. In the HBV-DNA monitoring group, one patient was treatment naïve and experienced only serological reactivation; 3 pts were previously treated with CIT, at least 12 months before the IBR, and experienced both serological (1) and clinical (2) activation Table1. Serological reactivation was only recorded on the HBV-DNA monitoring group as those were the only pts who underwent a systematic screening schedule in the following months, thus were diagnosed with HBV reactivation (and treated with lamivudine) in the absence of any clinical suspicion.

Conclusions: From the collected evidence, it seems reasonable to suggest that prophylactic treatment should be considered appropriate and started in pts who were previously treated with CIT. For the treatment naïve group, a clinical choice could be performed, knowing that reactivation could seldomly occur and be detected in time to promptly treat the pts, but prophylaxis is not mandatory for a favourable clinical course.

D117

SUCCESSFUL TREATMENT WITH IMATINIB FOR SYSTEMIC MASTOCYTOSIS ASSOCIATED WITH MDS/MPN

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Systemic mastocytosis (SM) is a rare hematological neoplasm characterized by the abnormal proliferation and accumulation of mast cells. Clinical manifestations are heterogeneous depending on the tissue infiltration and mast cell mediators released by their degranulation. The gain-of-function point mutations at codon 816 of KIT gene, high serum tryptase level, and expression of CD25 represent minor diagnostic criteria, however, the unique major one is depicted by bone marrow (BM) biopsy. A subset of SM occurs with other hematological neoplasms, most frequently myeloid malignancies such as myeloproliferative neoplasms (MPN), chronic myelomonocytic leukemia and myelodysplasia (MDS). Here we report the case of a 56-year-old female patient affected by SM associated with a hematological neoplasm: she presented with thorax skin rash and a blood test revealed white blood cell count of 12.3 x 10^9/L, increased basophils (3.08 x 10^9/L) and platelets 567 x 10^9/L. Screening of JAK2, CALR, MPL and BCR/ABL1 mutations was negative. Therefore, the patient underwent a BM biopsy, which showed the typical clusters of mast cells associated with an MDS/MPN and a grade 2 reticulin fibrosis. Second-level analyses showed an increased serum tryptase level (148 ng/mL) and peripheral CD34+ cells (172/µL). The abdominal ultrasonography showed a spleen diameter of 19 cm. NGS myeloid panel (Illumina MiSeqTM) detected no mutations in any of the 30 genes analyzed, among them, KIT mutations were negative. Therefore, imatinib 400 mg daily was started, and after 3 months of therapy the patient achieved a significant symptoms improvement. In addition, the BM biopsy showed an outstanding response of SM and an improvement in both MDS/MPN and the grade of fibrosis (MF-1) (Figure 1). Serum tryptase level decreased up to 3 ng/ml and spleen diameter up to 16 cm. The therapy with imatinib was well tolerated, except for grade 3 thrombocytopenia. Platelet count was restored after 2 weeks of imatinib interruption; treatment was resumed at lower dosage with no thrombocytopenia recurrence. To our knowledge, there are no data in the

literature concerning effective therapeutic option in this specific setting. Being aware of the limits of the present report, mainly the short follow-up, we can speculate that imatinib may represent a safe and effective option, not only in the context of non-KIT D816V mutated SM, but also leading to a clinical and histological improvement as far as MDS/MPN is concerned.

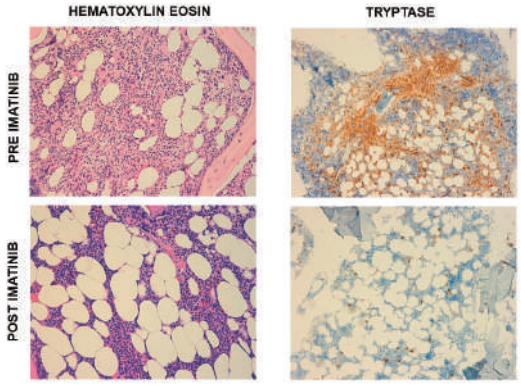

Figure 1.

D118

IBRUTINIB IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE-CENTER LONG-TERM ANALYSIS

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The covalent inhibition of Bruton tyrosine kinase with ibrutinib has demonstrated a significant clinical impact in patients with de novo and relapsed/refractory chronic lymphocytic leukemia (CLL) in need of treatment, with benefits in progression-free survival (PFS) and overall survival (OS) even in cases with unfavorable cytogenetics and molecular markers. All patients records with symptomatic CLL treated with ibrutinib have been retrospectively reviewed. Forty-six patients received ibrutinib either as frontline (N=10) or second or more advanced treatment (N=36). Median age at disease diagnosis was 62 years, with 41 male and 15 female patients. Median number of previous treatments for pretreated patients was 1 (range 1-4), mainly including chemoimmunotherapy. Eighteen patients presented with TP53 mutations; 17 had the deletion of chromosome 17p; 19 displayed an unmutated immunoglobulin variable heavy chain status. Median overall number of cycles was 26 (12-80). Among patients treated frontline, 4 patients were in Binet stage A, 5 in stage B and 1 in stage C, with a median cumulative illness rating scale (CIRS) of 5 (range 1-8). Best responses included 1 complete response (CR) and 6 partial responses (PR), for an overall response rate (ORR) of 70%. Patients receiving ibrutinib as a second or later line presented with Binet stage A in 9 cases, B in 14 and C in 10 (3 cases unavailable) and had a median CIRS of 5 (range 0-16). Best responses were 1 CR and 27 PR (ORR 72.2%). Median PFS was 28.8 and 21.1 months for patients treated frontline and as second/later line, respectively. Median OS was not reached for those treated frontline and 4.9 years for patients treated as second/later line. Richter transformation occurred in 5 patients (11%) at a median time of 16 months since the initial dose. At a median time of 3.8 years, 12 patients required further therapy (10 patients shifted to venetoclax). Hematological adverse events (AEs) consisted of grade 4 neutropenia, thrombocytopenia and anemia in 6, 4 and 1 case; grade 3 neutropenia in 6 cases and grade 1-2 thrombocytopenia in 2 cases. Grade 3-4 extrahematological AEs were: diarrhea, cutaneous rash, utero-vesical prolapse, vasculitis and urosepsis. No atrial fibrillation or bleeding were registered. Ibrutinib is effective and well tolerated in CLL patients treated frontline and with relapsed disease. Responses obtained in a real life setting are comparable with results from registration trials.

D119

FRONTLINE THERAPY WITH OBINOTUZUMAB CHLORAMBUCIL IN CLL PATIENTS: A REAL-LIFE EXPERIENCE

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Introduction: Obinutuzumab-Chlorambucil (G-Chl) actually is the standard of care in untreated chronic lymphocytic leukemia (CLL) patients (pts) with comorbidities. The treatment has been approved since 2017 and proved to be safe and effective with a good toxicity profile. Although chemo-immune treatment in target-therapy era plays a marginal role, the time-limited schedule of G-Chl represents a valid option for elderly unfit pts.

Aims: We conducted this retrospective study to evaluate efficacy and safety of G-Chl in a real-life setting.

Methods and patients: We enrolled 36 consecutives untreated CLL pts in six Tuscan centers, median age was 73 years (59-85). Twenty patients were male, 16 females. FISH status was available in 27/30 pts (14 negative, 7 deletion 13q, 4 trisomy 12 and 2 deletion 11q), IGHV status was analyzed in 22 pts only (13 mutated, 9 unmutated), TP53 mutation was investigated in 8 pts without any evidence of mutation. RAI stage at time of treatment was I in 5, II in 13, III in 12 and IV in 6 pts, respectively. CIRS>= 6 was in 14 pts. G-Chl was administered as normal clinical practice. Median follow-up was 23.4 months

Results: The overall response rate (ORR) was 75%: 12 pts (33%) achieved complete response, 15 pts (42%) partial response, 2 pts (6%) progression disease, in 7 pts (19%) the response was not available due to the ongoing treatment. Minimal residual disease (MRD) in peripheral blood was evaluated in 14 pts (9 negative, 5 positive). Median PFS was 28 months (16-40 months). We did not observe any significant impact on PFS by FISH status, IGHV status, RAI stage, CIRS>=6 and age>=70 years, respectively (p=0.882; p=0.181; p=0.848; p=0.501; p=0.305). In our cohort, MRD status was the only statistically significant prognostic factor on PFS (median PFS: 41 months for MRD- and 26 months for MRD+; p=0.049). Median time to next treatment was 36 months: as second-line therapy 7 pts received BTKi, 1 venetoclax, 1 idelalisib and 1 chlorambucil. We observed 1 clinical TLS, 7 infusion reactions, 7 thrombocytopenia (29% G>=3), 10 neutropenia (33% G>=3), 2 febrile neutropenia and 1 pneumonia (G2).

Conclusions: Our experience is consistent with PFS and ORR data, as reported in literature. G-Chl seems to maintain a good safety and tolerability profile. The time-limited schedule makes this treatment a valid option for elderly patients, especially for those who did not have a caregiver or did not display a good treatment compliance.

D120

SUBCUTANEOUS IMMUNOGLOBULINS IN CHRONIC LYMPHOCYTIC LEUKAEMIA WITH SECONDARY IMMUNODEFICIENCY. A MONOCENTRIC EXPERIENCE IN COVID-19 ERA

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Background: Secondary immunodeficiency was observed in 25-85% of patients (pts) with chronic lymphocytic leukaemia (CLL), increasing the risk of infections, morbidity and mortality. No real guideline leads the eligibility for prophylaxis, but many indications warrant immunoglobulins replacement therapy (IgRT) in selected pts without clear indications about delivery route (intravenous or subcutaneous), dosage, frequency of administration and duration.

Aims: The aim of this study is to assess efficacy and safety of subcutaneous IgRT (SCIg) and its impact on quality of life (QoL) for CLL pts in Covid-19 era.

Table 1.

Table 1. Patients characteristics and results.

Characteristics	Number of patients
Median age (years, range)	66 (56-88)
Median body weight (Kg, range)	68 (52-86)
Comorbidities, n	- 1 thyroiditis - 4 hypertension - 4 diabetes mellitus - 5 lung diseases (fibrosis, COPD etc)
Disease status at ScIg, n	- 1 CR - 6 PR - 3 SD
FISH, n	- 5 del13q - 1 del 11q - 1 del 17p - 1 trisomy 12 - 2 negative
IgVH status, n	- 5 mutated - 5 unmutated
Previous therapy median and type, n	2 (1-9) - 5 pts IBR - 4 pts BR - 4 pts Chl-antiCD20 - 3 pts FCR
Continuous and fixed-time therapies at ScIg replacement, n	- 4 IBR - 1 Ven - 2 Alkylating agents
Infection prophylaxis, n	- 6 Bactrim - 4 Bactrim + Klacid - 10 influenza vaccine
Neutropenia, n	None pts
Median baseline IgG gr/l, range (700-1600)	485 (118-817)
Number and type of infection/year, n	3 (1-5) pneumonia, UTI

Results

Parameter Normal range	Baseline Median value	1° month Median value	3° months Median value	6° months Median value	9° months Median value	12° months Median value
Gamma % (10-18)	7 (2-11)	7 (4-11)	7 (6-12)	8 (7-12)	9 (8-12)	9 (7-11)
IgG mg/dl (700-1600)	485 (118-817)	491 (259-808)	471 (436-818)	615 (436-865)	621 (578-839)	602 (538-915)
IgA mg/dl (70-400)	32 (2-85)	26 (2-55)	29 (5-84)	32 (2-82)	39 (2-73)	60 (5-71)
IgM mg/dl (40-230)	17 (9-28)	16 (9-28)	15 (6-48)	18 (7-80)	25 (11-78)	10 (9-46)
CD4 x10^9/L (630-1400)	429 (292-1056)	458 (262-962)	448 (434-463)	430 (373-487)	nd	nd
CD8 x10^9/L (350-810)	854 (227-1539)	888 (218-1220)	889 (600-1179)	966 (523-1410)	nd	nd
CD19 x10^9/L (100-410)	202 (2-52488)	55 (4-2847)	43 (3-3060)	11 (8-8926)	nd	nd
CD16/56 x10^9/L (140-420)	180 (61-541)	303 (59-383)	107 (67-353)	107 (104-330)	nd	nd

Methods: Ten CLL pts have been treated with SCIg from October 2019 to December 2020. Median age and body weight were 66 years and 68 Kg. Comorbidities were present in 5 pts. Median number of prior therapies was 2. At that time, 7 pts were on therapy. None presented neutropenia. All pts underwent antibiotic prophylaxis and influenza vaccinations. Median baseline IgG level was 485 mg/dl, with a median of 3 infections/year. Table 1. All pts received 10 g total dose of hyaluronidase-free SCIg, self-administered at home with a personal pump every 15 days, independently from body weight. The IgG level and CD4/CD8, CD19 and CD16/56 (natural killer, NK) lymphocytes subset were recorded both at baseline and during the observation period to monitor the immunological reconstitution.

Results: No patient experienced infectious events nor Covid-19 mediated interstitial pneumonia. Nobody interrupted nor modified the dosage and only one patient presented a skin rash (grade 2). Dealing with humoral immunity, IgG levels arose to a stable median value >600 mg/dl from 6 months onward. About cellular immunity, T-cells including CD4 and CD8 and NK cells displayed a stable fashion until 6 months. The CD19 B cells values reflect both the disease status and the ongoing treatment effects. Table 1. Finally, we observed advantages on both QoL and costs, since pts did not need to go to the hospital nor the help of a caregiver, rather they could comfortably get their SCIg at home without any assistance.

Conclusions: SCIg administration in CLL pts is safe and efficacious as infectious prophylaxis, with higher median IgG levels, thanks to its pharmacokinetic advantages and improved adherence to treatment. Especially in the Covid-19 era, the subcutaneous route is preferred to the intravenous one, because of the self-administration at home and the granted availability to the drug itself.

D121

A CAMPUS CML ANALYSIS: 1 YEAR OF THE PANDEMIC COVID-19 INFECTION IN CHRONIC MYELOID LEUKEMIA IN ITALY

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Hematological diseases are at increased risk of SARS-CoV2 infection but limited information has been reported in chronic myeloid leukemia (CML) patients. The Campus CML Italian group carried out a survey in order to observe the temporal course of the infection and the characteristics of positive patients. Retrospective information on 8665 CML patients followed at 46 centers throughout the country were recorded. Within this cohort, 217 Covid-19-positive patients (2.5%) were reported. Most patients (57%) were diagnosed as having Covid-19 infection between September 2020 and January 2021; 30% were diagnosed in phase 1 (March-April 2020) and 13% between May and August. Most of the positive patients were between 50 and 65 years (35%), while 26% had less than 50 years, 18.8% were between 65 and 75 years, and 11% had more than 75 years. A male prevalence was observed (73%). The median time from CML diagnosis to Covid-19 infection was 6 years (3 months-18 years). Fifty-six percent of patients presented concomitant comorbidities at the time of infection. When Covid-19 was diagnosed, 27% of patients were receiving imatinib, 26% nilotinib, 18% dasatinib, 8% ponatinib, 8% bosutinib, 2% asciminib, while 11% were not receive on treatment. At the time of infection, 74% of patients were in molecular remission, 6% in complete cytogenetic remission, 3% in partial cytogenetic remission, 6% in complete hematological response and 11% in treatment-free remission. At diagnosis, 28% of patients presented fever and respiratory symptoms, 13% cough, 10% isolated fever, 13% ageusia, 12% anosmia, 4% had more than 1 symptom, while 20% were completely asymptomatic. Twenty-one patients (9.6%) required hospitalization without the need of respiratory assistance, 18 (8.2%) were hospitalized for respiratory assistance, 8 (3.6%) were admitted to an ICU, while 150 patients (69%) were only quarantined; 23% of patients discontinued TKI therapy during the infection. The source of contagion was familiar in 49% of patients, 18% due to work, 3% in healthcare professionals, whereas in 30% was not known. Twelve patients died due to Covid-19 infection with a mortality rate of 5.5% in the positive cohort and of 0.13% in the whole cohort. This study reports the 1-year of data on the Covid-19 infection in a specific hematological malignancy in the European country first hit by the pandemic. A longer follow-up is needed to further define the impact of Covid-19 infection sequelae in CML patients.

D122

ZANUBRUTINIB WAS ACTIVE IN HEAVILY TREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA OR WALDENSTRÖM MACROGLOBULINEMIA. EARLY OBSERVATIONS FROM THE ITALIAN NAMED PATIENTS PROGRAM

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Introduction: Zanubrutinib is a second generation, highly specific BTK inhibitor that has proved to be active in patients with chronic lymphocytic leukemia (CLL, SEQUOIA trial) and Waldenström macroglobulinemia (WM, ASPEN trial). A phase 2 clinical trial of zanubrutinib in ibrutinib intolerant patients is also ongoing (NCT04116437).

Aims: To describe the clinical features and early observations of patients with CLL or WM treated with zanubrutinib within the named patient program (NPP).

Methods: Inclusion criteria for NPP were patients with relapsed or refractory CLL or WM, either previously untreated with a BTK inhibitor due to comorbidities or previously treated with ibrutinib but discontinued due to an adverse event (AE). Patients must have adequate liver and kidney functions. Patients were excluded if they had disease progression with a BTK inhibitor, had active central nervous system disease, or were not able to read and sign the informed consent form. All patients received 160 mg zanubrutinib twice daily in 28-day cycles until disease progression or intolerance. All treatments were made available by BeiGene company and authorized by company local ethics committees.

Results: Six Italian patients were enrolled, 4 were affected by CLL and 2 with WM. The median age was 71 years (range 69-75), median CIRS score and creatinine clearance were 6 (range 3-11) and 69ml/min (range 41-113), respectively. The median line of previous treatments was 4, ranging from 2 to 7. Four patients previously discontinued ibrutinib due to AEs (1 infection, 2 atrial fibrillation), while the other 2 did not receive ibrutinib due to severe comorbidities (1 hypokinetic cardiomyopathy and 1 atrial fibrillation needing anticoagulation). Three/4 CLL patients harbored TP53 mutation or complex karyotype. At last follow-up (15 mar 2021), 4 patients started zanubrutinib and 2 were still on treatment (Figure 1A). The median treatment duration was 69 days (range 12-79 days). Two patients discontinued for AEs (1 invasive fungal infection and 1 acute myeloid leukemia). At the best response assessment, 2/3 CLL and the WM patient showed at least a 70% decrease of lymphocyte count and monoclonal component, respectively (Figure 1B).

Conclusion: Preliminary observations from the NPP showed that zanubrutinib was a highly active and feasible drug, in heavily treated patients either intolerant to or not-candidates for ibrutinib, with CLL or WM. Updated results observations on all the patients will be presented.

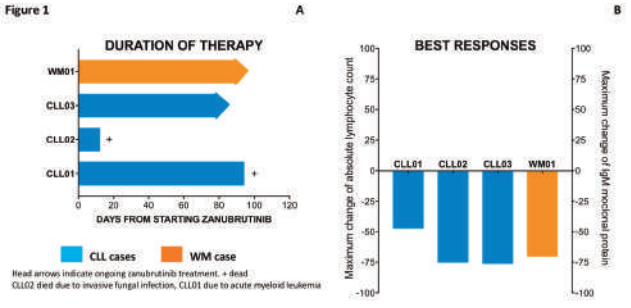

Figure 1.

D123

DE-ESCALATION AND TREATMENT-FREE REMISSION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE TREATED WITH FIRST-LINE NILOTINIB: THE ITALIAN DANTE STUDY

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Background: Treatment-free remission (TFR) is an important goal for chronic myeloid leukemia (CML) treatment, with 40-60% of patients (pts) in sustained deep molecular response (sDMR) remaining in TFR after stopping first-line therapy, with novel strategies being explored to optimize these *Results:* The UK DESTINY study investigated tyrosine kinase inhibitor de-escalation before TFR in mainly imatinib-treated pts. Here, we report the design of phase 2, multicenter DANTE study (NCT03874858) aimed at investigating de-escalation and TFR in Italian pts with CML in chronic phase (CML-CP) treated with nilotinib (NIL).

Methods: Adults with CML-CP treated with NIL 300 mg BID in first-line for ≥3 years who achieved sDMR for ≥1 year (≥MR 4.0; BCR-ABL level ≤0.01% IS) are enrolled. The study consists of 4 phases: screening (week [wk] −4-0), consolidation (wk 0-48), TFR (wk 48-144), and follow-up (until wk 144). Ongoing treatment with ≥400 mg/day dose is allowed at study entry. During consolidation phase, pts are treated with NIL 300 mg QD. At the end of consolidation phase, pts with sDMR enter TFR phase and NIL is discontinued; pts with loss of major molecular response (MMR; BCR-ABL ≤0.1% IS) at any time return to NIL 300 mg BID; and pts with ≥MMR, but without sDMR, continue NIL 300 mg QD. During TFR phase, BCR-ABL levels are monitored monthly from wk 52-96, and then every 3 months. Patients who remain on half-dose treatment after wk 48 and pts with loss of MMR during study are monitored every 3 months. Primary endpoint is the percentage of pts in full treatment-free remission (FTFR) 96 wks after the start of consolidation phase. FTFR is defined as pts with MMR or better, including those who discontinued treatment during TFR phase and those who are treated with half the standard dose. Key secondary endpoints include percentage of pts with sDMR at wk 48; TFR rate at wk 96 and 144; BCR-ABL kinetics and safety. Efficiency of digital droplet PCR is also evaluated.

Conclusions: Currently, the patient enrollment target has been reached (104 pts recruited from 27 centers in Italy). DANTE is the first study evaluating TFR optimization with NIL, and informs on feasibility and safety of de-escalation before discontinuation in CML-CP pts who have achieved sDMR with ≥3-year NIL treatment. The study also appraises if maintaining NIL at half the standard dose for pts with ≥MMR, but not eligible for TFR, is safe. Promising results are expected to bring further advance in CML management.

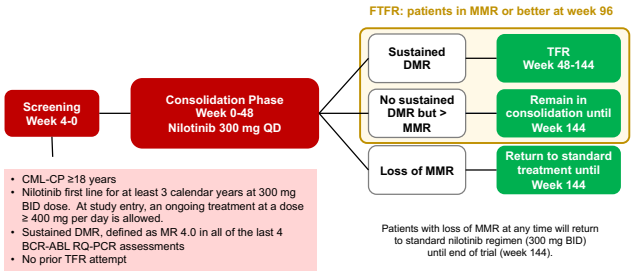

Figure 1.

D124

FAMILIAL ESSENTIAL THROMBOCYTHEMIA: SAME PATHOLOGY AND DIFFERENT MUTATIONAL STATE

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Essential thrombocythemia is chronic myeloproliferative neoplasia, as defined by WHO in 2016, with the best prognosis. A small percentage of ET cases can be considered familial ET. In this report we describe 6 cases of Familial ET, evaluating the heterogeneity of the mutational state and the clinical presentation.In all cases diagnosis of ET was performed by Bone medullary Biopsy.

Case 1: Patient A: A 52.2 year old male patient with hypertension and dyslipidemia, the diagnosis was made in 2002 . Subsequently in 2014 mutation of JAK2, CALR and MPL were absent. Negative search for BCR-ABL transcript.The patient is at high risk (R-IPSET): Patient B: 66.9-year-old male patient, brother of patient A, Presence of JAK2 mutation V617F with burden 39.1%,.Patient High risk (R-IPSET).

Case 2: Patient C. A 39.6-year-old woman came t with the presence of a V617F mutation of Jak2 with 28% burden, According to the R-IPSET the patient was classified as low Risk. Patient D. 37.8-year-old male, brother of patient C (with a father in common and a different mother), came to our observation in 2016. Is positive to the mutation of exon 9 of calreticulin type 2 like.Is classified with very low risk. (R-IPSET).

Case 3. Patient E. 38.8-year-old woman ,she came to our observation in 2007. The search for the V617F mutation of JAK2 was negative, Subsequently in 2016 a mutation search was performed for CALR and MPL who were absent, the BCR-ABL transcript search was also absent.The patient was classified as very low Risk. (R-IPSET). Patient F. 44.9-year-old female sister of patient E , negative for JAK2 mutations, CALR and MPL, BCR-ABL transcript search negative .. High Risk (R.IPSET) (DVT IN 2013).

Conclusion: The consideration that the same mutation can be put in relation to three different diseases (ET, PV, MF) arouses the plausible suspicion that other mechanisms may contribute to determining the phenotypic aspects of the pathologies. Several authors have hypothesized

that the underlying germline may predispose to the acquisition of oncogenic mutations. In this regard Harutyunyan, AS et al report the presence of the three different driver mutations in 3 subjects belonging to the same family.The cases of ET reported by us despite all presenting the same pathology, confirmed by the histological examination, show extreme variability from the point of view of the presence of driver mutations, confirming what was reported by the various authors.

D125

HIGH RATE OF DEATH IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS WHO DEVELOP COVID-19

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Several authors reviewed possible causes of poor prognosis of patients with chronic Ph-negative myeloproliferative diseases diagnosed with SARS-COV2 infection. The dysregulation of the cell-mediated immunity, which may worsen the antiviral response in these patients, and the overproduction of cytokines, which could cause an altered inflammatory response towards CoV2 infection and cause more severe clinical manifestations and possible complications leading to death. Finally, ruxolitinib treatment may have a role in the response to CoV2, via the known alterations of the cell-mediated immunity, consisting in the suppression of NK and T-cells activity. Another important factor may contribute to a worse prognosis of MPN patients who develop COVID19: we refer to the higher incidence of cardiovascular risk factors that characterize this group of patients. It is known as the coexistence of cardiovascular risk factors such as diabetes, hypertension and obesity significantly worsen the prognosis of patients with COVID19 . In our patients , cardiovascular risk factors are present in 74.7% of patients with ET. , in 165 patients with polycythemia vera cardiovascular risk factors are reported in 77.6%: . in patients with prefibrotic myelofibrosis (preMF) (N = 48) risk factors are present in 75% and in patients with overt myelofibrosis (overtMF) (N = 138). Overall, these risk factors are present in 75% of patients with prePMF, and in 75.4% of patients with overtMF (Table 1). Our data show overall the high frequency of cardiovascular risk factors in patients with myeloproliferative diseases probably related also to the advanced age of these patients at the time of diagnosis. In a retrospective analysis, the presence of cardiovascular risk factors was demonstrated to increase the thrombotic risk in patients with MPNs and negatively affect survival In conclusion, we believe that in addition to immunological factors and to the immunodepressive role of ruxolitinib, the high frequency of cardiovascular risk factors in MPNs patients could significantly contribute to the poorer prognosis of COVID19 infection in this subset of subjects.

Table 1.

	ET N=238 (%)	PV N = 165 (%)	PrePMF N = 48 (%)	PMF N = 138 (%)
Cigarette smoking	34 (14.6)	25 (15.6)	11 (22.9)	20 (14.5)
Hypertension	152 63.9	105 63,7	26 54,2	88 63,8
Diabetes	34 14,6	28 16,9	5 10,4	20 14,5
Dyslipidemia	59 24,9	47 28,6	7 14,5	13 9,4
Obesity	23 9,5	12 7,5	4 8,3	11 8,0

Table 1. Frequency of individual cardiovascular risk factors (CVR) in 589 MPNs patients

D126

ESSENTIAL THROMBOCYTEMIA TRIPLE NEGATIVE, CLINICAL FEATURE AND OUTCOME

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Essential thrombocythemia (ET) as defined by WHO in 2016, is a philadelphia negative chronic myeloproliferative neoplasm showing a better prognosis than polycythemia vera and myelofibrosis. In a variable percentage, patients with Essential Thrombocythemia show none of the known drivers mutations (JAK2, CALR and MPL). Such patients are classified as triple negative (TN). In this study we evaluated some of the characteristics of this population by comparing them with those of ET patients with driver mutations.The estimated survival in patients with ET is about 20 years. In our experience TN patients show a survival of 24.5 years higher than that found in patients with driver mutations. (21.66 years) could be related to the younger age at the diagnosis of TN patients. This finding is confirmed in other previous experiences. Patients with ET TN show significantly a lower symptom load evaluated by MPN 10 score, significant appears furthermore, the lower frequency of splenomegaly in TN patients. Splenomegaly is present in about 15% in patients with ET and appears to correlate with a worse prognosis. Our data do not show in the two groups of patients with Et a different frequency of thrombotic and cardiovascular events, this occurrence is instead reported in other similar studies.Finally, the role of subdriver mutations occurring in several genes (TET2, SH2B3, and ASXL1) that may be present in TN patients in fact the correlation with thrombotic events and survival has not been demonstrated. Atypical JAK2 CALR and MPL mutations have been identified in some TN patients .Overall, our data, even within the limits of a mono-institutional case series, show a better prognosis in patients with TN ET. Atypical JAK2 CALR and MPL mutations have been identified in some TN patients .In the literature there are no studies with large case series that address in detail the clinical and molecular characteristics of patients with triple negative ET.The hypothesis that ET TN patients represent a population with clinical characteristics different from those of patients with driver mutations and with a better prognosis must be supported by prospeptic studies with an adequate number of patients.

Myeloma and Monoclonal Gammopathies

D127

ORAL IXAZOMIB, LENALIDOMIDE, AND DEXAMETHASONE FOR R/R MULTIPLE MYELOMA. EXPERIENCE OF REP (RETE EMATOLOGICA PUGLIESE)

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Outcomes of multiple myeloma have improved substantially over the past 15 years with the introduction of proteasome inhibitors and immunomodulatory drugs. The Tourmaline study showed that in patients with relapsed and/or refractory multiple myeloma (R/R MM), treatment with oral ixazomib plus lenalidomide–dexamethasone (IRd regimen) was associated with significantly longer progression-free survival (PFS) by a median duration of approximately 6 months than the PFS observed with the use of placebo plus lenalidomide–dexamethasone (20.6 months in the ixazomib group and 14.7 months in the placebo group).

Aims: With the aim to verify the efficacy of IRd regimen in heavily pre-treated R/R MM patients and/or with poor cytogenetic profile, we retrospectively analysed 31 patients from hematological centers of Rete Ematologica Pugliese (REP).

Results: From May 2019 to January 2021, 31 patients were treated with IRd regimen. Table 1 reports baseline characteristic of all patients: median age was 76 years.

Table 1.

Baseline Characteristics of patients (no. 31)	**No.**	**%**
Medianage - years (range)	76 (54-87)	
>75 years	24	77.4
Gender male/female	13/18	
Creatinine Clearance		
<30 ml/min	0	
30-60 ml/min	5	16.1
>60 mil/min	26	83.9
Cytogenetics		
Standard-risk	13	41.9
High-risk	7	22.6
Notavailable	11	35.5
Priortherapies		
1	3	9.7
2	13	41.9
3	5	16.1
>3	10	32.3
Priorstemcelltransplant	9	29
Diseasecategory		
Relapsed	17	54.8
Refractory	14	45.2
Type of priorregimen		
Bortezomib-containing	31	100
Carfilzomib-containing	6	19.3
Daratumumab-containing	10	32.2

Cytogenetic features were available in 20 (64,5%) of the patients and showed that 22.6% patient population had high-risk, including 4 del(17p). All patients received prior regimens Bortezomib-based and 25 prior regimens Lenalidomide-containing; 10 and 6 patients also received prior Daratumumab and Carlfizomib, respectively. Nine patients underwent SCT. The median time between diagnosis and IRd treatment has been 66 months (9-156). The median follow-up was 16 months (2-23 months). Twenty (64.5%) of the 31 patients obtained a response and 8 (25.8%) of these patients obtained a VGPR at least (Table 2).

Table 2.

Best confirmed response		%
Diagnosis to IRD (months)	66 (9-156)	
Overal Response Rate	20	64.5
CR	4	12.9
sCR	1	3.2
VGPR	3	9.7
PR	10	32.2
MR	2	12.9
SD	2	
PD	7	22.6
NE	2	6.4
Deaths	14	45.2
Median no. of treatment cyclesto response (range)	1 (1-3)	
Median no. of treatment cyclesto best response (range)	5 (1-15)	
Median follow up - months (range)	16(2-23)	
Therapyongoing	14	45.2

AdverseEvents (> 3/4 gr)	No. (%)
Hematologicevents	10 (32.2)
Infections	6 (19.3)
Gastrointestinalevents	3 (9.7)
Thromboembolism	1 (3.2)
Second neoplasm	2 (6.4)

Five, out of 7 patients with cytogenetic high-risk, achieved at least a PR (2 CR e 3 PR). Only one cycle was necessary to obtain a response while a median of 5 (range 1-15) cycles was necessary to achieve the best response. Hematological and not hematological toxicity profile was acceptable and the therapy was stopped in 3 patients due to adverse events. To date, 17 patients are alive and IRd-treatment is ongoing for 14 patients.

Conclusion: Our experience shows that the IRd regimen has a good safety profile and an high response rate in heavily pretreated patients with advanced disease. This retrospective analysis confirms that this oral regimen is also effective in high –risk cytogenetic group.

D128

UTILITY OF SFLC ASSAY IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS. A REAL-LIFE UNICENTRIC RETROSPECTIVE STUDY

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Introduction: Serum free light chain (sFLC) assays represents a tool in diagnosis, prognosis and response assessment in multiple myeloma (MM) patients. Its use in response monitoring in course of treatment and predictive value at disease relapse outside of oligosecretory/micromolecular MM setting is still uncertain.

Methods: A total of 130 RRMM patients treated with at least three lines of therapy at our center between 2000 and 2020 were investigated in a retrospective cohort. In this work, we have focused on the predictive role of sFLC ratio and involved sFLC in MM patients beyond the first

two lines of treatment.

Results: Median age at diagnosis was 64 years (range 31-80 years), more than half of patients being male. Median number of treatment lines was 4 (range 3-8). Forty patients had oligosecretory/ micromolecular disease at diagnosis, while around 25% of t normosecretory patients underwent oligosecretory switch at disease relapse. Around 60-70% of the patients had altered sFLC values at disease relapse. Predictive role of involved sFLC at second disease relapse on PFS3 was demonstrated with value >100 mg/ml (p=0.04). The analysis showed that both involved sFLC <250 mg/ml (p=0.001) and ratio <25 (p=0.0009) at second disease relapse ("*pre-sFLC*") were associated with longer PFS3, based on roc analysis. Involved sFLC <100 mg/ml and ratio >25 at third disease relapse ("*post-sFLC*") had negative impact on PFS3 as well, p=0.03 each respectively. Post-sFLC ratio >25 at fourth disease relapse was also significant in terms of PFS4 (p=0.01). Statistical significance on PFS3 of both involved pre-sFLC and its ratio at second disease relapse was confirmed in patients between 65 and 75 years, patients treated with immunotherapy-based regimens, in clinical relapse, including both normosecretory and oligo/micromolecular subcohort. As for PFS4 predictive utility of pre-sFLC at third disease relapse was shown in proteasoma inhibitor-based regimen, p=0.004 for sFLC >250 and p=0.02 for ratio >25 respectively. On the other hand post-sFLC ratio at fourth disease relapse was significant in terms of PFS4 in patients with clinical relapse (p=0.003) and those treated with immunotherapy (p=0.01).

Conclusions: With growing number of treatment lines, monoclonal component dosage could underestimate disease evolution. Periodical monitoring of sFLC could be of aid, not only as predictive factor prior to treatment change, but also in order to evaluate response in course of treatment.

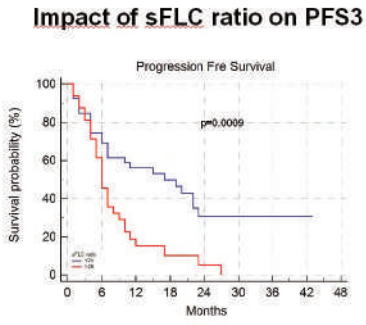

Figure 1.

D129

A REAL LIFE SINGLE CENTER EXPERIENCE OF DARATUMUMAB, LENALDOMIDE AND DEXAMETHASONE COMBINATION IN MULTIPLE MYELOMA PATIENTS

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Background: The introduction of monoclonal antibodies represented a significant breakthrough in the myeloma therapy scenario. Among the MoAbs currently approved for myeloma treatment, daratumumab is the most advanced in terms of clinical development. The combination daratumumab-lenalidomide-dexamethasone (Dara-Rd) has shown promising results in the treatment of relapsed/refractory disease (POLLUX) and subsequently also in newly diagnosed myeloma (MAIA).

Aims: Clinical data regarding the efficacy and tolerability of this drug combination are relatively scarce in frail patients, as well as in those with renal failure or amyloidosis. Against this background, we retrospectively evaluated the safety and efficacy of Dara-Rd combination in a cohort of real-life patients.

Methods: We collected baseline data at diagnosis and at the time of daratumumab therapy initiation of each patient. All patients received Dara-Rd treatment according to POLLUX/MAIA schedule.

Results: Our observational retrospective study included 62 patients, M/F=29/33, median age 66, high-risk cytogenetic in 26%, amyloidotic involvement in 13%, renal impairment in 16%, and a single case of PC leukemia. After a median follow up of 14 months, the overall response rate was 87%, with high-quality response (≥VGPR) in 62% of the patients. Nine patients (15%) underwent ASCT after a median of 9 cycles of Dara-Rd, obtaining a CR in 67% of them. The 12-month progression-free survival rate was 72%, whereas the overall survival rate was 83%. By multivariable analysis, the achievement of at least a VGPR (HR: 0,10; IC 95%: 0,04-0,5; p=0,002), absence of high-risk cytogenetics (HR: 0,06; IC 95%: 0,01-0,20; p<0,001) and ECOG value <2 (HR: 0,05; IC 95%: 0,01-0,22; p<0,001) were found to be associated with increased PFS. Meaningful factors for the overall survival were: response ≥VGPR (HR: 0,21; IC 95%: 0,05-0,79; p=0,021) and high-risk cytogenetics absence (HR: 0,3; IC 95%: 0,09-0,98; p=0,047). The most common G3-4 adverse events were hematological ones: neutropenia (54%), lymphopenia (21%), thrombocytopenia (8%) and anemia (6,5%). Infections (G≥3) were a common occurrence among non-hematological adverse events (16%). A case of secondary primary prostatic cancer was reported. Daratumumab-associated IRR occurred in 68% of the patients and were mostly of G1-2.

Conclusions: In conclusion, even in real-life, Dara-Rd treatment is effective and well tolerated, even by patients with comorbidities

D130

ABSTRACT WITHDRAWN

D131

FLUORESCENT IN SITU HYBRIDIZATION (FISH) ABNORMALITIES IN LIGHT CHAIN AMYLOIDOSIS (AL) PATIENTS: VARIATION WITH THERAPY AND EFFECT ON PROGRESSION FREE SURVIVAL (PFS)

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Introduction: Cytogenetic aberrations and their patterns in systemic AL are still relatively unknown.

Aims: to evaluate FISH abnormalities in AL and assess the impact on PFS.

Material and methods: a prospective study on bone marrow biopsy (BMB) with interphase FISH at diagnosis and then after treatment if achieved at least a PR. The iFISH panel covered: t(11;14), t(4;14), t(14;16) and probes for 1q21, 11q22.3, 13q14, 17p13 and was performed after enrichment of plasma cells using magnetic activated cell sorting with CD138 immunobeads.

Results: AL patients attending our Department from January 2020 to April 2021: 18 patients (median age 60, 57% male), 2 with localized and 16 with systemic AL (sAL), were enrolled. According to PS and age, sAL patients received CYBORD (n=10) and VMD (n=6). Currently, 4 patients still haven't achieved a PR; 5 died before receiving BMB re-evaluation: 4 in the first cycle for PD (median age 73) and one after 3 cycles of CYBORD due to Sars-COV19; 7 patients achieved PR after a median of 4 cycles (3-6). In total 25 BMB were performed and fully analysed. At diagnosis, median bone marrow plasma cells count (PC) was 5% (0-26); 4/18 were>10%; median CD38+,138+,56+,19-,45- PC was 3.5%(1.2-8). CD138+ enriched PC was inadequate (<10%) for FISH in 5/18; abnormal findings in 10/13 and negative in 3/13. Translocation

(11;14) in 6/13 [3 had a translocation with an unknown partner: "t(11;14) variant"] and hyperdiploidy-overall in 5/13 were the most prevalent. We also found trisomies of 1q21, 11q13 and 17p and deletion of 13q and 14q32. BMB re-evaluation after treatment was performed in 7 patients. After 6 cycles, two patients with t(11;14) variant had a CR and a VGPR but FISH couldn't be performed for inadequate PC; after 3 cycles, t(11;14) persisted in 1patient with VGPR; after 6 cycles, trisomies persisted in one patient with VGPR; median PC [2% (0-3)] resulted still inadequate for FISH in one case with PR after 6 cycles and in 2 CR cases after a median of 4 cycles (negative and inadequate at diagnosis). OS and PFS of the entire population [median FUP 5 months (1-16)] were 68% and 43%, respectively. PFS according to the presence/absence FISH alteration, type and PC at diagnosis were analysed (Figure 1).

Discussion: We observed a favourable trend of PFS e ORR in our patients with both t(11;14) and variant at diagnosis (in comparison with negative FISH) was observed, although yielding adequate CD138+ PC for FISH represents a limit for cytogenetic abnormalities identification and MRD evaluation in AL patients.

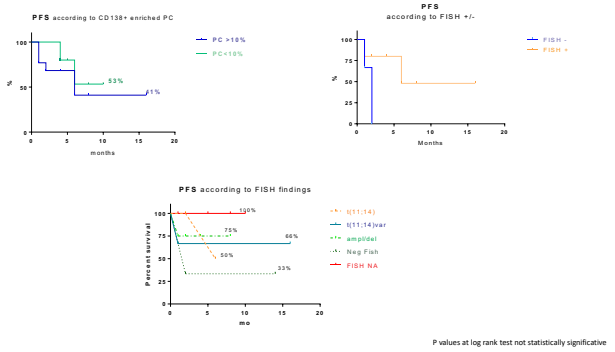

Figure 1. PFS according to FISH findings at diagnosis.

D132

COMPREHENSIVE EVALUATION BY NEXT-GENERATION FLOW ANALYSIS OF CIRCULATING PLASMA CELLS, EXPRESSION OF PD-L1, BCL-2 AND CORRELATION WITH FISH ABNORMALITIES IN PATIENTS AFFECTED BY SMOLDERING MULTIPLE MYELOMA AT THE DIAGNOSIS

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Background: Smoldering myeloma (SMM) represents an intermediate disease in a spectrum of step-wise progressive diseases termed plasma cell dyscrasias.

Aim: The aim of the present study is to evaluate by next-generation flow (NGF) the characteristics of PCs in bone marrow (BM) and the presence of circulating PCs in SMM and MM patients at diagnosis and during follow-up, studying the expression of BCL-2 and PD-L1 on BM and correlating the results with FISH analysis (IgH translocations, del 17p). NGF is performed using the two 8 colours tubes panel developed by the EuroFlow Consortium (BD OneFLOW Tm PCST and BD OneFLOW Tm PCD. BD BioSciences). Data analysis is performed on a FASCantoII cytometer (BD BioSciences).

Results: From October, 2019 to February, 2021 we selected 38 cases of suspected SMM, and only in 24 of these diagnosis was confirmed. The median age of patients is 66,5 years old, blood cell count showed normal values, serum kappa/lambda ratio was abnormal in 16/20 cases. Currently circulating PCs were not detected. Flow Analysis showed that the most expressed markers were CD56/CD27/CD81/CD28^{dim}/CD117^{dim}. CD200 was expressed in 2/24 cases (8.3%), CD20 in 4/24 cases (17%), CD19 was low expressed in 1/24 (4.1%) and CD45 was negative in 23/24 cases (96%). BCL-2 (MFI) was highly expressed in all cases (Mean 14,18 ±5,26; Median 13,5; ≥ 13,5 10/24 42%) while PD-L1 was positive in 8/24 (Median 23,5). FISH analysis was performed and resulted negative in 9 cases. 8/24 (33%) SMM patients, who had diagnosis from at least 2015 and that were in stable disease, had the same results of expression of BCL-2 and PD-L1.

Conclusions: Our preliminary results demonstrated a variable expression of PD-L1, while BCL-2 is highly expressed (not all studies demonstrated early expression of these markers) and all cases did not have FISH abnormalities, demonstrating a less genetic instability compared to MM cases. The diversified expression of analyzed markers confirms the high heterogeneity and complexity of the smoldering phase in MM. NGF and genetic status could correlate with clinical characteristics, explaining the heterogeneous clinical course of this disease, improving the prognostic risk stratification. In the future the assessment of aberrant CPCs with NGF can be a powerful, minimally-invasive blood test to discriminate both SMM cases at high-risk of progression to MM, becoming a surrogate marker for progression to MM.

D133

LENALIDOMIDE (R) AND DESAMETASONE (D) IS A VALID THERAPEUTIC OPTION AS BRIDGE TO ASCT AND TO SALVAGE TREATMENT IN RELAPSED/REFRACTORY (R/R) TO BORTEZOMIB LIGHT-CHAIN AMYLOIDOSIS (AL) PATIENTS

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Introduction: Studies on intermediate dose of R for AL treatment are scanty and few are in ASCT eligible patients.

Aim: assess RD effect and toxicity as bridge to ASCT and to salvage therapy in R/R to bortezomib AL patients.

Table 1.

Patients characteristics at RD treatment (n=13)	Values
Age, median (range)	59 (48-82)
Time from AL diagnosis, median (range)	9 mo (3-21)
Previous lines received	
CYBORD	13 (100)
ASCT	1 (7)
Lambda isotype	12 (92)
Monoclonal component g/l, median (range)	0.5 (0-0.8)
Dflc mg/l, median (range)	0,23 (0,20-0,90)
Cardiac involvement	9 (70)
Revised Mayo clinic stage I	-
Revised Mayo clinic stage II	1 (0.12)
Revised Mayo clinic stage III	4 (0.44)
Revised Mayo clinic stage IV	4 (0.44)
Troponin (median,ng/l)	2469 (48-15060)
NT-ProBNP (median,ng/l)	4815(1550-15440)
Renal involvement	7 (53)
Proteinuria g/24h, median (range)	2.9 (1.4-14.5)
EGFR ml/min median (range)	65 (45-80)
Neurological involvement	1 (7)
Liver involvement	1 (7)
Gastrointestinal involvement	1 (7)
Soft tissue involvement	4 (30)
Number of organs involved	
1 organ	5 (41)
2 organs	5 (41)
3 organs	2 (16)
Patients' responses and evolution with RD treatment	
Hematologic responses	
CR	3(23)
VGPR	4 (30)
PR	3(23)
SD	3 (23)
RD cycles, median (range)	6 (4-13)
ASCT procedure	3 (23)
Re-treament	5 (38)

Values are n (%) unless otherwise notified. CYBORD: Cyclophosfamide, bortezomib, desametasone. Median cycles 5, range 1-8.
ASCT: autologous stem cell transplant; EGFR: Estimated Glomerular Filtration Rate.
CR: complete remission; VGPR: very good partial response; PR: partial response; SD: stable disease; (according to the updated international amyloidosis consensus criteria, Comenzo et al, 2012).

Material and Methods: A retrospective analysis of 13 patients with R/R AL (median age 59 years) treated with RD in our department from

March 2017 to March 2021 was performed.

Results: ORR (≥ PR) was 76%. Three had a CR: two received ASCT while the other suspended after 6 cycles and died at 10 months, in off therapy, for pulmonary embolism following atrial fibrillation. Four had a VGPR after a median of 5 cycles: one received ASCT while 3 maintained the response for a median of 13 months; 2/3 relapsed and died at 6 months from Pomalidomide treatment for PD. Three PR after a median of 4 cycles: 2 not eligible for therapy shift died at a median of 24 months (heart failure following atrial fibrillation and sepsis fatal episode); the last one, due to loss of response, is now treated with Pomalidomide. Organ responses were recorded in 5 patients (4 had cardiac involvement as single organ or with renal/nervous system). After a median of 5 cycles, 3 refractory patients suspended RD (one for renal failure who died short after, while the other two died within the start of Pomalidomide treatment for AL progression). Median Freedom-From-Treatment-Failure was 8 months (3-60). Median survival from enrollment was 14 months (3-60).

All received anti-thrombotic prophylaxis, antihistamines prophylaxis in the first cycle and closely monitoring with frequent blood cell count to promptly start anti-infective prophylaxis (growth factors, antibacterial and anti-fungal treatment). R starting dose was 15 mg in 6 patients and 10 mg in 7 patients. Rash was quite common without need of dose modifications; 45% of grade 1/2 anemia and neutropenia without need of delay, 23% of R reduction until episodes resolution (to 5mg in two grade 2 infections and to 10mg for grade 2 diarrhea) and one case of treatment discontinuation due to renal failure were recorded.

Conclusions: RD is a valid therapeutic option. Three patients (2 with MAYO stage III) achieved a VGPR and RC (n=2) and organ responses after 6 cycles becoming unexpectedly ASCT eligible since 2/3 patients received scant CYBORD treatment (one for rapid intolerance to Bortezomib while the other shifted for progression after 2 cycles).

D134

CARFILZOMIB – LENALIDOMIDE – DEXAMETHASONE (KRD) IN PATIENTS WITH MULTIPLE MYELOMA REFRACTORY TO FIRST LINE THERAPY WITH VTD: FOCUS ON STEM CELL MOBILIZATION AND OVERALL RESPONSE RATE BEFORE AUTOLOGOUS TRANSPLANT

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VTD plus ASCT is the standard treatment for fit patients with new diagnosis of MM. Patients not achieving ≥PR are not considered good candidates for ASCT and this can lead to poor OS. There is no standard therapy in this subset of patients: the alternative of second line induction *vs* mobilization without further treatment is still matter of debate. We are trying to investigate the role of KRD before mobilization in these patients, due to good ORR in the refractory setting, with its ability to preserve the collection of CD34+ cells. From May 2017 to March 2021, we treated 12 patients with MM and refractory to first line VTD, with 2-4 courses of KRD. Median age was 58 years with male sex 75% (Table 1). All patients were considered refractory to VTD if they did not achieve ≥PR at the third cycle or in PD. Nine patients (75%) had stage III at diagnosis. Seven patients had cytogenetic evaluation (58%) with high cytogenetic risk in 4 patients (57%). Two patients (29%) had del17p. All patients were evaluable for treatment response. Median cycles of VTD were 2 (r. 1-3). Response to VTD were MR in 3 patients (25%), SD in 7 patients (58%) and PD in 2 patients (17%). Carfizomib was administered at 27 mg/m^2 days 1-2, 8-9, 15-16 in cycle of 28 days with lenalidomide at 25 mg for 14 days and dexamethasone at 40 mg each week. After a median of 3 KRD cycles (r. 2-4), ORR was 75% with 1 CR, 4 VGPR and 5 PR. Two patients had progressive disease and received salvage chemotherapy: both patients had del17p observed by cytogenetics before VTD. One patient refused ASCT, hence nine patients proceeded to CD34 mobilization after induction therapy with navelbine at 25 mg/m^2 d+1 and cyclophosphamide 1,5 gr/m^2 d+2 followed by G-CSF from d+4. No patients needed plerixafor. All patients reached the goal of mobilization in single day apheresis with a median CD34+ of 6,7x10^6/Kg (r. 1,9–10,2). Seven patients underwent to single ASCT and 2 patients to a tandem ASCT. After a median follow up of 22 months (r. 5-30) 6 patients (67%) were in remission, while 3 patients relapsed and died for refractory disease. Median OS from diagnosis was 22 months (r. 5-30), PFS after ASCT was 12 months (r. 2-21). Our data suggest that KRD can be an effective salvage induction therapy. This scheme did not compromise CD34+ mobilization and allowed patients to receive ASCT. Controlled clinical trials could investigate this scheme in a subset of patients still characterized by uncertain prognosis, also to define the real impact on OS.

Table 1.

Caratteristiche dei pazienti	
Sesso M/F	9/3 (75)
Età mediana (range)	58 (35 – 68)
Stadio III	9 (75)
Caratteristiche del trattamento	
Mediana cicli VTD (range)	2 (1 - 3)
Risposte dopo VTD	
• MR	3 (25)
• SD	7 (58)
• PD	2 (17)
Mediana KRD effettuati (range)	3 (2 – 4)
Best response a KRD	
• CR	1 (8)
• VGPR	4 (33)
• PR	5 (42)
• SD	0
• MR	0
• PD	2 (17)
Pazienti sottoposti a mobilizzazione CD34+	9 (75)
Esclusi dalla mobilizzazione	3 (25)
• Malattia refrattaria	2 (67)
• Decisione del paziente	1 (33)
CD34+ mobilizzate x10^6/kg, mediana (range)	6,7 (1,9 –10,2)
Pazienti sottoposti ad ASCT	9 (75)
• Singolo ASCT	7 (78)
• Doppio ASCT	2 (22)
Mantenimento post ASCT	3 (33)
Follow up mediano post ASCT (range)	4,33 (2,07 – 21,25)
Mediana PFS dopo ASCT, mesi (range)	12 (2 – 21)
Mediana OS, mesi (range)	22 (5 – 30)

D135

A NON-INTERVENTIONAL OBSERVATIONAL RETROSPECTIVE STUDY OF SECOND-LINE TREATMENT WITH THE COMBINATION DARATUMUMAB – BORTEZOMIB - DEXAMETHASONE (DARAVD) IN MULTIPLE MYELOMA PATIENTS REFRACTORY TO LENALIDOMIDE

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With the progressively increasing use of lenalidomide (len) as maintenance therapy after ASCT or as continuous treatment for newly diagnosed multiple myeloma (MM) patients (pts), the condition of len-refractoriness at first relapse has become an unmet clinical need in the last years, characterized by the lack of availability of the most effective combinations. Nowadays, one of the few alternatives available in clinical practice is the triplet daratumumab(dara)-bortezomib(B)-dexamethasone (DaraVd). This regimen has been explored so far in a small fraction of pts refractory to upfront lenalidomide. We conducted a retrospective observational study aimed at defining the outcomes of len-refractory pts treated with DaraVd at first relapse. Eighteen pts were

included in the present analysis; additional pts, with a prolonged follow-up, will be available and presented at the meeting. Baseline characteristics were comparable with general MM population; pts with high risk (HR) cytogenetics (t(4;14) and/or t(14;16) and/or del17) were 4 (22%). B dose-reduction, due to toxicity, was needed in 5 pts (28%). All the pts showed at least one adverse event (AE) of grade ≥ 2. Infusion related reactions (IRR) were 39%, always at first infusion, of grade 1 or 2. Most common toxicities were infections (39% of grade 2-3, no herpes zoster), pneumonia (6%), gastrointestinal disorders (22% diarrhea and 17% constipation) and asthenia (33%). Haematological AEs presented in 100% of the pts (89% thrombocytopenia of which 56% grade ≥3, 28% neutropenia of which 6% grade 3). Peripheral neuropathy (PN) rate was 44% (8 pts), of which 16% grade 3. No pts discontinued the treatment for toxicity. The overall response rate (ORR) was 83% (44% ≥VGPR). With a median follow-up of 8.5 months, median PFS was 8 months (5.5 and 8.5 months in HR and SR pts, respectively) and median OS was not reached. PFS wasn't influenced by dose and duration of previous len exposure, while it was positively influenced by the absence of del17 (p=0.007), normal platelets count (p=0,029) and a response ≥CR (p=0.058) or ≥VGPR (p=0.048). Our data show that, even when used at first relapse, DaraVd has limited efficacy in len-refractory pts, in comparison to the general population treated with the same regimen. Newer more effective triplets, such as combination of monoclonal antibodies with carfilzomib and/or pomalidomide, are eagerly awaited.

D136

NEXT GENERATION FLOW CYTOMETRY FOR MEASURABLE RESIDUAL DISEASE DETECTION AT PREFIXED TIME IN 26 MULTIPLE MYELOMA PATIENTS TREATED WITH VTD AND DOUBLE AUTOLOGOUS CSE TRANSPLANTATIONS: A UNICENTRIC REAL-LIFE EXPERIENCE

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Background: The clinical value of measurable residual disease (MRD) in Multiple Myeloma (MM) still deserves to be defined clearly. In present study we verify MRD status in 26 MM patients treated up-front with bortezomib, thalidomide, and dexamethasone (VTD) plus tandem autologous stem-cell transplantation with high dose melphalan (HDM/ASCT), to assess whether significant differences in MRD depth exist after 4 or 6 VTD cycles, or after the end of induction and after the first HDM/ASCT or after first and second HDM/ASCT.

Methods: We evaluated MRD in 26 consecutive MM patients who underwent induction with 6 courses of VTD followed by up-front tandem ASCT, treated at our institution starting from 2017. Bone marrow MM plasma cells were detected using a single eight colours tube (PCST one flow, Becton Dickinson) flow cytometry assay (FC), allowing to identify the aberrant phenotype CD38+/CD138+/CD19-/CD56+/Cy Kappa/Cy Lambda.

Results: the median age of the 26 MM patients was 61.92 years (range 41-71 years). Seven (46.7%) of the 15 patients with an evaluable ISS could be classified as advanced clinical stage. Non IgG type M-protein was found in 9 patients (36.0 %). A lambda light chain was detected in 10 cases (41.7%). Cytogenetic was available in 13 patients. An adverse risk karyotype was detected in 11 cases. At the prefixed time points, MRD levels were expressed as the ratio between clonal and normal plasmacells (PC/P ratio) and were 46.1 (± SD 32.6) *vs* 48.5 (± SD 38.3) (p = n.s.) in the 19 patients assessable at the 4 and 6 VTD cycles, 40.2 (± SD 38.6) *vs* 14.0 (± SD 14.1) (p = 0.01) in 15 patients receiving 6 VTD and the first HDM/ASCT, and 12.9 (± SD 10.1) *vs* 15.9 (± SD 15.7) (p = n.s.) in 10 patients assessed after the first and second HDM/ASCT. No MRD negative patients were observed; 9 cases (34.6%) achieved a PC/P ratio ≤5 at some points during their clinical course. At this time none of these 9 patients progressed, while 5 of the 15 patients with a ratio ≥5 progressed at 5, 5, 6, 6 and 9 months, respectively.

Conclusions: Our data confirm the effectiveness of HDM/ASCT in MM patients to improve not just the clinical response but also MRD levels. The detection of a PC/P ratio ≤ 5 (MGUS like) seems to predict a better prognosis. In addition, the similar MRD levels detected after 4 or 6 VTD cycles might suggest the possibility of early mobilization of HSCs.

D137

ANTITHROMBOTIC TREATMENTS IN THE REAL LIFE OF PATIENTS WITH MULTIPLE MYELOMA IN THERAPY WITH IMMUNOMODULANTS: A MONOCENTRIC EXPERIENCE

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Venous thromboembolism (VTE) is a common complication in patients with Multiple Myeloma (MM), with more than 10% of patients still developing thromboembolic events. Particularly, treatment with immunomodulating agents (IMiDs) is characterized by frequent thrombotic complications. Although the International Myeloma Working Group (IMWG) since 2014 has developed the thrombotic risk stratification model and the recommendations for thromboprophylaxis (TPX), the frequency of VTE remains high, prompting more appropriate risk stratification tools and revised TPX strategies. Our aim was to determine in the real-life the prophylaxis treatments adopted to avoid VTE during IMIDs therapy. At Sant'Andrea University Hospital – Sapienza a total of 119 MM patients were treated with different IMIDs-based regimens from October 2013 to February 2020. Anti-MM treatment included combinations of bortezomib, thalidomide and dexamethasone (VTD, N=35), lenalidomide and dexamethasone (Rd, N=63) pomalidomide and dexamethasone (PomaD, N=8), carfilzomib, lenalidomide and dexamethasone (KRd, N=6), and elotuzumab, lenalidomide and dexamethasone (EloRd, N=1) and daratumumab, lenalidomide and dexamethasone (DaraRd, N=6). All Patients treated with IMIDs underwent TPX to prevent the risk of VTE. In particular, Low Molecular Weight Heparin (LMWH) was used in 116/119 patients. The remaining three patients were already on different TPX: Cardioaspirin, Rivaroxaban and Dabigatran. VTE (without pulmonary embolism) was recorded in six patients (5%), all of them during LMWH prophylaxis. In particular, two patients were newly diagnosed on VTD therapy and four were relapsing patients treated with Lenalidomide-based combination therapy. No VTE occurred in patients treated with Pomalidomide. All patients suffering from VTE episodes were characterized by high thromboembolic risk according to IMWG. Interestingly, in our experience, the bleeding risk was low (1.68%): two minor bleeding events during LMWH treatment. In summary, in our monocentric experience the LMWH antithrombotic prophylaxis resulted safety and effective with low incidence of thrombotic and bleeding events. However, the subcutaneously administration of LMWH was not well perceived affecting daily quality of life and compliance. Therefore, we are now opening for further investigate a clinical trial aimed to evaluate the efficacy and safety of direct oral anticoagulants (DOACs) for prophylaxis of VTE during IMIDs treatment.

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RAPID AND PERSISTENT EFFICACY OF CARFILZOMIB-LENALIDOMIDE-DEXAMETHASONE (KRD) IN AGGRESSIVE MULTIPLE MYELOMA DISEASE

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Aggressive multiple myeloma (MM) is defined as presence of renal failure or hypercalcemia, extramedullary disease (EMD), elevated LDH, skeletal-related complications, presence of peripheral blood plasma cells, high increase in M protein rate, adverse cytogenetic abnormalities and short duration of response to prior therapy or progression while on current therapy. Randomized controlled clinical trials usually do not include aggressive disease except for high cytogenetic risk. Here we describe five MM patients characterized by an aggressive presentation successfully treated with Carfilzomib-Lenalidomide-Dexamethasone regimen (KRd). Patient #1 presented with severe hypercalcemia at the time of diagnosis, patient #2 developed acute renal failure in course of first line treatment, patient #3 had a massive disabling plasmacytoma, patient #4 experienced intracranial EMD at first relapse and patient #5 was diagnosed with primary plasma cell leukemia (pPCL). When aggressive disease occurred, patients were suddenly treated with KRd. Treatment is ongoing for patients #2, #3, #4 and #5, while patient #1 is on lenalidomide maintenance after KRd induction therapy and tandem ASCT. Two VGPR (patient #3 and #4) and three PR (#1, #2, #5) were achieved after only one cycle. In addition to biochemical response a prompt resolution of neurological symptoms in patient #1, a reduction of EMD in patients #3 and #4 and the normalization of complete blood count in patient #5 were obtained. FISH analyses revealed high risk cytogenetic alterations in 4 out of 5 patients (#1, #2, #3, #5). KRd is known to improve PFS and OS regardless of cytogenetic risk. ENDEAVOR trial sub-analysis showed that a quick disease response can be achieved with proteasome inhibitors (PIs) in patients with aggressive renal impairment. In a similar way PIs induced a quick remission in patient with EMD, also in those with soft-tissue and CNS involvement who usually have worse OS than those who developed bone-related EMD (5.59 *vs* 4 *vs* 9.21 months respectively). Preliminary data of EMN12/HO129 trial investigating carfilzomib and lenalidomide-based treatment for PCL showed that KRd induced deep hematologic responses after 4 cycles (≥VGPR in 80% and ≥CR in 33%). Our reported data suggest that KRd regimen could be an excellent choice in a cohort of patients with aggressive disease usually excluded from pivotal registrative studies because of its quick efficacy with an acceptable toxicity profile.

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A SINGLE-CENTER REAL-LIFE EXPERIENCE WITH BELANTAMAB MAFODOTIN IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Although the therapeutic landscape for multiple myeloma (MM) has expanded, it remains an incurable disease. Novel, well-tolerated and highly effective therapies in the relapsed/refractory (RRMM) setting represent a real hope. Belantamab mafodotin is a first-in-class monoclonal antibody-drug conjugate whose target is B-cell maturation antigen (BCMA) conjugated to the cytotoxic microtubule inhibitor monomethyl auristatin F. In the DREAMM-2 study involving patients with relapsed MM after at least three prior therapies, including an immunomodulatory drug, a proteasome inhibitor and an anti-CD38 monoclonal antibody, Belantamab mafodotin achieved an ORR of 32% with DoR of 11 months and a PFS of 2.9 months. Preliminary results from the DREAMM-2 trial allowed its approval in August 2020 in the USA and in Europe for the treatment of this setting of RRMM patients. In our center five heavily pre-treated patients with RRMM are currently in treatment with Belantamab mafodotin as monotherapy outside of clinical trials. The drug was obtained through a compassionate use request. Particularly, two patients completed eleven cycles of treatment achieving clinical and biochemical response without any grade 3-4 side effects. Patient #1 is a 69 year old patient diagnosed with MM in August 2009 that underwent extramedullary relapse of disease in the skin accompanied by an increased FLC ratio after seven previous lines of therapy. After only one cycle, the patient achieved a complete remission of disease as skin lesions regressed and FLC ratio returned within normal ranges. During treatment (third cycle) she experienced eye pain and photophobia accompanied by grade 2 corneal events. She was successfully treated with lipid tear substitutes and vitamin A oinment. Patient #2 was diagnosed with MM in April 2008 and underwent six lines of therapy before initiating treatment with Belantamab. Before treatment, she was dependent on analgesics and tranfusion support. After one cycle, hemoglobin values returned within normal values and bone pains notably improved and treatment with morphine was stopped. The patient obtained a reduction of more than 50% of the monoclonal component after five cycles. Treatment was well tolerated. From our experience, Belantamab mafodotin is a safe and effective therapy for heavily pretreated RRMM patients. In addition, ocular toxicity is manageable.

Quality of Life

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LATE TOXICITIES AND LONG-TERM MONITORING IN CLASSICAL HODGKIN LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA SURVIVORS: A SERIES OF SYSTEMATIC REVIEWS OF THE FONDAZIONE ITALIANA LINFOMI

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Background: In the current years it is estimated that cancer survivors are more than 16 million and it is predicted to reach 22 million by 2030. Up today there is a paucity of data on monitoring of late toxicities affecting lymphoma survivors, thus we still need more information to set up an evidence based follow-up strategy.

Aim: Researchers of the Fondazione Italiana Linfomi (FIL) drawn a series of systematic reviews with the aim to: i) evaluate evidence for an homogeneous monitoring of lymphoma survivors; ii) find a balance between the unmet medical need and the sustainability of healthcare system.

Methods: The research work was carried out by a multidisciplinary team of 16 FIL Researchers under the methodological supervision of the Istituto di Ricerche Farmacologiche Mario Negri (Milan). The systematic reviews focused on 6 topics: cardiotoxicity, secondary cancers, endocrine-metabolic sequelae, fertility and neurological/ cognitive toxicities, healthy lifestyles. The following questions were analyzed, considering the population of classical Hodgkin lymphoma (cHL) and Diffuse large B-cell lymphoma (DLBCL) survivors treated at $\geq$ 18 years old: i) incidence of the long-term toxicity; ii) comparison with recent therapies (e.i. modern radiation therapy); iii) best monitoring. The search was carry out on PubMed, Embase and the Cochrane Library, and hand searching, up to December, 2020. Selection process and data extraction were conducted according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines.

Results: Six independent systematic reviews were conducted. 170 full text papers were included in the final analysis. They concerned: cardiotoxicity (n=22), secondary cancers (n=21), endocrine-metabolic sequelae (n=9), fertility (n=46), neurologic and cognitive toxicity (n=62), healthy lifestyles (n=10). The optimal monitoring was found out for: i) early left ventricular ejection fraction dysfunction, coronary artery disease, valvular heart disease; ii) secondary acute myeloid leukemias/myelodisplastic syndromes and solid tumors; iii) metabolic syndrome, thyroid, gonadal, and mineral bone disorders; iv) fatigue, cognitive impairment, anxiety and depression. Fertility preservation and correction of unhealthy lifestyles were also examined.

Conclusion: The final documents could be a reasonable bridge from evidence to decision in order to improve the clinical practice and customize the general follow-up approach of cHL and DLBCL survivors.

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HOMECARE FOR HEMATOLOGICAL PATIENTS (PTS): A GROUNDED THEORY STUDY

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Background: The Reggio Emilia Haematological Home Care (HHC)programme was implemented in 2012.The success of this service is strongly connected to the way in which all the actors live and experience it.Grasping the different perspectives means understanding HHC in its complexity without reducing it to a mere organizational issue.

Aim: The study aimed to understand the HHC process through the perspectives of pts, caregivers(CGS) and healthcare professionals (HPs). The generative research question was: "What happens when a haematological pt is cared for at home"? We wanted to understand the various points of view of the actors included in this type of assistance to define its limits and positive aspects.

Materials and Methods: We conducted a qualitative study according to the Grounded Theory methodology through semi-structured interviews. We identified 22 participants (4 pts,14 CGS,4 HPs). The interviews were transcribed, analysed through the initial coding and finally a conceptual framework was built to do focused coding (identification and definition of conceptual macro-categories).On the basis of the focused coding, 3 additional participants were selected (theoretical sampling). From the macro-areas identified,the core category of the research was extracted and condensed through theoretical coding.

Results: The results demonstrated the existence of 3 phases of the care process: "knowledge", "learning", and "routinization" of HHC. In phase 1, the pts were offered HHC, and the therapeutic plan was explained to them together with their CGS. HHC guarantees comfort because the pts can live in their home environment,but on the other hand it is frightening,because HHC is different form hospital care the pt is used to. Phase 2 is "learning": the pts and the CGS gradually learn to manage clinical practices at home, but they also learn to recognize signs and symptoms to report to HPs and to have a constant confrontation with them. In phase 3, the pts, the CGS and the HPs struggled to find a balance in which home assistance becomes part of the daily routine and a trusting bond. The core category is the implementation of pts' wishes for assistance.

Conclusion: HHC require strong mutual understanding among actors.HHC reaches its main goal when the pt and family are prepared and when their care needs are met. Accompaniment in the various stages of the disease and being close to family members and pts increases their quality of life and guarantees personalized assistance.

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FERTILITY ANALYSIS BEFORE AND AFTER TREATMENT IN LONG SURVIVING MALE WITH HODGKIN'S LYMPHOMA

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Most males diagnosed with Hodgkin lymphoma (HL) are of reproductive age, and as average paternal age in many industrialized countries rises, proportion of men diagnosed with HL before having children will increase. Furthermore, reproductive function is impaired in a considerable number of patients at the time of diagnosis, compromising possibility of spontaneous fertilization and opportunity for sperm cryopreservation before treatment. This study aimed to analyze the influence of HL and its treatment on spermatogenic status of 46 male patients with HL treated between 2008 and 2016, with spermiogram available at diagnosis; in 24 spermiogram was also available after treatment. All patients underwent ABVD as first-line chemotherapy, of which 12 patients with relapsed/refractory disease underwent haematopoietic stem cell transplantation (HSCT). Analyzing prognostic factors, we found that number of spermatozoa at diagnosis was reduced in stage 3-4 ($19x10^6$/ml vs. $49x10^6$/ml; p=0.001); motility and vitality were reduced in stage 3-4 (motility: 29% *vs*. 43%, p=0.015; vitality: 49% *vs* 71%, p=0.011) and in presence of B symptoms (motility: 30% *vs*. 44%, p = 0.016; vitality: 53% vs. 69%, p=0.04;) abnormal forms were increased in patients with high ESR (84% *vs*. 75%, p=0.017) and albumin <4 gr/dl (87% *vs* 75%, p=0.002). Analyzing the sperm before and after treatment we observed that in 8 (33%) there was a worsening of the number of spermatozoa (of these 50% underwent HSCT), while in 16 there was an increase in spermatozoa (of these 87% did not undergo HSCT). The number of normal forms was reduced in 20 patients, motility in 9 (of these 56% underwent HSCT), vitality in 15 (of these 33% underwent HSCT). Furthermore, we found that patients undergoing HSCT were associated with severe impairment of fertility in terms of sperm motility (74% at diagnosis *vs* 22% after HSCT; p=0.025). In patients who did not undergo HSCT we found a statistically significant improvement in fertility in terms of motility (35% at diagnosis vs. 50% after treatment; p = 0.009). In this study, we found that HSCT induced infertility in majority of male patients with HL and that first line treatment could improve fertility status caused by disease. Further studies are needed in a larger case series to investigate risk factors for impaired fertility at diagnosis and after treatment.

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DEPRESSION-AND-ANXIETY DISORDER IN LYMPHOMA PATIENTS AND SURVIVORS: A SYSTEMATIC REVIEW

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Introduction: Lymphomas are rising in incidence whereas new therapies are under development. As therapies improve, so survival rates do, highlighting long-term complications such as anxiety-depressive disorder and decreased quality of life in lymphoma patients. The complications above, underdiagnosed and undertreated, can negatively affect patients' lives and interfere with their treatment compliance.

Purpose: This systematic review investigates the association between the development of anxiety-depressive disorder and lymphoma summarizing the current literature on prevalence, incidence, the impact of the disease on patients and survivors. Moreover, this study highlights patients at high risks of developing anxiety-depressive disorder and sensitizes health practitioners.

Materials and Methods: PubMed, Embase, and Cochrane Library databases were screened to perform an extensive review. Inclusion criteria were studies of any level of evidence published, from 1986 to 2020, in peer-reviewed journals reporting clinical and written in English. Relative data were extracted and critically analyzed. PRISMA guidelines were applied, and the risk of bias was assessed, as was the methodological quality of the included studies. Twenty-three studies were included after applying the inclusion and exclusion criteria. Of these, all were human clinical studies.

Results: Twenty-three studies were included in our systematic review for a total of more than 27.000 patients. Among lymphoma patients, the prevalence of anxiety was between 8% and 25%, the prevalence of depression was between 9% and 18%. We found that age, sex, comorbidities, cancer stage, type of treatment, unmet needs, depression, type of hospital, doctor-patient relationship, educational status, previous psychiatric issues were significantly associated with anxiety. Moreover, time from diagnosis, age, sex, comorbidities, physical health scores, anxiety, unmet needs, type of hospital, internet use, doctor-patient relationship, educational status, previous psychiatric issues were significantly associated with depression. Anxiety and depression were also found to reduce survival and quality of life in hematological cancer survivors.

Conclusion: Anxious-depressive symptoms are highly prevalent among lymphoma patients and their development is strongly linked to the risk factors aforementioned. Health practitioners should be sensitized to these complications frequently underdiagnosed.

D144

PSYCHOLOGICAL DISTRESS IN OUTPATIENTS WITH LYMPHOMA DURING THE COVID-19 PANDEMIC

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Cancer patients are a population at high risk of contracting COVID-19 and, also of developing severe complications due to the infection, which is especially true when they are undergoing immunosuppressive treatment. Despite this, they had still to go to hospital to receive chemotherapy during lockdown. In this context, we have evaluated the psychological status of onco-hematological outpatients receiving infusion and not deferrable anti-neoplastic treatment for lymphoproliferative neoplasms, with the aim of both measuring the levels of post-traumatic symptoms, depression, and anxiety during the pandemic and also of investigating the perception of risk of potential nosocomial infection. The Impact of Event Scale-Revised (IES-R) and the Hospital Anxiety and Depression Scale (HADS) were administered to all patients. Moreover, patients were investigated about their worries regarding the impact of COVID-19 on their lives as onco-hematologic patients. Since the 2nd to the 29th April 2020 (during the first phase of the lockdown period in Italy), 77 outpatients were prospectively evaluated. They were diagnosed with non-Hodgkin's lymphoma, classical Hodgkin lymphoma, and Chronic lymphocytic leukemia/Small lymphocytic lymphoma. The mean age was 56.6 (range 22-85). We found that 36% of patients had anxiety (HADS-A), 31% depression (HADS-D), and 43% were above the cut-off for the HADS-General Scale; 36% fulfilled the diagnostic criteria for post-traumatic stress disorder (PTSD). Women and younger patients were found to be more vulnerable to anxiety and PTSD. The study firstly analyzes the psychological impact of the COVID-19 pandemic on the frail population of patients affected by lymphoproliferative neoplasms, to underly the importance of screening patients for emotional and distress conditions and then offering them psychological support.

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ERECTILE DYSFUNCTION (ED) IN PATIENTS WITH LIGHT-CHAIN AMYLOIDOSIS (AL): DIAGNOSIS AND CORRELATION WITH THE HEMATOLOGIC DISEASE

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ED can be an early AL feature. Aim: investigate the incidence and evaluate the presence and possible causes of ED. *Methods:* All male patients with AL attending the Haematology Department of Federico II University were enrolled, from July to November 2020. Patients older than 70y and/or with ECOG ≥ 2 were excluded. Andrological assessment was performed by G.F and P.R and consisted in physical examination, power Doppler ultra-sound evaluation and a questionnaire concerning sexual activity (IIEF-15). Mayo clinic staging assessment was reported for all patients at diagnosis and enrolment.

Results: 13 patients were enrolled: 5 at AL diagnosis ("treatment naïve") while 8 in follow-up after AL treatment ("Off-therapy"). In the latter, hematologic CR and VGPR were recorded in 5/8 and 3/8 patients, respectively (median 38 and 57 mo); complete organ response was recorded in all. Hormonal dosages resulted within the reference limit for all patients and nobody suffered from pathological depression according to our psychologist consultant (S.A). ED prevalence was 92%: 9 severe, 1 moderate and 2 mild ED. The flowmetric indices showed a reduction of peak systolic value (PSV) in 77% of patients and a pathological acceleration (Acc) in 4/13. Patients with pathological Acc showed a higher age (p=0.05) and worse scores at IIEF-TOT (p=0.006), at Desire Function (p=0.01), at Overall Satisfaction (p=0.03); patients with severe ED showed a higher age (p=0.003) and a pathological left Acc value (p=0.04); patients with mild ED and normal erectile function (localized AL) showed normal right PSV (p= 0.004) and normal left PSV (p=0.006) compared with patients with moderate and severe ED. For the two groups ED prevalence and ED stage was homogenous as the flowmetric characteristics. In the "off-therapy" group no differences were found according to hematologic response. Conclusion: a strong association between arterial inflow deficit, penile hemodynamic alteration, and ED was outlined. A possible cardiotoxic effect of extracellular light chain was disclosed by the presence of early endothelial dysfunction in systemic AL independently of treatment received, time from AL diagnosis and organ involvement. A possible indication our study may provide is that any patient seeking medical advice for unexplained ED should undergo AL screening.

Table 1.

Table 1: Patients clinical and haematologic characteristics

	Treatment naive group (n=5)	Off-therapy group (n=8)	p value
Age, median (range)	53 (41-62)	61 (55-69)	0,49
Time from diagnosis, median (range)	5 days (2-8)	66 months (10-112)	
Characteristics at AL diagnosis			
Localized AL	1 (0,2)	0	
Lambda isotype	2 (0,4)	6 (0,75)	0,28
Monoclonal component g/l, median (range)	3 (4,1-12,3)	4 (3,5-7,2)	0,63
Dflc mg/l, median (range)	223,4 (2,7-560)	77,9 (0,32-248)	0,17
Cardiac involvement	4 (0,7)	5 (0,6)	0,21
Revised Mayo clinic stage III	1 (0,2)	2 (0,25)	
Revised Mayo clinic stage IV	3 (0,6)	3 (0,37)	0,47
Troponin (median,ng/l)	96 (10-100)	90 (20-420)	0,93
NT-ProBNP (median,ng/l)	6037 (80-9588)	4829 (80-29133)	0,92
Renal involvement	3 (0,6)	7 (0,87)	0,07
Proteinuria g/24h, median (range)	1474 (140-14250)	5169 (30-7193)	0,01
Neurological involvement	0	1 (0,12)	
Liver involvement	1 (0,2)	0	
Gastrointestinal involvement	0	1 (0,12)	
Number of organs involved			
1 organ	3 (0,6)	2 (0,25)	
2 organs	0	5 (0,6)	0,04
3 organs	2 (0,4)	1 (0,12)	

Values are n (%) unless otherwise specified

Myeloproliferative Disorders

D146

MUTATIONAL ANALYSIS IN LOW RISK MYELODYSPLASTIC SYNDROMES: A SINGLE CENTER REPORT

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Background: Myelodysplastic syndromes (MDS) are a very heterogeneous group of diseases. Recently, a deeper knowledge of low risk MDS (LR-MDS) molecular landscape lead to novel treatments, such as luspatercept for SF3B1 mutated patients with ring sideroblasts. Less is known about the prevalence and type of other mutations and their clinical significance.

Table 1.

Table	NGS neg (n=14)	Any mutation (n= 36)
Median age, years (range)	70 (41.3-82.3)	74.6 (48.2-86) *
Male, N(%)	5 (35.7)	21 (58.3)
Female, N(%)	9 (64.3)	15 (41.7)
MDS type, N(%)		
MDS-SLD	1 (7.1)	7 (19.4)
MDS-MLD	9 (64.3)	10 (27.7)
MDS with isolated 5q-	0 (0)	2 (5.5)
MDS-RS-SLD	2 (14.3)	4 (11.1)
MDS-RS-MLD	0	10 (27.7)
MDS-EB-1	0	1 (2.7)
MDS/MPN	0	2 (5.5)
ICUS/IDUS	2 (14.3)	0
Laboratory values, median (range)		
Hb, g/dL	10.5 (6.9-14.8)	9.8 (7.6-13.9)
ANC x10^9/L	2.1 (0.9-4.4)	2.2 (0.3-5.8)
PLTx10^9/L	149 (32-320)	162 (25-564)
Endogenous EPO U/L	80 (8.2-229)	79 (15.5-566)
LDH, UI/L	225 (178-287)	194 (118-323) *
creatinine, mg/dL	0.8 (0.5-1.3)	0.9 (0.5-1.53)
Bone marrow evaluation		
Median cellularity, %(range)	40 (20-70)	40 (20-90)
hypocellular, N(%)	4 (28.5)	1 (2.7)
hypercellular, N(%)	3 (21.4)	8 (22.2)**
normocellular, N(%)	7 (50)	26 (72.2)**
Reticulin fibrosis, N(%)	4 (28.5)	2 (5.5)
Risk scores		
IPSS, N(%)		
low	12 (85.7)	25 (69.4)
int-1	2 (14.3)	11 (30.5)
IPSS-R, N(%)		
very low	10 (71.4)	17 (56.6)
low	4 (28.6)	16 (40)
Int	0	2 (3.3)
high	0	1 (2.7)
Treatment		
Treated, N(%)	6 (42.8)	27 (75)
Transfusion dependent, N(%)	5 (35.7)	18 (50)
Erythropoietin, N(%)	6 (42.8)	27 (50)
HI, N (%)	3 (50%)	19 (70.3)
TTR, months (range)	5.3 (2.3-9.4)	5.9 (1.4-11.21)

*p=<0.01, **p=0.01

MDS SLD myelodysplastic syndrome single lineage disease; MDS MLD myelodysplastic syndrome multilineage disease; MDS-RS myelodysplastic syndrome with ring sideroblasts MDS/MPN myelodysplastic syndrome/myeloproliferative neoplasm; ICUS/IDUS Idiopathic cytopenia of undetermined significance and idiopathic dysplasia of uncertain significance PLT platelets; ANC absolute neutrophile count; Hb hemoglobin; EPO erythropoietin. HI hematologic improvement. TTR time to best response

Aim: To evaluate the prevalence of somatic mutations of myeloid genes in LR-MDS patients and their relationship with clinical/laboratory features and outcome.

Methods: 50 LR-MDS patients have been evaluated by next generation sequence (NGS) technology Ion Reporters software 5.2 (Ion Torrents5), screening 69 potentially oncogenic genes present in the Oncomine Myeloid Research Assay diagnostic panel. Only variants with an allelic frequency (VAF) > 5% were reported.

Results: Table 1 shows the clinical features in mutated and unmutated patients. Seventy-two % of cases showed at least one mutation involving genes such as SF3B1 (N=16), TET2 (N=10), SRSF2 (N=6), ASXL1 (N=5), DNMT3A (N=4) and many others. Subjects harbouring at least one mutation were older as compared to NGS negative cases (p<0.01), and more frequently displayed a normo/hypercellular bone marrow (p=0.01). Interestingly, mutated cases showed a higher response rate to recombinant erythropoietin (70.3% *versus* 50%), although not significantly. Subsequent analyses were made according to the number and type of genetic defects: harbouring 2 or more mutations was associated with older age [76 (41-86) versus 71 (48-85) years, p< 0.05], lower neutrophil counts at diagnosis [1.47 (0.4-4.2) versus 2.71 (0.4-5.8) x10^9/L, p<0.01], and to shorter time to response to recombinant EPO [2.2 (1.5-8.8) months versus 6.3 (1.5-16) months, p=0.06]. Mutations involving the splicing pathway (*i.e.* SF3B1, U2AF1, and ZRSR2) were the most common, and correlated with older age (76 versus 71, p=0.01), increased bone marrow cellularity [50 (25-90) versus 40 (20-50)%, p<0.01], higher platelet [209 (41-564) versus 105 (20-332) x10^9/L, p=0.02] and neutrophil counts [2.8 (0.37-5.8 versus 1.8 (0.4-3.5) x10^9/L, p=0.03].

Conclusions: somatic mutations involving myeloid genes were frequent in patients with LR-MDS and mainly correlated with older age and deeper cytopenias. Considering mutation types, expectedly, those involving splicing pathway correlated with a more proliferative phenotype. Of note, somatic mutations did not negatively impact on response to recombinant erythropoietin.

D147

SWITCHING TO AN ALTERNATIVE RECOMBINANT ERYTHROPOIETIN AGENT MAY BE EFFECTIVE IN PATIENTS WITH LOW-RISK MYELODYSPLASTIC SYNDROME

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Low risk myelodysplastic syndromes (MDS) mainly present with anemia that may benefit from recombinant erythropoietin (rEPO) in about 70% of cases. Several types of rEPO are available, and it is a matter of debate which one is the most effective and at what dose. Moreover, median duration of response is limited, and subsequent therapeutic options are scanty, so that most subjects would finally become transfusion dependent. We evaluated the efficacy of an alternative rEPO product in patients with MDS refractory or relapsed after the first rEPO course. MDS patients followed at two tertiary hematologic centers in Milan, Italy, subsequently treated with two different rEPO products have been included, and response rates have been evaluated according to the International Working Group 2006 criteria. A total of 25 patients with a median age of 74 years (59-85), followed for a median of 51 months (12-225) have been included. Considering the first course, median endogenous (e)EPO was 59 U/L (3-257) and 12 patients were transfusion dependent. The first product utilized was mainly epoetin alpha (N=16, biosimilar in 9), followed by epoetin zeta (N=4), beta (N=3), and darbepoietin (N=2), resulting in an overall response rate of 60% after 2.4 months (0.8-18). Median treatment duration was 20 months (2.4-81) with the first product, and patients were switched to an alternative compound due to loss of response (N=17), inefficacy (N=8). At switching, 14 patients were transfusion dependant, and pre-dose eEPO was 142 U/L (43-390). Most patients shifted from alpha biosimilar to epoetin alpha (N=9) or vice versa (N=2), from alpha to zeta (N=4) or vice versa (N=4), from beta to alpha (N=3) or vice vera (N=1), and from darbepoietin to alpha (N=2). 44% of patients responded after a median of 1.9 months (0.7-5.2), including 3 cases refractory to the first rEPO. Interestingly, 10/11 responders had been switched to epoetin alpha (p=0.03), and only 27% were transfusion dependent before the switch (versus 78% in non-responders p=0.01). At the last follow up, 3 patients were still on rEPO, whilst 8 stopped it due to loss of response (N=6) or intolerance (N=2) after a median of 15.8 months (11.6-17.5). Switching to an alternative rEPO was effective in 44% of cases, particularly in transfusion independent patients shifted to epoetin alpha. These results may suggest a try of an alternative rEPO product in both primary refractory MDS patients and in those relapsing after a first agent.

D148

REAL LIFE DATA ON AZACITIDINE THERAPY FOR INTERMEDIATE-2/HIGH-RISK MDS, AML WITH MDS-RELATED CHANGES AND CMML-2: AN UPDATE OF A SINGLE CENTRE EXPERIENCE

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Azacitidine is currently the most common treatment in patients (pts) with intermediate-2/high risk MDS, AML with MDS related changes and CMML-2 ineligible for intensive chemotherapy or transplantation. Compared to registration studies, real life data often performed worse or contradictory results. In 2019 we published our data in line with the best results in the literature. Now we propose an update with more pts and longer follow up. From march 2007 to june 2020 we treated 44 pts: 4 MDS-EB-1, 13 MDS-EB-2, 24 AML with MDS-related changes, 3 CMML-2. 36 were male, 8 female. Median age was 77 years (range 64-90). ECOG PS was 0 or 1. Diagnosis and risk stratification were established according to 2016 WHO and IPSS. Treatment were carried out on a outpatient regimen, limiting hospitalization to complications only. Azacitidine was administred according to the usual schedule of 75 mg/sqm for 7 days every 28 and continued without dose reductions up to tolerance or progression. Response was evaluated in 32 pts who have performed at least 4 cycles and classified according the 2006 IWG criteria: complete remission (CR), marrow CR (mCR), partial remission (PR), hematological improvement (HI), stable disease (SD), failure (F) for progression or death. Therapy was prematurely interrupted in 12 pts due to infection (5), intolerance (2), progression (3), cardiovascular cause (1), lost to follow up (1). The 32 pts evaluable for response received a median of 16 cycles (range 4-56). ORR (CR, mCR, PR, HI) was 54,5% (24 out of 44) of all pts and 75% (24 out of 32) of pts who have carried out at least 4 cycles; 5 among these pts (15.6%) had a SD and 3 (9.4%) a F; 20 out 32 were trasfusion dependent and 9 of them achieved trasfusion independence. Of the 24 responders pts, 9 got CR, 7 mCR, 2 PR, 6 HI. Response was observed after a median of 6 cycles (range 4-18); in 7 pts (29.2%) was achieved after 12 or more cycles. Median duration of response was 7 months (range 1-30). Median survival of 29 responders + SD was 20 months (range 4-65); in the failure pts was 3 months (range 1-30). In conclusioin therapy with azacitidine is safe and effective in pts with intermediate-2/high risk MDS, AML with MDS related changes and CMML-2 ineligible for intensive chemotherapy or transplantation. The late responses observed in almost 30% of responders confirm the importance of continuing therapy beyond 6 cycles until tolerance or progression in order to increase number and quality of responses.

D149

POLIGENIC PREDISPOSITION TO LATE ONSET SEVERE HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN A PATIENT WITH MDS

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Haemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by uncontrolled hyperinflammatory response, caused by hereditary genetic defects (primary HLH) or associated with infections, malignancies, rheumatological or immunological conditions (secondary HLH). We report a case of secondary HLH in a 64-year-old man, referred to our Centre in January 2020. He had a history of leuko/thrombocytopenia, splenomegaly and psoriatic arthritis, treated with etanercept between January 2018 and November 2019. Haematologic workup (bone marrow morphology, biopsy, karyotype) led to diagnosis of myelodysplastic syndrome with multilineage dysplasia (WHO2016 MDS-ML) with no blast excess, chromosome 12 inversion (p11q13), absence of fibrosis, IPSS-R intermediate 1. Since November 2019, the patient had been complaining of persistent fever and night sweats; infections and immunological causes were ruled out. As fever was considered a symptom of the hematologic condition, in August 2020 5-azacitidine was started. Shortly after the first cycle, fever and pancytopenia worsened, leading to hospital admission. Subsequently, laboratory data showed hepatic impairment (total/direct bilirubin 8.6/6.6 mg/dl), hypofibrinogenemia (0.89 g/L), markedly increased ferritin (>24000 μg/L), triglycerides (316 mg/dl) and LDH (> 720 U/L) and bone marrow features of haemophagocytosis, with no evidence of infection. H-score resulted in 80-88% probability of HLH/MAS. Clinical scenario also included a severe ulcerative hemorrhagic esophago-gastro-duodenitis unresponsive to antimicrobial therapy, considered as secondary to the haemophagocytic process. As treatment with dexamethasone 40 mg/day led to no improvement, HLH-94 protocol was started (etoposide, dexamethasone, cyclosporine). Exome sequencing analysis showed the A91V monoallelic mutation in the PRF1 gene, known as a possible predisposing factor for HLH. Two additional potentially pathogenetic variants of uncertain significance were identified in the AK2 (P205E) and the GATA2 gene (P161A), the latter associated with genetic susceptibility to myelodysplastic syndromes. These findings support the diagnosis of late onset HLH syndrome, possibly triggered by immunosuppressive therapy administered for MDS and psoriatic arthritis, in the presence of pre-existing predisposing genetic factors. At the time of writing, the patient is well, in complete remission, tapering immunosuppressive therapy.

Allogeneic and Autologous Transplantation

D150

TOTAL MARROW IRRADIATION FOR SECOND ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ADVANCED ACUTE LEUKEMIA

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Background: Relapse after allogeneic haematopoietic stem cell transplantation (HSCT) is associated with very poor outcomes. Total Marrow Irradiation (TMI) is a novel high precision radiation treatment allows to deliver therapeutic radiation doses over extensive selected targets while substantially reducing radiation to vital organs to preserve their functions.

Aim of the pilot study: To evaluate the efficacy of high dose per fraction TMI in combination with thiotepa, fludarabine and alkeran as conditioning regimen in 9 patients with acute leukemia relapsed after a first allogeneic HSCT.

Patients and Methods: The conditioning regimen consisted of: TMI 8 Gy in 5 patients on day -8 -7 or TMI 12 Gy in 4 patients on day -9 -8 -7, plus Thiotepa 5 mg/Kg on day -6, Fludarabine 50 mg/mq on day -5 -4 -3, alkeran 140 mg/mq on day -2. TMI was delivered in daily single fraction dose of 4 Gy. The median age was 45 years (range, 19-70 years); 3 patients were in remission, 6 had active disease at the time of the second allogeneic HSCT. The median number of nucleated cells infused was $4.3x10^8$/Kg (range 2.6-7.7).

Results: The median time to neutrophil counts of > $0.5x10^9$/L was 16 days (range 13-22) and to platelet counts of > $20x10^9$/L was 19 days (range 11-27) respectively. None of the patients had any rejection; all the patients showed a full donor chimerism on day 30 after transplant. The cumulative incidence of grade I II acute GVHD (aGVHD) was 30%, and of moderate chronic GVHD (cGVHD) 11%. Neutropenic fever was shown in 7 patients, only one patient developed a sepsis from Pseudomonas Aeruginosa; one patient had pericardial effusion; one patient had mucositis grade II. The median follow up was 528 days (range 227-858). Day +30 and day +100 transplant related mortality was 0. The overall cumulative incidence of transplant related mortality was 22%; both patients died of interstitial pneumonia received TMI 12 Gy. The relapse rate was 22% and the two patients died of leukemia were not in remission at the time of the transplant. The actuarial 17 months disease free-survival (DFS) was 53%.

Conclusions: This is the first report demonstrating the safety and the efficacy of the TMI conditioning regimen in patients with advanced acute leukemia receiving second allogeneic transplantation with encouraging outcome in terms of engraftment, early toxocity, GvHD and relapse.

D151

ALLOGENEIC STEM CELL TRANSPLATANTION WITH THIOTEPA BUSULFAN AND FLUDARABINE (TBF) FOR MYELOFIBROSIS: A RETROSPECTIVE ANALYSIS

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Allogeneic hematopoietic stem-cell transplantation (HSCT) currently remains the only curative therapy for intermediate or high risk disease.myelofibrosis (MF). We are reporting 56 patients who underwent an allogeneic HSCT in our Centre between 2016 and 2020, and assessed factors predictive of outcome. The median age was 59 years (36-72). Most patients (72%) were JAK2+ and had int2-high DIPSS (92%). The conditioning regimen consisted of thiotepa, busulfan , fludarabine (TBF). All pts received thiotepa 10 mg/kg and fludarabine 150 mg/m^2. The dose of busulfan was adjusted considering the age and the comorbidity score. One patient received 3 days of busulfan (total dose 9.6 mg/kg); 47 received 2 days (total dose 6.4 mg/kg) and 8 received one day of busulfan iv (3.2 mg/kg). Donor was an identical sibling in 13 pt, haploidentical in 18, matched unrelated donor (UD) in 18 and a mismatchedUD in 7. Thus we had 31 HLA matched and 25 HLA mismatched grafts. Fortytwo patients received post-transplant cyclophosphamide (PTCy)-based GVHD (Graft versus host disease) prophylaxis with cyclosporine and mycophenolate mofetil only 14 received a standard GvHD prophylaxis. The 2 year survival (OS) was 73 % and disease free survival (DFS) was 66 % and the cumulative incidence (CI) of TRM was 23% and of relapse 11%. The incidence of acute GvHD grade II-IV was 22% in HLA matched and 50% in HLA mismatched pts (p=0.022), grade III-IV was 6% and 25% respectively (p=0.042) . The incidence of moderate-severe chronic GvHD was 25% in HLA matched and 36% in HLA mismatched grafts (p=0.36). HLA had a major impact on survival : 85% *vs* 49% survival for matched *vs* mismatched patients (p=0.01). Also age >60 years had a major impact : 51% 2 year survival in patients over 60 years of age (n=24) and 88% in younger patients (n=32) (p=0.007, with a DFS of 46 % and 80% respectively and a CI of TRM of 42% *vs* 9%. As to the total dose of busulfan, we found 26% TRM in patients receiving busulfan for 2 days (n=47) and 0% in patients receiving 1 day only (n=8) ; relapse rate was 10% and 20% respectively. In multivariate cox analysis including age, spleen size ,DIPSS score, number of transfusion received and donor type, only HLA identical donor influences the incidence of acute GvHD, transfusion burden and age plays a role in NRM and OS, but no variables predict relapse. In conclusion: older patients with MF have a high NRM and need to be prepared with a milder conditioning regimen.

D152

MOBILIZATION AND APHERESIS OF HAEMATOPOIETIC STEM AND PROGENITOR CELLS IN PEDIATRIC CANDIDATES FOR GENE THERAPY: A 10-YEAR, 45 PATIENT SERIES

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Background: Gene therapy usually requires the collection of a greater number of hematopoietic stem and progenitor cells (HSPCs) than conventional HSPC transplantation, to account for cell manipulation and for unmanipulated backup storage. While pediatric donors usually undergo bone marrow (BM) harvest, HSPC mobilization and apheresis may more suitable for autologous GT.

Methods: We analyzed the mobilization and apheresis procedures of all GT patients <18 years treated at IRCCS Ospedale San Raffaele between April 1st 2010 and March 31st 2020. Patients were affected by adenosine deaminase deficiency (ADA-SCID), b-thalassemia, metachromatic leukodystrophy (MLD), mucopolysaccharidosis 1 Hurler (MPSIH), or Wiskott-Aldrich syndrome (WAS).

Results: Forty-five consecutive patients (ADA-SCID=4, b-thalassemia=7, MLD=10, MPSIH=8, WAS=16) underwent mobilization with lenograstim, alone (n=4) or with plerixafor (n=41), and 1-3 cycles of apheresis. Median weight was 15.8 kg (range 7-54.1); median age was 3.7 years (range 0.4-14.4). Forty patients were enrolled upfront for collection of both the drug product (DP) starting material and an unmanipulated backup, whilst 5 were enrolled for reasons of potential or actual limitations of BM harvest. HSPCs were used as a starting material for DP manufacture (n = 2), cryopreserved for backup (n=2), or both (n=41). We recorded 108 adverse events in the 14 days following the last apheresis, mostly of grade 1-2 (87%). Minimum collection targets were usually 7-13x10^6 CD34+ cells/kg; median total apheresis yield was 37x10^6 CD34+ cells/kg (range of 3.3-63.8 x10^6). 3/40 that underwent mobilization upfront required an additional HSPC collection. 42/43 backups were >2x10^6 CD34+ cells/kg; 41/42 patients received a DP dose in the target infusion range (4 to 30.9x10^6 CD34+ cells/kg), and all those that received the DP engrafted. As compared to our historical BM harvest cohort, mobilization and apheresis allowed the collection of more HSPCs, in a short period of time. Shorter anesthesia, lower fluctuations in intravascular volume and reduced pain are additional advantages.

Conclusions: Mobilization and leukapheresis allow the safe collection of a large number of HSPCs, even in young pediatric donors, meeting GT requirements. Beyond GT, high HSPC yields may allow to overcome significant weight discrepancies between a pediatric donor and a familial HSCT recipient, and prospectively allow to implement HSPC selection strategies.

D153

POOR RESPONSE TO FIRST BNT162B2 SARS-COV-2 VACCINE DOSE IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT

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On March 2021, the Italian National COVID-19 vaccination campaign was extended to patients with high risk chronic medical conditions, including recipients of allogeneic stem cell transplant (allo-SCT). In the present study, we prospectively assessed serological response following the first BNT162b2 SARS-CoV-2 vaccine dose in recipients of allo-SCT in our centre. Inclusion criteria for the participation in this study included: (1) age above 18 years; (2) allo-SCT for any hematological disease; (3) eligibility for vaccination. Patient serum was collected on day 1 (D1; before the first BNT162b2 dose), and on day 21 (D21; before the second dose of the vaccine). IgG antibodies to the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2 were analyzed by CMIA assay. The study population included 34 recipients of allo-SCT (22 males/12 females; median age: 59 years, range 28-70 years). Patients characteristics are depicted in Table 1. Thirty-one patients were evaluable for serological assessments on D1 and D21. On D1, only 1 patient with known previous exposure to SARS-CoV-2 had detectable anti-S and anti-N IgG antibodies. After the first dose of vaccine, on D21, 4/31 (13%) patients had detectable anti-S IgG antibodies above the cut-off of 7,1 BAU, excluding the patient who had experienced natural infection. Median anti-S IgG titer of responders was 121,6 BAU (range 32,8-481). Interestingly, all these four patients were receiving tyrosine kinase (TKI) inhibitors for an ongoing cGVHD (Ruxolitinib, 2 pts; Imatinib, 2 pts) at the time of vaccination. Median time from transplant to vaccination was 51 months (range 32-74) in responders, as compared to 10 months (range 4-142) in non responders. In fact, no patients who had undergone allo-SCT from less than 2 years showed an antibody

response. Our preliminary data suggest that the first dose of BNT162b2 vaccine leads to production of SARS-CoV-2 IgG antibodies in a minority of recipients of allogeneic stem cell transplant. Further, patients who received vaccination later after transplant seem more likely to show antibody production.

Table 1. Patient characteristics.

Number of patients (M/F)	34 (22/12)
Age in years, median (range)	59 (28-70)
Diagnosis	
AML	17
ALL	4
MDS	3
NHL	7
CLL	1
CML	2
Conditioning regimen	
MAC	21
RIC	11
nMAC	2
GVHD prophylaxys	
ATG/CNI based	19
ptCY based	15
Donor	
MSD	9
UD	23
Haplo	2
Time from SCT to Vaccination (months), median (range)	17 (2-141)
Ongoing cGVHD	9
Ongoing immunosuppressive treatment	9
Ongoing TKI treatment	9
Ruxolitinib	4
Imatinib	2
Nilotinib	1
Ponatinib	1
Gilteritinib	1

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; ATG, anti-thymocyte globulin; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CNI, calcineurin inhibitors; Haplo, haploidentical donor; MAC, myeloablative; MDS, myelodisplastic syndrome; MSD, matched sibling donor; NHL, non-hodgkin lymphoma; nMAC, non myeloablative; ptCY, post transplant cyclophosphamide; RIC, reduced intensity; UD, unrelated donor; TKI, tyrosine kinase inhibitors

D154

ABSTRACT WITHDRAWN

D155

IMPACT OF CRYOPRESERVATION OF PERIPHERAL BLOOD STEM CELLS (PBSC) IN TRANSPLANTION FROM UNRELATED DONOR

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Background: Cryopreservation of PBSC for allogenic hematopoietic stem cell transplantation (allo-HSCT) was implemented due to the current Coronavirus Disease 2019 pandemic. Impact of unrelated donor (UD) graft freezing on outcome of allo-HSCT in terms of hematological recovery, graft versus host disease (GVHD) and survival is still controversial.

Methods: In this study we compared graft composition, clinical characteristics and outcome of 23 allo-HSCT from UD cryopreserved PBSC (Cryo Group) with 23 matched-pair allo-HSCT from fresh UD PBSC (Fresh Group) performed in our Center between January 2020 and March 2021.

Results: Table 1 shows no significant differences in clinical characteristics of patients, donors and transplants between the Cryo and the Fresh group. In Cryo Group median time from apheresis to cryopreservation was 1.78 days (range 0.99-2.23) while median time from cells collection and reinfusion was 15.04 days (range 7.66-25.45). In the Fresh Group median time from apheresis to reinfusion was 1.57 days (range 0.89-2.4). The number of viable (7-AAD negative) CD34+ and CD3+ cells per kg patient infused were significantly lower in Cryo Group ($4.98x10^6$/kg *vs* $7.02x10^6$/kg; p=0.001 and $15,29x10^8$/kg *vs* $20,75x10^8$/kg, p=0.034 respectively). Indeed, there was a 37% median loss of viable CD34+/kg cells after freezing. All patients engrafted. Median time to neutrophil engraftment (>0.5 $x10^9$ /uL) was 14 days for both groups. Median time to reach $20x10^9$/uL platelets was 15 days for the Cryo Group and 16 days for the Fresh group (p=0.224), while median time for platelet engraftment >$50x10^9$/uL was 19 days in both series. No differences in the need of red blood cells and platelets transfusions were recorded (p=0.169; p=0.429). No differences in acute GVHD grade ≥ 2 incidence was observed (36% Cryo Group *vs* 39% Fresh Group; p=0.463). All 22 evaluable patients were alive 100 days after transplant in Cryo Group, of which 21 were in complete remission. Two out 23 patients died due to infections in Fresh Group and 18 of 21 alive patients were in complete molecular remission.

Conclusions: In our series no differences between Cryo and Fresh groups were found in engraftment, acute GVHD ≥ 2 incidence and 100 days survival, despite a lower CD34+ infused dose in Cryo Group. Frozen PBSCs could be considered a safe option also for allo-HSCT from MUD but higher amount of PBSC should be collected to warrant an adequate viable CD34+ post-thawing.

Table 1. Patient characteristics.

	CRYOPRESERVED (N = 23)	FRESH (N = 23)	p value
MEDIAN AGE	53 (29-72)	57 (25-73)	0.266
SEX:			
• MALE	15 (65%)	14 (61%)	0.760
• FEMALE	8 (35%)	9 (39%)	
MEDIAN PATIENT WEIGHT	74 (52-100)	75 (53-98)	0.706
DISEASE			
• AML**	12 (52%)	14 (61%)	0.802
• ALL	5 (22%)	3 (13%)	
• NHL	2 (8%)	3 (13%)	
• Other	4 (18%)	3 (13%)	
DISEASE STATUS			
• CR***	15 (65%)	16 (69%)	0.409
• R/R	9 (39%)	4 (17%)	
• Other	5 (21%)	3 (13%)	
HCT-CI			
• 0	11 (48%)	8 (35%)	0.170
• 1-2	7 (30%)	4 (17%)	
• >2	5 (22%)	11 (48%)	
KARNOFSKY			
• < 90%	10 (43%)	14 (61%)	0.238
• >90%	13 (57%)	9 (39%)	
ECOG			
• >= 2	3 (13%)	8 (35%)	0.084
• < 2	20 (87%)	15 (65%)	
ABO			
• Major incompatibility	9 (39%)	6 (26%)	0.072
• Minor incompatibility	11 (48%)	7 (30%)	
• Compatible	3 (13%)	10 (44%)	
DONOR MEDIAN AGE	30 (19-51)	30 (18-53)	0.796
DONOR/RECIPIENT SEX MISMATCH	12 (52%)	9 (39%)	0.374
DONOR/RECIPIENT WEIGHT DISCREPANCY (>10Kg body weight)	13 (56%)	16 (69%)	0.359
MISMATCH	6 (26%)	9 (39%)	0.345
CONDITION REGIMEN			
• Myeloablative	12 (55%)	15 (65%)	0.465
• Reduce intensity	10 (45%)	8 (35%)	
MEAN WBC/kg INFUSED	8.3x10^8	7.80x10^8	0.516
MEAN CD34/kg INFUSED	**4.98x10^6**	**7.02x10^6**	**0.001**
MEAN CD3/kg INFUSED	**15.29x10^7**	**20.75x10^7**	**0.034**

** AML = Acute Myeloid Leukemia; ALL = Acute Limphoid Leukemia; NHL = Non Hodgkin Limphoma
***CR = complete remission; R/R=relapsed/refractory

D156

REAL-WORLD EXPERIENCE IN 251 CONSECUTIVE – SINGLE CENTER, TRANSPLANTS FOR ACUTE MYELOID LEUKEMIA

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Here we report outcomes of 251 consecutive patients with intermediate-high risk AML (primary: 202, secondary:49) who received a 1st allogeneic hematopoietic stem cell transplant (HSCT) in our Unit (from Jan-2012 to Oct-2020). Median age was 51 yrs (range 18 – 74). 133 pts were in 1st Complete Remission (CR), 66 in 2ndCR and 52 in active disease at HSCT. The donor was HLA identical sibling, haploidentical related (haplo), matched unrelated donor (MUD), or mismatched unrelated donor (MMUD) in 42 (17%), 179 (71%), 24 (10%) and 6 (2%) pts, respectively. The myeloablative conditioning regimen consisted of thiotepa, fludarabine and busulfan (TBF) (n.103) or TBI with fludarabine

or cyclophosphamide (n.43). Patients over 60yrs or with comorbidities received TBF with 2 or 1 days of busulfan (n.105) as reduced intensity conditioning. The GvHD prophylaxis was performed with cyclosporine (CS), methotrexate and ATG for HLA identical sibling donor (without ATG in bone marrow related) and high dose post transplant cyclophosphamide, CS and mycophenolate for haplo. Median FU was 1262 days (range 171 – 3088). Median time to neutrophil engraftment was 17 days (range: 9 – 56). 11 pts (4%) rejected the graft (9 haplo, 2 MMUD). Engraftment was achieved in all others (95%). Incidence of acute gr.II-IV GvHD was 16% (n.41). Incidence of relapse and non-relapse mortality was 26% (n.67) and 18% (n.46), respectively. The relapse incidence was higher for pts transplanted in active disease (50% *vs* 18%in CR1, 22% in CR2) (p=0.05). Major causes of death were relapse (54%), infections (23%) and GvHD (16%). The 5yrs OS is 55% and the 5yrs PFS is 50%.

The 5yrs OS is 64% in CR1, 58% in CR2 pts (p=n.s.) and 22% in those transplanted in active disease (p<0,001). No significant differences were detected among primary and secondary AML, 5yrs OS 56% versus 48% (p=n.s.). We detected a worse outcome in pts receiving HSCT from MMUD: 3 yrs OS was 72%,55%,58% and 28% for HLA-identical, MUD, haplo and MMUD respectively (p<0,05). This large real world-experience confirms that allo-HSCT is a feasible, long-term curative option for int-high risk AML patients and that an haplo donor can be safely considered. As expected, better results were obtained in pts in remission. Moreover, in the latter, even if relapse was the major detrimental factor, engraftment and remission were achieved, demonstrating that transplant can be a platform for further targeted prophylactic / MRD based / treatment strategies

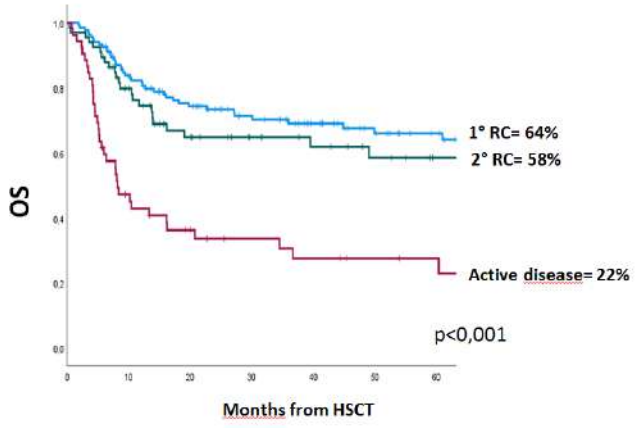

Figure 1.

D157

FLIT3-INHIBITORS IN COMBINATION WITH CHEMOTHERAPY IN NEWLY DIAGNOSED AML-FLIT3+ , OR AS SINGLE AGENT IN RELAPSED/REFRACTORY AML-FLT3+ SHOULDE BE FOLLOWED BY ALLO-SCT IN ALL ELEGIBLE PATIENTS. REAL LIFE EXPERIENCE OF "RETE EMATOLOGICA PUGLIESE"

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Background: Mutations in the FMS-like tyrosine kinase3 (FLIT3) gene are present in 25%-30% of all AML. Patients with FLIT3 mutations have a high relapse risk and inferior cure rates. In the last years three multi-kinase inhibitor, midostaurin, gilteritinib and quizartinib become available as single agent or in combination for FLT3-mutated AML patients. Here we report the outcome of patients with newly diagnosis (ND)- or relapse/refractory (R/R) AML-FLIT3 mutated treated with FLT3 inhibitor in combination or as single agent in order to evaluate its efficacy and safety and its role as bridge to transplant in a real life setting.

Method: From November 2018 to April 2021, 57 patients (Pts) with AML-FLT3+ were selected in the haematology department belonging to the REP. Median age was 54 years (range 27-87), 43 patients (75%) had ND-AML-FLIT3+ and 14 (25%) R/R-AML-FLIT3+. Among R/R Pts, 6 relapsed after 3+7 associated to midostaurin, 3 after FLAG +/- Idarubicin, 3 after HMA, 1 after fixed combination of daunorubicin and cytarabine and 1 after HSCT. The ND-AML-FLIT3+ received induction therapy 3+7 plus midostaurin 50 mg twice daily on day 8 through 21 followed by consolidation therapy with HD-ARA-C plus midostaurin and maintenance therapy (n=3). Among 14 R/R-AML-FLIT3+, 12 (72%) Pts received gilteritinib 120 mg orally once daily and 2 quizartinib 60 mg orally once daily. All eligible Pts received allo-SCT in first or second remission.

Results: ORR (CR+iCR and PR) was 71,9 % including CR+iCR rate of 72,9% in ND- and 35,7% in R/R-AML-FLIT3+. The median time to response was 1 mo. (range 1-2). After median follow-up of 10 mo. (range 1-28), 39 Pts (68,4%) are alive, including 3 Pts still on maintenance therapy post allo-SCT with midostaurin. Nine out of 18 died of progressive disease. Overall 31 pts became eligible for allo-SCT (54,3%), 27 with ND- (62,7%) and 4 with R/R-AML-FLIT3+(28,3%). Grade 3/4 hematological toxicity was observed in 30% of Pts and 42% experienced non-hematological toxicity (FN 20%, sepsis 16% and invasive fungal infection 6%). The median EFS and OS was not reached in ND- *vs* 3 mo. in R/R-AML-FLIT3+ (p=0.0001). The median OS for all transplanted Pts was NR *vs* 10 mo. (p=0.059). The estimate 24 mo. OS rate among ND-AML-FLIT3+ was better for transplanted Pts 71% *vs* 41%, (p=0.071) Figure 1.

Conclusion: After FLIT3 inhibitors therapy allo-SCT is feasible and highly effective mainly in patients with ND-AML-FLIT3+ improving EFS and OS in this high risk population.

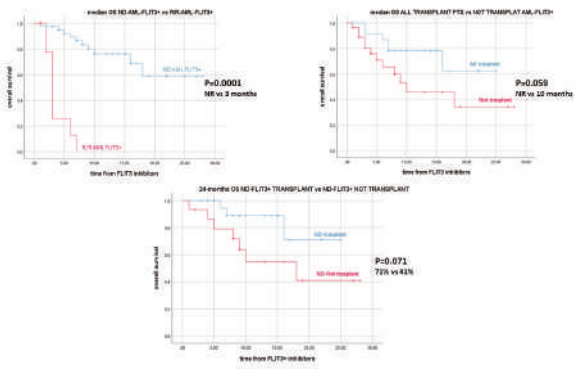

Figure 1.

D158

GVHD PREVENTION IN ALLOGENEIC HSCT FOR LYMPHOMAS: A SINGLE CENTER RETROSPECTIVE ANALYSIS

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Introduction: cGvHD is the major complication after allogeneic HSCT. Intensification of GvHD prevention, without losing GVL effect is particularly important when PBSC are used. While the Addition of ATLG to the standard CNI based GVHD prophylaxis has already been demonstrated to be efficacious in several hematological diseases, no focused trial has been ever run in the setting of lymphomas.

Methods: We retrospectively evaluated the role of low doses ATLG in addition to cyclosporine and either short-term methotrexate or mycophenolate as GvHD prevention in HSCT in 75 patients affected by Hodgkin (45) and non-Hodgkin lymphomas (30) consecutively performed in our Center. ATLG dose ranged from 15 to 30 mg/kg according to stem cell source and HLA mismatching.

Results: Patients (median age 39) underwent allogeneic HSCT (PBSC 51, BM 24) from MRD (10) and URD (only 32/65 were well matched) after a thiotepa-based RIC regimen. Cumulative incidence of grade 3-4 aGvHD was 10.6% at 1 year. In multivariate analysis the only prognostic factor for aGvHD was HLA mismatching (p=0.021). Two-year incidence of cGvHD was 22.7%. cGvHD was mild in 17%, moderate in 57% and severe in 26% of patients. It was correlated in multivariate analysis with female donor sex (p=0.01), HLA mismatching (p=0.05) and PBSC source (p=0.05). Two-year cumulative incidence of NRM and progression/relapse was 17.5% and 26.2%. OS and PFS at 2 years were 75.2% and 56.6%, respectively. Disease status at transplant was the most significant prognostic factor for both OS (p=0.043) and PFS (p=0.003).

Conclusions: This study shows that low doses ATLG are associated with a low incidence of cGvHD without increasing the risk of disease recurrence. Moreover, our results suggest that the major effect of ATLG is prevention of severe GvHD.

D159

EXTRACORPOREAL PHOTOPHERESIS AS RELIABLE SECOND LINE THERAPY FOR STEROID-RESISTANT CHRONIC GRAFT-VERSUS-HOST DISEASE

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Chronic graft-versus-host disease (cGvHD) is a major complication after allogeneic hematopoietic cell transplantation. The incidence of steroid refractory chronic GvHD is approximatively of 50%. Extracorporeal photopheresis (ECP) represents one of the possible option as second line therapy for steroid-resistant GvHD (SR-cGvHD). We reported data of 46 patients submitted to allogeneic stem cell transplantation in our Department between april 1998 and april 2020 who received ECP for SR-cGvHD. The total number of ECP performed until December 2020 was of 1800, with approximatively 360 procedures performed for year. Each patients received a median number of 39 procedures (4-116) starting at a median time of 311 days after transplant (112-7738). Median age of the patients was 51 years (22-73). Donor source was as follows: matched related donor (n=20), matched unrelated donor (n=13), mismatched unrelated donor (n=4) and haplidentical donor (n=9). Chronic GvHD score was mild in 16 patients, moderate in 23 patients and severe in 7 patients. Before starting ECP treatment, 33 patients were receiving a median dose of 37 mg of prednisone (5-140), whereas 35 patients were receiving a median dose of 150 mg of cyclosporine A (20-400). At the observation time fixed at December 2020, ECP treatment was ongoing in 22 patients, after a median duration of 475 days, with an overall response rate of 91% divided as follows: complete response (CR, n=1), partial response (PR, n=9) and very good partial response (VGPR, n=10). On the other hand, ECP treatment was stopped in 16 patients. Among them the overall response rate was of 94% (CR n=10, PR n=2, VGPR n=3), whereas only one patient maintained a stable disease (SD). Finally, eight patients died after transplant while ECP was ongoing and the death causes were GvHD in 5 patients (PD n=4, SD n=1) and underlying disease relapse in 3 patients (GvHD response CR n=1, SD n=2). Regardless of the final GvHD response, median dose of prednisone after ECP treatment was significantly knocked down as compared to that assumed before starting ECP (6 mg vs. 30 mg, Wilcoxon signed rank p<0.0001, Figure 1A). Similarly to that, cyclosporine dose after ECP treatment was significantly reduced as compared to that administered before ECP initiation (35 mg vs. 140 mg, Wilcoxon signed rank p<0.0001, Figure 1B). In conclusion, ECP represents in our centre a reliable option for chronic SR-GvHD with an overall response of approximatively 78%.

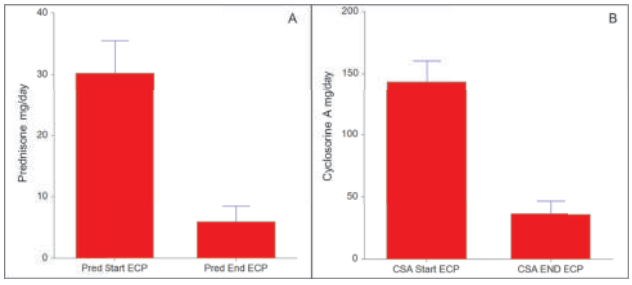

Figure 1. Reduction of steroid (A) and cyclosporine A (B) dose induced by ECP treatment.

D160

ABSTRACT WITHDRAWN

D161

ANTI HLA DONOR SPECIFIC ANTIBODIES (DAS) DOES NOT AFFECT GRAFT AND SURVIVAL IN UNMANIPULATED HAPLOIDENTICAL HAEMATOPOIETIC STEM CELL TRANSPLANT (HAPLO-HSCT) WITH ORIGINAL POST-TRANSPLANT CYCLOFOSFAMIDE (PT-CY) SCHEDULE. A SINGLE CENTRE EXPERIENCE

A. Proia, M. Santopietro, F. Fanelli, F. Blandino, F. Ipsevich, A. Matteocci, M.B. Pinazzi, A. Severino, M.D. Simone, L. Pierelli, L. Rigacci

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In haplo-HSCT using PT-Cy, according to Luznik regimen, CSA started at the day +5. Recently, Raiola *et al*., on a large cohort of patients (treated early with CSA at day 0), demonstrated that HLA disparity in haplo-HSC with PT-Cy did not affect neither engraftment or relapse. The aim of our monocentric retrospective study was to evaluate the impact of anti-HLA DSA on graft and survival in haplo-HSCT with PT-CY, delaying start of CSA prophylaxis (day +5), as original schedule. From 2008, 52 patients received TBF MAC (n=47) or RIC (n =5) conditioning: Thiotepa (10 or 5 mg/kg), iv Busulfan (9,6 or 6,4 mg/kg) and Fludarabine (120 mg/mq). GVHD prophylaxis was PT-Cy (50 mg/kg) at day +3 and +4, CSA 2 mg/kg/day and MMF 45 mg/kg/day from day +5. In 32 patients stem cells source was BM, while in 19 was PB, and in 1 case BM plus PB. Forty-nine adults (median age 45 yrs, range 19-69) and 3 children (age 2, 4 and 6 yrs), with 11 high risk ALL, 26 AML/MDS, 1 myelofibrosis, 11 Lymphomas, 2 multiple myeloma, and 1 Richter evolution of CLL underwent unmanipulated haplo-HSCT. Disease status at transplant was CR1 in 22 patients (42%), CR2 in 11(21%), 6 resistent/refractory (11.5%), 8 PR1(15.5%) and 5 PR2(10%). From 2011, DAS were analyzed in 45 consecutive cases. Sustained donor engraftment occurred in 44 patients (85%), with a median time to neutrophils (>0,5x10^9/L) and

platelets recovery (>20x10^9/L) of 19 and 25 days from HSCT, respectively. Eight patients were not evaluable for engraftment: 5 early deaths, 1 early relapse and 2 graft-failure. The incidence of grade II-IV aGVHD and cGVHD was 29% and 25%, respectively. With a median follow-up of 33 months (range, 1-103), 28 patients are alive and 25 are disease free. Causes of deaths were relapse for 7 patients (13%) and transplant-related mortality(TRM) for 17 patients(33%) (infections 8; aGVHD 8; cardiac failure 1). Considering only 33 patients in CR at transplant TRM was 25%. The overall survival (OS) was 52.1% and disease free survival (DFS) was 47.4%. DAS were detected in 6 (11,5%) patients, 4/6 treated with plasma-exchange based protocol. No differences were observed in OS, DFS, engraftment or development of acute or chronic GVHD according to DSA levels or detection. In haplo-HSCT with PT-Cy, starting CSA prophylaxis at day +5, no significative influence on engraftment or GVHD incidence was observed, even in case of patients with positive anti-HLA DSA.

D162

PERFORMANCE OF HIGH-FREQUENCY ULTRASOUND IN THE EVALUATION OF SKIN INVOLVEMENT IN CUTANEOUS CHRONIC GRAFT-VERSUS-HOST DISEASE

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Introduction: The availability of sensitive and standardized tools for detecting the subclinical fibrotic skin alterations of cGVHD is crucial both for an early intervention and for a standardized response evaluation. High frequency ultrasound (HFUS), currently used in the assessment of several inflammatory cutaneneous disorders (e.g. hidradenitis suppurativa), represents an easy diagnostic tool characterized by low cost and high performance.

Materials and Methods: Eighteen patients with cutaneous cGVHD and 10 healthy controls have been evaluated with HFUS between June 2018 and June 2020. 16 patients had active cGVHD (3 severe, 11 moderate, 2 mild cGVHD) and 2 had previous history of cGVHD: 9 had skin score < 2; 6 skin score 2 (n = 3 with superficial scleroderma; n= 3 without scleroderma); 3 had severe cGvHD with skin score 3. A standard US technique was used (B-mode and color Doppler US) of MyLabOne ultrasound unit (Esaote, Genova, Italy) with high-resolution (>18MHz) by the same physicians. We measured the epidermal, dermal, and hypodermal thickness (in mm) and echogenicity (as percentage density: hypoechogenic 0–15%, isoechogenic 15–20%, hyperechogenic >20%) of the right side abdomen, ventral surface of the forearm, and neck sternocleidomastoid region.

Results: Overall a statistically significant reduction of the epidermis thickness has been observed in cGVHD patients, compared to the normal controls, while a significant increase in dermal thickness was observed in all evaluated areas ($p<0.05$). The increase in dermal thickness and hypoechogenicity was more evident in moderate skin cGVHD (with skin score 2), compared to patients with mild and severe cutaneous cGVHD, probably due to an increase deposition of collagen and in the amount of inflammatory cells and oedema in this phase of cGVHD (but pathological samples were not available). In addition in the 3 patients with cGVHD without clinically overt skin involvement, HFUS were able to show a reduction of the 3 skin layers; and indeed after 3 months of follow up they developed cutaneous cGVHD.

Conclusions: Our findings suggest that HFUS can represent a promising tool both for the early detection and for the quantitative assessment of sclerodermatous changes in skin cGVHD; interestingly, in the early SSc, US abnormalities can anticipate clinical signs of skin involvement.

However, this tool should be prospectively tested in larger trials, for assessing the efficacy of novel therapies.

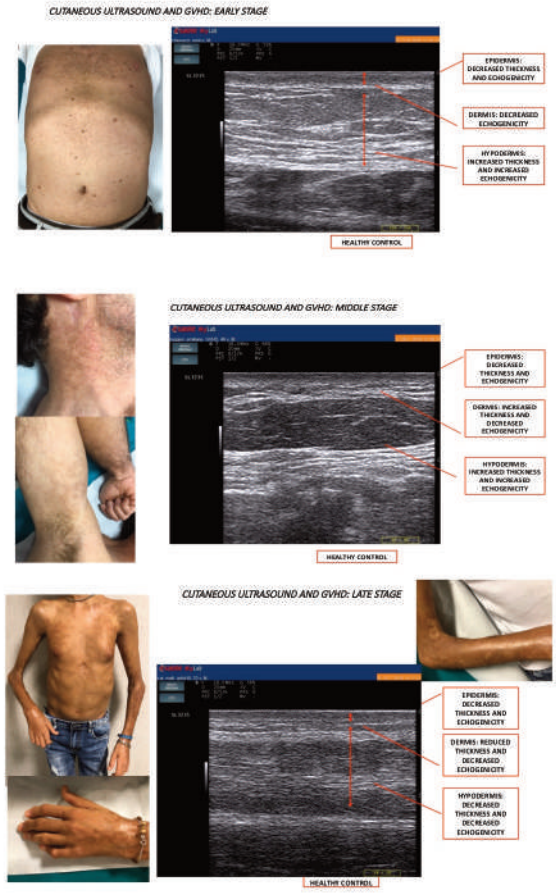

Figure 1.

D163

GVHD PROPHYLAXIS WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE (PTCY) IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: In hematopoietic stem cell transplantation (HSCT) from both sibling and mismatched related donors, Graft Versus Host Disease (GVHD) remains the most feared complication. Use of post-transplant cyclophosphamide (PTCY) in GVHD prophylaxis has allowed safe procedures even across the HLA barrier.

Methods: We studied 14 patients undergoing HSCT from sibling donors who practiced PTCY (Cohort1=A); there were 2 comparison cohorts: Cohort2=B (n=47 sibling donors, standard prophylaxis with cyclosporine±methotrexate±anti-thymocyte globulin) and Cohort3=C (n=54, mismatched related donors, PTCY+cyclosporine+mycophenolate mofetil prophylaxis). All transplants were performed between 2011 and 2020 at transplant centers in Avellino (n=14, A), Naples, and Salerno (n=71 and n=30). The outcomes were overall survival (OS), non-relapse mortality (NRM), acute and chronic GVHD and relapse.

Results: PTCY was well tolerated in HSCT without recurrent, clinically meaningful treatment-emerging adverse event. Myeloablative con-

ditioning regimens were given to 71.4, 91.5 and 96.3% of patients in A,B,C, acute leukemias were 91.5, 100 and 81.5%, the stem cell source was bone marrow in 64.3, 31.9 and 83.3% in the 3 cohorts. Median age was 52.5 (range 20-62), 52 (range 18-64) and 47.6 (range 19-71) in A,B and C. The OS at 36 months was 43% in A, not statistically different from B (45%; p>0.05) and C (54%; p>0.05). The day-100 NRM rate was 14% in A, not statistically different from B (21%; p>0.05) and C (20%; p>0.05). The rate of grade II-IV and III-IV acute GVHD were 42.9% and 14% in A, which were not statistically different from B (29.8% and 8.5%; p>0.05) and C (31.5% and 13%; p>0.05). The chronic GVHD was 15.4% in A, which was significantly reduced as compared to B (68.4%; p=0.01) and C (57.5%; p=0.011). The rate of relapse was 21.4% in A, which was overlapping to that of C (15.2%; p=0.685), and slightly lower, not statistically different, to that of B (36.6%; p=0.3). The leading cause of death was relapse, which accounted for 50, 63 and 40% of events in A, B and C.

Conclusions: PTCY gave a good NRM and survival with reduction of chronic GvHD, it was safe and effective as single agent GvHD prophylaxis in HSCT from sibling donors, but PTCY alone was unable to reduce acute GvHD rate as compared with standard Csa+MTX±ATG regimens, for this reason we want to start a prospective trial with PTCY-CsA double-agent immunosuppressive regimen for HSCT from HLA-matched sibling donors.

D164

THE DISASTER PLAN ADJUSTMENT TO THE COVID-19 PANDEMIC AT AORN CARDARELLI TRANSPLANT PROGRAM IN NAPLES, ITALY

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[1]UOSC Ematologia con Trapianto CSE, AORN Antonio Cardarelli.; [2]UOSC Ematologia, AORN Antonio Cardarelli, Italy

Background: Covid-19 pandemic had a significant impact on Transplant Program (TP), often demanding the disaster plans adjustment in the clinical practice, according to JACIE Standards. We report the disaster plan adopted at AORN Cardarelli TP.

Methods: From June 2019, TP of AORN Cardarelli has started the allogeneic activity. From January 2019 to date, 171 transplant procedures has been carried out (126 auto/45 allo). Since March 2020, no visitors were allowed in our clinical unit and Covid-19 swabs were performed 48 hours before transplant admission and anamnestic questionnaire and capillary serologic test were performed in outpatients. Subsequently, National and EBMT guidelines were followed. Outcomes: 17 cases of Covid-19 infection occurred. Among outpatients, 4 were in post-allogeneic, 1 in post-autologous transplantation follow-up and 11 (5 pts and 6 stem cell donors) were in pre-transplant screening. During hospitalization for allogeneic transplantation, one AML patient experienced Covid-19 pneumonia at day +32. Coronavirus spread among healthcare workers too: 3 physicians, 6 nurses,3 cleaning and 2 health workers ,with shortage of trained staff. Nobody of transplant team need hospitalization, 2 pts (1 NHL/1MM) died for Covid-19 pneumonia at 6 months from allogeneic and autologous HSCT, respectively; the patient who developed Covid-19 pneumonia during hospitalitation, is well and was discharged on d +62.

Discussion: The occurrence of Covid-19 pneumonia in a hospitalized patient with inability of transfer to the Covid unit, led to the disaster plan adjustment including: switching the positive in negative pressure of the HEPA filtered room in which patient was hospitalized; implementation of clean and dirty path for accessing to the patient's room; training of all personnel on the Covid-19 dressing procedure; block of admissions; communication of the emergency to the National and Regional competent authority to transfer patients on the waiting list to other TP; FFP2 mask use for hospitalized patients, during the contact with health care workers; screening for Covid-19 through molecular swab for staff (weekly) and hospitalized patients (twice a week).

Conclusion: The prompt adjustment of the disaster plan allowed no further spread of Covid19 infection among patients and staff. JACIE accreditation system represents a useful tool for the transplant programs allowing the management even of unprecedented clinical condition as Covid19 pandemic.

D165

IS ALLOGENEIC TRANPLANTATION AN OPTION IN PATIENTS AFFECTED BY CONCURRENT MYELOFIBROSIS AND CHRONIC MYELOID LEUKEMIA (CML)?

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Classification of Chronic Myeloproliferative Disease (CMPD) is based on hematologic, histopathologic and molecular characteristics including the presence of the BCR-ABL1 and JAK2 V617F or MPL and CALR. Although the different gene mutations ought to be mutually exclusive, a number of cases with co-occurring BCR-ABL1 and JAK2 V617F or CALR, have been identified with a frequency of 0.2-2.5%in the European population. We describe 4 patients treated in our institution with coexistence of BCR/ABL chronic myeloid leukemia and another Ph− CMPD. The median age was 52 years (41-58), 2 were females. Two patients developed myelofibrosis (MF) first and CML afterwards, and patients the oppositve:the median interval between the 2 diseases was 31 months. Characteristic "driver mutations" detected were Jak2 in 3 patients and Calreticulin (CALR) gene, type1 in 1. Two patients receveid imatinib and 2 nilotinib as treatment for CML obtaining an optimal molecular response according to ELN criteria. Ph−CMPDs were managed with conventional therapy, including hydroxiurea, transfusions and also JAK 2 inhibitors such as ruxolitinib in all patients, with temporary symptom control. A dose reduction of ruxolitinib and imatinib or nilotinib was required in order to manage extra–hematologic and hematologic toxicity. Despite deep control of CML, all of them were eligible for allogenic hemapoietic stem cell transplant (HSCT) as the only curative option in intermediate- or high risk MF. All but one patient showed a normal karyotype before transplant. Three patients received Thiotepa, Busulfan and fludarabine based conditioning regimen and peripheral blood HSCT from a matched unrelated donor. One patient died from multi-organ failure 7 months after HSCT and no evidence of diseases. Two patients are alive in CR after a median of 12 months. The last patient is waiting for transplant, with a high comorbidity score.

This is a rare condition of CMPD, which should be considered : evolution to myelofibrosis prevails over CML, making an allogeneic HSCT a therapeutic option.

D166

DECITABINE PLUS VENETOCLAX IN REFRACTORY ACUTE MYELOID LEUKEMIA AS BRIDGE TO ALLOGENIC STEM CELL TRANSPLANT: A SINGLE ISTITUTION EXPERIENCE

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Acute Myeloid Leukemia (AML) is a hematopoietic stem cell disorder that is characterized by the clonal expansion of myeloid blast and suppression of normal hematopoiesis. In the relapsed or refractory AML (R/R AML) adult patients (pts), the prognosis remains poor and the allogeneic stem cell transplant (alloSCT) is possible for only a minor pro-

portion of patients. We describe a single centre experience of 5 patients with R/R AML treated with Decitabine plus Venetoclax regimen (DEC-VEN) as salvage therapy followed by an alloSCT. Patients were 42, 48, 50, 65 and 68 years old respectively. Four pts had de novo AML and 1 patient s-AML. Complex cariotype was detected in 2 pts while molecular mutations were undetected. Patients showed to be refractory after FLAG-IDA (n=3) and 3+7 regimen (n=2) as previous treatment. Decitabine 20 mg/mq intravenously daily on days 1-5 and oral Venetoclax 400 mg daily were used as a salvage treatment. The median number of cycles was 3 (range 2-5). All pts achieved a complete remission (blast count < 5% in bone marrow, ACN ≥ $10x10^9$/L; platelet count≥ 100 x 109/L) before alloSCT but minimal residual disease (MRD) negativity by flow cytometry was achieved in only one of them. Between February and August 2020, pts subsequently received allogenic transplant: 2 full-matched related donor PBSCT and 3 full-matched unrelated donor (MUD) PBSCT; 3 and 2 patients after a MAC (TBF) and after a RIC regimen (TBF Ric) respectively. GVHD prophylaxis was based on the use of methotrexate and cyclosporine with 2 days of anti-thymocyte globulin in MUD-transplants. The main complications post-transplant were nonESBL-E. Coli sepsis occurred in one patient during aplastic phase, and Cytomegalovirus colitis in another one at day 180 after alloSCT. No patients experienced acute and/or chronic GvHD. All pts achieved successful engraftment and stable full donor chimerism. Nowdays four pts are CR (MRD negative) 1 year (2 pts) and 6 months (2 pts) after transplant, while one patient relapsed at day 90. In conclusion our real-world experience showed the DEC-VEN may be an active and a well tollerated regimen for R/R AML patients. This combination allowed achieving a complete remission before alloSCT, thus showing to be also a favorable treatment option as bridge to alloSCT in R/R AML. AlloSCT was able to eradicate MRD from patients, thus confirming its central role in the treatment of R/R AML. However, further studies with larger numbers of patients are needed to confirm these data.

D167

CELLULE T DEL DONATORE ATTIVATE DA "CHECKPOINT INHIBITORS" PER ERADICARE UN LINFOMA DELLA ZONA GRIGIA DEL MEDIASTINO CHE CAUSAVA INSUFFICIENZA RESPIRATORIA DOPO TRAPIANTO ALLOGENICO

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Grey-Zone Lymphoma (GZL) is a rare lymphoma with histological features that are intermediate between classical Hodgkin's Lymphoma (cHL) and diffuse large B-cell lymphoma (DLBCL). GZL has a poor prognosis and there are no standard treatments. Chemotherapy regimens used for DLBCL, autologous (auto-) and allogeneic (allo-) stem cell transplantations (SCT) are effective therapeutic options. Nivolumab, an anti-PD1 checkpoint inhibitor (CPI) that rescues T cell activity against lymphoma, is approved for cHL. There are only few reports with CPIs for GZL. Also, nivolumab before or after allo-SCT increases the risk of acute graft-versus-host disease (aGvHD), a life-threatening complication (Merryman, Blood 2017). Here we report a peculiar case of a 37 years old female with refractory GZL who was successfully treated with nivolumab after allo-SCT. She had supraclavicular and mediastinal disease at presentation (IIB Ann-Arbor stage).

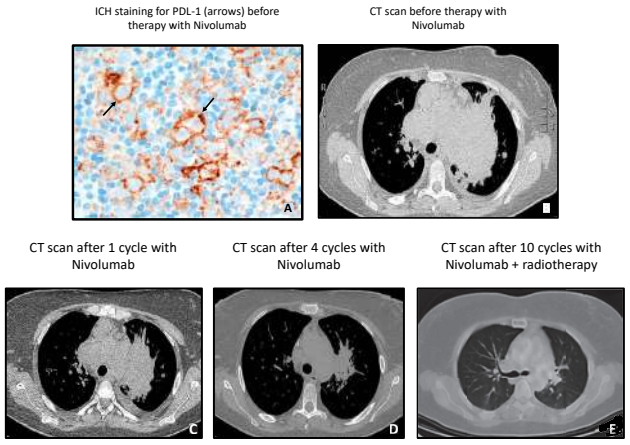

Figure 1.

Biopsy of the supraclavicular node showed GZL infiltration. The disease was refractory to 4 chemotherapy lines including auto-SCT. Upon progression of the disease (mediastinal mass, lung nodes and disease-related symptoms), reduced-intensity conditioning HLA-haploidentical allo-SCT with post-transplant cyclophosphamide was performed. Dyspnea with tirage, hypoxemia, and respiratory failure due to further disease progression (12x7 cm mediastinal mass) occurred 80 days after allo-SCT. Biopsy revision showed PDL-1 expression on 70% of the neoplastic cells (mainly on Hodgkin-like cells, Figure 1A). Thus, post-transplant immune suppression was suspended and nivolumab 3 mg/kg every 21 days was started. Symptoms resolved in less than one week after the first drug infusion and the mediastinal mass gradually disappeared (Figure 1B-E). Radiotherapy (RT; 36 Gy, 15 fractions) was also given to the mediastinum and to the only lung node still active after 6 nivolumab infusions. Nivolumab was used early after transplant and it was followed by a very fast immune recovery (CD4+ T cells 16-195/mmc, CD8+ T cells 83-352/mmc). However, no aGvHD occur. The patient is currently in complete remission and is continuing nivolumab (12 infusions) with no GvHD so far. This case suggests that nivolumab may be a valid and safe option in patients with refractory GZL when delivered early after allo-SCT. Indeed, arming donor T cells with nivolumab after allo-SCT boosted their anti-lymphoma activity with no aGvHD. RT delivered after nivolumab may have increased its efficacy.

D168

IMPACT OF COVID 19 PANDEMIC ON HSCT ACTIVITIES: REPORT FROM A SINGLE CENTER

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Background: The current COVID-19 pandemic, caused by SARS-CoV-2, is responsible of a severe acute respiratory syndrome. This pandemic poses unprecedented stress on the health care system including HSCT. Several international organization such as EBMT, WBMT, CIBMTR, produced guidelines for the management of different aspects of HSCT.

*Aim of the study: T*o assess how COVID-19 pandemic has modified internal management of different steps of HSCT, during pandemic.

Methods: We compared HSCT activity between 2019 and 2020, taking into account the same six months period from March to September.

Results: During pandemic Covid19, our transplant center has modified his procedures and activities according to the EBMT guidelines. Non-

urgent transplants were deferred as much as possible, especially for non-malignant disorders. The decision was made based on individual considerations. All patients were tested for SARS-CoV-2 before start of the conditioning and all donors too before start of donation. We started to cryopreserve all stem cell product before start of conditioning. Comparing HSCT activity between 2019 and 2020, we performed the same numbers of HSCT. In both periods, patients submitted to HSCT were predominantly with acute leukemia, so we respected the urgency criteria. Sibling donors and cord blood unit remained the same, but we increased MUD donors, in particular from European registry and we reduced the haploidentical ones. This change is due to mandatory cryopreservation for all apheresis products. We have avoided to cryopreserve bone marrow products due to the higher risk to drastically reduce CD34+ cell count during the process. For urgent patients with only haploidentical donors, we decide to use PBSC after G-CSF stimulation and so we modified GVHD prophylaxis. We used PTCY on day+3 +5, cyclosporine, tapering dose from day+100 and mycophenolic acid until day+90 post HSCT. So use of bone marrow as stem cell source was drastically reduced. Despite this changes, outcome post transplant were not affected: graft failure, sepsis and acute GVHD did not differ between the two time period. (Table 1). We stopped Car-T infusion after the beginning of lockdown on March 2020, due to logistic difficulties and we started again on September 2020. For the outpatient follow up, we increased telehealth method, using telephone and/or televideo conferences for patients over six months after transplant, without serious complications.

Conclusion: According to the international guidelines, we were able to continue HSCT activities in the order to ensure a lifesaving treatment for patients for whom this procedure cannot be postponed

Table 1. Patients' characteristics.

	2019 34	2020 33
Underlying disease		
Acute leukemia	**22**	**23**
Myelodisplatic syndrome	**3**	**1**
Myeloproliferative neoplasms (MFI)	**4**	**5**
Lymphoproliferative disease	**3**	**4**
Multiple myeloma	**0**	**0**
others	**2**	**0**
Donor type		
Mud	**10**	**14**
Sibling donor	**11**	**12**
Haploidentical donors	**10**	**4**
CBU	**3**	**3**
Stem cell source		
PBSC	**17**	**28**
BM	**14**	**2**
CBU	**3**	**3**
Conditioning regimen		
Myeloablative	**29**	**26**
Reduced intensity regimen	**5**	**7**
Gvhd prophylaxis		
PTCY	**31**	**32**
none	**3**	**1**
Graft failure n°(%)	**2/34 (6%)**	**2/33 (6%)**
Sepsis n°(%)	**18/33 (54%)**	**19/34 (56%)**
Relapse n°(%)	**8/33 (24%)**	**10/34 (29%)**
Overall Survival n°(%)	**27/33 (82%)**	**27/34 (79%)**

D169

A CASE OF RELAPSED/REFRACTORY PRIMARY CUTANEOUS CD8-POSITIVE CYTOTOXIC T CELL LYMPHOMA AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANT

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Primary cutaneous aggressive epidermotropic cytotoxic CD8-positive T-cell lymphoma (CD8+ PCAETL) is a rare T-cell lymphoma characterized by disseminated ulcerative lesions, frequent extracutaneous involvement and poor prognosis. Several retrospective series generally suggest resistance to chemotherapy agents while hematopoietic stem cell transplantation (HSCT) might represent the definitive curative therapy. However, as those patients receive HSCT after several lines of treatment the relapse after HSCT still represents an unmeet challenge. A 56-year old man presented with extensive annular erythematous plaques with ulceration on the trunk, inguinal region, and lower extremities. Skin biopsy showed an epidermotropic CD3+, predominantly CD8+ lymphoid infiltrate with expression of cytotoxic markers (granzyme B, TIA1, perforin, βf1) and expression of CD30. Before HSCT the patient underwent several systemic chemotherapy, without achieving a significant response; finally the patient received MTX and α-INF achieving a partial response. He received a HSCT from HLA-matched unrelated donor after a reduced intensity conditioning (RIC) with cyclophosphamide, fludarabine and low dose total body irradiation. Post-transplantation course was complicated by skin aGVHD (grade II), responsive to prednisone. Three month after HSCT, a new onset of annular plaques with erosive features were positive at the skin biopsy for CD8+ PCAETL, suggesting a relapse with loss of CD30 expression. Skins lesions improved after early tapering of immunosuppression, followed by the combination of chemotherapy with MTX and α-INF and multiple donor lymphocyte infusions (DLI). After a 9-month remission, a second relapse has been documented, in association with moderate chronic GVHD, requiring oral steroids and extracorporeal photopheresis achieving a partial response of cGVHD and the disappearance of lymphoma. At 2-years after the HSCT the patient has neither lymphoma lesions nor skin cGVHD. In this setting HSCT with RIC has shown promising activity thanks to the reduced toxicity and to a potent GVL effect; indeed the hematopoietic graft creates a chimeric marrow with an immunological platform allowing an immune boost with the DLI. When relapse occurs after HSCT, rapid withdrawal of immunosuppressive therapy and DLI could be a valid therapeutic option for recurring disease in CD8+ PCAETL and interestingly in this case the concomitant cGVHD treatment did not adversely influence the lymphoma relapse.

D170

CATASTROPHIC COMPLEMENT-MEDIATED ENDOTELIAL DAMAGE IN A PATIENT WITH NEUTROPHILIC LEUKEMIA RECEIVING ALLOGENEIC TRANSPLANTATION

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A 52-year old man presented with splenomegaly, anemia and a severe neutrophilic leukocytosis. The diagnostic work up concluded for accelerated phase chronic neutrophilic leukemia with mutated CSFR3; he started therapy with Hydroxyurea. He suddenly developed acute renal failure with severe tubular damage, worsening of anemia and thrombocytopenia, without signs of autoimmunity or microangiopathy. The patient was treated with dialysis, steroids and IVHDIG with progressive recovery of renal function and platelet count. However, the chest CT scan showed a "ground glass" micronodules spread bilaterally, confluent, with "patching" distribution.The bronchoalveolar lavage was negative while the lung biopsy showed destruction of the basement membrane of the alveolar capillaries with hemorrhagic and macrophage infarction suggesting hemorrhagic alveolitis; the kidney biopsy showed signs of fibroyaline glomerulosclerosis, deposits of complement fractions, compatible with C3-glomerulopathy. We concluded for a diffuse complement-mediated endothelial damage secondary to neutrophilic leukemia, improving after increase of cytoreduction therapy. The patient

undergo allogeneic transplantation from HLA-matched unrelated donor, with reduced toxicity conditioning including Treosulphane-Fludarabine. Post-transplantation course was complicated by a flare of the previous microangiopathy injury, requiring dialysis plus Eculizumab administration, weekly. After a transient improvement, simultaneously with the neutrophils recovery, we observed a severe capillary leak syndrome associated to PERDS (periengraftment respiratory distress syndrome); despite the intensification of therapy and support, the patient died. The study of complement gene mutations, performed before transplant, did not reveal pathogenetic variants directly associated with aHUS/C3 glomerulopathy or with congenital purpura, but genetic variants have been identified in heterozygosity at the level of the C3 gene (a variant of unknown significance), in homozygous at the level of CFH and of THBD associated with C3-glomerulopathy. It is well known that neutrophils can trigger the inflammatory response of the endothelium; in this case, both the conditioning-induced injury and the concomitant presence of a myeloproliferative disease with abnormal neutrophils proliferation, associated with the genetic variants of the complement, probably triggered a severe endothelial damage with catastrophic evolution.

48° Congress of the Italian Society of Hematology
Milano, Italy, October 24-27, 2021

MAIN PROGRAM

PERIPHERAL T-CELL LYMPHOMA: MOLECULAR FEATURES AND IMPLICATIONS FOR FIRST-LINE TREATMENT STRATEGIES

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Peripheral T-cell lymphoma classification

Peripheral T-cell lymphomas (PTCLs) represent a rare and heterogeneous disease entity, accounting fo only 6% to 10% of all non-Hogkin lymphomas. The 2017 World Health Organization (WHO) classification describes 29 different subtypes of PTCLs[1].

These entities are now defined as Mature T-cell lymphomas (MTCLs), the term "mature" indicating that in these lymphoproliferative disorders, the neoplastic T-cells have undergone T-cell receptor (TCR) rearrangement. MTCLs are currently divided in 4 categories reflecting the predominant disease sites and clinical manifestations: 1) Nodal, 2) Extranodal, 3) Cutaneous and 4) Leukemic MTCLs[2].

The present review will focus on first line treatments of the the most common forms of Nodal and Extranodal MTCLs in adults, i.e. PTCL not otherwise specified (PTCL-NOS), systemic Anaplastic Large Cell Lymphoma (ALCL), Nodal PTCL with T-follicular helper (TFH) phenotype which includes Angioimmunoblastic T-cell lymphoma (AITL), TFH lymphoma and nodal PTCL with TFH phenotype, (the latter 2 categories being previously considered under the PTCL-NOS subtype), extranodal natural-killer T-cell lymphoma nasal-type (ENKTCL nasal-type), Enteropathy associated T-cell lymhoma (EATL), Hepatosplenic T-cell lymphoma (HSTCL), breast implant - associated ALCL (BI-ALCL). It should be noted that PTCL-NOS represents a heterogeneous category constituted by MTCLs which do not meet the criteria for being included in precise disease entities.

However, despite this intrinsic diversity MTCLs have historically been uniformly treated with the same therapeutic approach used for aggressive B-cell non-Hodgkin lymphomas (NHLs) such as Diffuse Large B-cell lymphoma (DLBCL), i.e. antracycline-based chemotherapy. As compared to DLBCL which can be cured in a substantial fraction of cases, the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, prednisone) is now considered largely ineffective in most MTCL subtypes, with the exception of ALCL carrying *NPM1-ALK* and *DUSP22* rearrangements. Despite the lack of significant clinical activity, several efforts have been made in the past to build novel therapeutic strategies based on the ineffective CHOP backbone, trying to derive treatment paradigms and clinical research strategies from the B-cell NHL field. As expected, this strategy has not produced relevant improvements in outcome over the past 20 years. However, recent advances in the understanding of the biology of PTCL have led to the development and approval of novel targeted agents specifically active in MTCL, such as epigenetic modifiers and small molecule inhibitors, which could represent the backbone for more effective therapies and for a chemofree treatment era in the MTCL field.

Biology of MTCL: implications for chemosensitivity and first-line treatment strategies

Given the clinical and histopathologic heterogeneity of MTCLs it is no surprise that recent biologic and genomic studies uncovered precise genomic signatures underlying specific MTCL subtypes, which in some cases translated in a better risk stratification and rationale for targeted therapeutic approaches. Mechanistically, it is well known that antracycline-based chemotherapy induces a p53-mediated response by directly hindering ribosome biogenesis through inhibition of rRNA synthesis. In normal proliferating cells, the level of p53 is maintained low because of the binding with MDM2 with consequent p53 ubiquitination and proteasome degradation. When rRNA synthesis is inhibited, it results in the binding of several ribosomal proteins, no longer used for rRNA binding and ribosome building, to MDM2. This binding relieves the inhibitory activity of MDM2 toward p53[3].

ALCLs carrying *NPM1-ALK* and *DUSP22* rearrangements represent the MTCL subtypes characterized by the most favorable outcome following standard antracycline-based chemotherapy, with cure rates exceeding 80%. Much attention has been given to the pathogenetic role of the pro-survival signaling cascades triggered by the NPM-ALK fusion kinase. It is important to note that NPM-ALK mediated signaling also modulates the activity of the Murine Double minute 2 (MDM2) protein, which in turn targets p53 for proteasome-dependent protein degradation, thus inhibiting p53-mediated responses. According to this model, *TP53* mutations are relatively rare in *ALK* rearranged ALCL. Given these premises it is clear that inhibition of MDM2 activity through rRNA synthesis blockade represents a potential vulnerability for *TP53* wild-type ALCL, which may explain the high efficacy of CHOP chemotherapy in this specific ALCL subset. Similar considerations could apply for *DUSP22* rearranged ALCL, which represent another ALCL subtype characterized by good outcome following antracycline based chemotherapy. One of the main consequences of *DUSP22* rearrangement is DUSP22 protein dowregulation. Since DUSP22 has been reported to negatively regulate mitogen-activated protein kinase (MAPK) kinase signaling, which in turn increases MDM2 phosphorylation and activity, one could hypothesize a similar inhibition of p53-mediated response as a common mechanism underlying lymphomagenesis in *NPM1-ALK* and *DUSP22* rearranged ALCLs. This model of activity provides the rationale for exploring chemofree treatment alternatives to doxorubicin, based on MDM2 and rRNA synthesis inhibitors, in *NPM1-ALK* and *DUSP22* rearranged ALCL. Besides these 2 "outliers", the outcome of MTCL following CHOP chemotherapy is generally poor. The recently described mutational landscape of MTCL provides mechanistic explanation of these unsatisfactory results. Being an intact p53-mediated response a requirement for the efficacy of antracycline-based chemotherapy, it is no surprise that ALCL carrying *TP53* genomic alterations have a dismal outcome. Similarly, *TP63* mutations predict poor prognosis in ALCL, being *TP63* implicated in the apoptotic response to oxidative stress. Notably *NPM1-ALK*, *DUSP22*, and *TP63* alterations are mutually exclusive in ALCL. As described above PTCL-NOS is now considered an heterogeneous disease entity including those MTCL which do not meet the diagnostic criteria for other specific subtypes. PTCL-NOS are characterized by high genetic complexity, and frequent *TP53* genomic alterations. Gene expression profiling

studies identified 2 PTCL-NOS subtypes characterized by overexpression of *TBX21* and *GATA-3* transcription factors, the latter characterized by poor outcome and frequent genomic alterations of the *CDKN2A-TP53* axis and the PTEN-PI3K pathway. Epigenetic dysregulation represents a main unifying feature of MTCL. Mutations of epigenetic genes such as *DNMT3A, TET2, IDH2* are common in AITL, PTCL-NOS and PTCL with TFH phenotype. *SETD2* mutations are prevalent in rare MTCL subtypes characterized by poor outcome such as EATL and HSTCL. Notably *DNMT3A* and *SETD2* are directly involved in anthracycline resistance, which may provide molecular explanation for the poor outcome observed in those MTCL subtypes. In conclusion, these data demostrate that most MTCLs are currently treated with a suboptimal and molecularly flawed treatment approach. On the contrary, the good outcome of *ALK* and *DUSP22* rearranged ALCL with DNA damaging agents and drugs inhibiting ribosome biogenesis seems to have a precise molecular explanation (Figure 1).

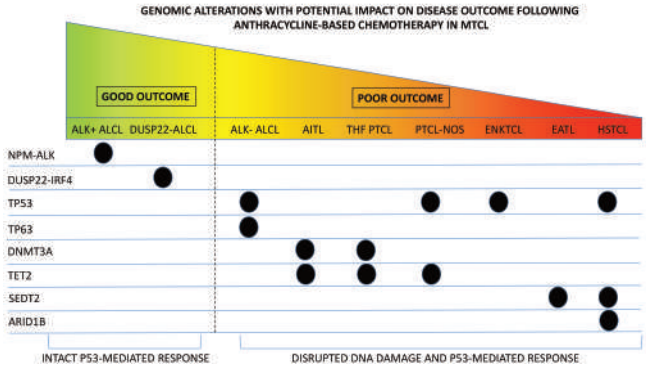

Figure 1. Genomic alterations with potential impact on disease outcome following anthracycline-based chemotherapy in MTCL. *ALK* and *DUSP22*-rearranged ALCL are characterized by good curability following anthracycline-based induction therapy, in the absence of genomic alterations impairing p53-mediated responses. On the contrary ALK-negative ALCL and the remaining MTCL subtypes frequently show genomic alterations involved in disruption of p53-mediated responses and resistance to DNA damage, thus explaining the inferior prognosis compared to *ALK* and *DUSP22*-rearranged ALCL.

Data supporting the inclusion of etoposide in first-line chemotherapy regimens

Current MTCL treatment guidelines recommend the addition of etoposide to standard CHOP (CHOEP regimen) as first line therapy in young (< 60 years od age) fit patients (Figure 2). This recommendation is based on data from a retrospective study on 289 patients with MTCL treated with 6-8 cycles of standard CHOP or CHOP plus etoposide, demostrating an event-free survival (EFS) advantage for patients treated with CHOEP with less 60 years (y) of age with normal lactate dehidrogenase (LDH) levels[4]. It should be noted that in this study the majority of patients had ALCL (n=191). A second retrospective study from the Swedish lymphoma registry demonstrated a similar progression-free survival (PFS) advantage for young (< 60 y) patients tretaed with CHOEP, but no difference in overall survival (OS) (reviwed in 2). These data could be explained at the molecular level by the fact that etoposide activates a p53-mediated response irrespective of ribosome biogenesis inhibition, thus generating an additive effect to standard anthracycline-based chemotherapy on p53 activation at best. Moreover from the clinical point of view, data from retrospective studies comprising heterogeneous MTCL categories are often of difficult interpretation and subjected to many biases. In any case, the addition of etoposide to CHOP did not dramatically change the poor outcome of MTCL; the fact that etoposide still requires an intact p53 function to induce apoptosis in target cells, provides molecular explanation of the suboptimal results obtained with this strategy.

Integration of novel agents in MCTL first-line treatment: the pitfalls of the "CHOP + X" strategy

Several attempts have been made to add novel agents to standard CHOP in order to improve the unsatisfactory results of standard chemotherapy, using a "CHOP + X" strategy mutuated from the B-cell NHL field. However, the idea to use CHOP as a backbone for designing novel combination strategies in MTCL is questionable, given the suboptimal results of this regimen in virtually all MTCL subtypes excluding *ALK* and *DUSP22* rearranged ALCL. In line with this and not suprisingly, most clinical trial investigating the efficacy of novel agents in combination with CHOP (such as romidepsin, belinostat, lenalidomide, pralatrexate, alemtuzumab) failed to show significant advantages over standard CHOP, often with additional toxicities. More recently a phase II trial of first-line oral azacitidine (CC-486) plus CHOP yelded promising results in 21 patients with MTCL (17 had PTCL with TFH phenotype)[5]. A randomized trial comparing oral azacitidine-CHO(E)P with duvelisib-CHO(E)P against CHO(E)P in CD30 negative PTCL is ongoing.

Brentuximab Vedotin plus CHP as first-line treatment for CD30 positive MTCL

So far the only positive randomized study employing the "CHOP + X" strategy, has been the ECHELON-2 study investigating the efficacy of the antibody drug-conjugate Brentuximab Vedotin (BV) in combination with CHP (CHOP without vincristine), in a population of CD30 positive (>10% of CD30+ cells) MCTL constituted predominantly by ALCL (75% of all patients included in the trial), which is ubiquitously and strongly CD30 positive[6]. The results, showing a clear PFS advantage for the CHP+ BV arm, were considered practice-changing only for the ALCL subtype. In fact, given the imbalance in the composition of the patient population (75% ALCL), the study was not powered enough to show the benefit of CHP + BV over CHOP in non-ALCL subtypes. In line with this, while the Food and Drug Administration (FDA) approved BV+CHP as first line therapy for CD30 + MTCL, the European Medicines Agency (EMA) approved this combination only for newly diagnosed ALCL. The results of this study established CHP+BV as the recommended first-line therapy for ALCL (Figure 2).

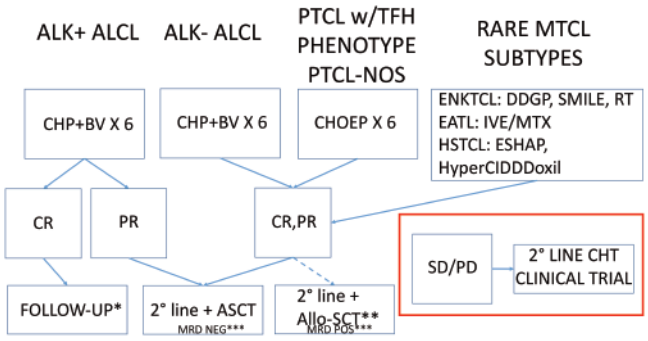

Figure 2. MCTL first-line treatment algorithm. *Follow-up if CR after induction in *ALK*-rearranged ALCL and in limited stage ENKTCL nasal-type; **Consider allo-SCT in first remission or maximal response after induction in case of HSTCL. * Minimal residual disease could be evaluated in leukapheresis products in case of CR before ASCT, in order to assess the suitability of ASCT vs allo-SCT consolidation strategies. ESHAP: Etoposide, cisplatin, high-dose cytarabine, metiprednisolone. IVE/MTX: ifosfamide, etoposide, epirubicin/methotrexate-ASCT. SMILE: dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide. DDGP: dexamethasone, cisplatin, gemcitabine, pegasparaginase. HyperCIDDDoxil: fractionated cyclophosphamide, liposomal doxorubicin, vincristine, and dexamethasone, alternating with methotrexate and high-dose cytarabine. MRD: minimal residual disease.**

Data supporting first-line intensification strategies: autologous and allogeneic stem cell transplantation

International guidelines recommend front-line intensification with autologous stem cell transplantation (ASCT) in MTCL responding to first-line induction, excluding *ALK*-rearranged ALCL (Figure 2). It is important to note that this recommendation comes from the interpretation of limited data from non-randomized studies composed of very heterogeneous patients populations. One of the largest prospective studies on ASCT consolidation in MTCL was published by D'Amore and coworkers in 2012[7]. The study included 160 MTCL patients excluding *ALK*-rearranged ALCL. With a 5-year OS of 51%, the results of this study supported the use of ASCT as first-line consolidation for MTCL patients responding to first-line induction chemotherapy. In this study the relative majority of patients had PTCL-NOS, however compared to all other MTCL subtypes a relative OS advantage was observed only for ALK-negative ALCL. The role of first-line intensification with ASCT in ALCL patients after CHP+BV is unclear, although available data seem to show a PFS advantage for ASCT consolidation. Despite its widespread use, the role of first-line ASCT consolidation in the MTCL is still debated. A recent study by Park and coworkers[8], showed that whether ASCT in first complete remission (CR) could be of value in high-risk MTCL patients considered as a whole, it did not provide significant PFS and OS advantages in specific MTCL subsets such as PTCL-NOS and ALK-negative ALCL. As opposite, ASCT intensification produced a clear OS benefit in the AITL subset.

Following the evidence of graft versus lymphoma effect in MTCL[9], the role of first-line allogeneic stem transplant (allo-SCT) consolidation has been evaluated in retrospective and prospective studies. A recent phase 3 randomized study from Schmitz and coworkers investigated the role of allo-SCT consolidation in first CR in PTCL[10]. While confirming a strong graft versus lymphoma (GvL) effect and a lower relapse rate, this study did not demonstrate a significant OS advantage for allo-SCT, due to increased transplant related mortality (TRM) compared to ASCT. However, the interpretation of this study is affected by the very limited available tools to evaluate the benefit-risk ratio in this particular patient's population. In this light, strategies based on minimal residual disease (MRD) detection in leukapheresis products could be of value in evaluating the suitability of ASCT consolidation. The risk of allo-SCT could be taken preferentially in MRD positive patients, who otherwise would be at very high risk of relapse following ASCT. Clinically applicable biomarkers for predicting allo-SCT outcome and thus for selecting those MTCL patients who would derive maximal benefit from allo-SCT are warranted. In any case first line intensification strategies are applicable only in the minority of patients responding to first line chemotherapy and a substantial fraction of patients does not achieve CR with regimens based on the CHOP-backbone. Thus, refractoriness to first line treatment remains the key unsolved problem in MTCL therapy.

Rare MTCL subtypes: current clinical management

Extranodal NK-T cell lymphoma nasal-type

Extranodal NK T-cell lymphoma (ENKTCL) nasal-type represents 1-2% of all NHL and has a very poor outcome with standard anthracycline-based chemotherapy. ENKTCL nasal-type is more frequenti in Asia and South America and more than two thirds of patients with ENKTCL have stage I or II disease localized in the upper aerodigestive tract. Two thirds of cases present with localized disease to the upper aerodigestive tract, tipically with nose lesions. This MTCL subtype is characterized by an intrinsic chemoresistance due to frequent overexpression of P-glycoprotein genes and frequent dysruption of the p53 pathway (*TP53* mutations/deletions). There is global hypermethylation in ENKTCL, leading to epigenetic inactivation of key genes regulating chemosensitivity such as *TP53*, *BIM*, *DDX3X*. The frequent inactivation of the asparaginase synthetase gene *ASNS* explains the sensitivity of this particular subtype to L-asparaginase therapy. In fact the combination of radiotherapy plus L-asparaginase containing regimens such as SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide) or more recently DDGP (dexamethasone, cisplatin, gemcitabine, pegasparaginase) represents the standard of care for localized-stage disease. In particular, the DDGP regimen seems to be more effective and less toxic compared to the SMILE regimen (Figure 2).

Intestinal T-cell lymphoma

These lymphoma subtypes are derived from intraepitheliod lymphocytes and express the mucosal homing receptor CD103. There are 3 types of intestinal T-cell lymphoma: Enteropathy associated t-cell lymphoma (EATL), monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) and indolent T-cell lymphoma. EATL and MEITL are considered aggressive MTCL subtypes, the first being associated with gluten sensitivity. Due to the extremely poor prognosis with CHOP-based chemotherapy, alternative regimens are currently employed. The Newcastle Regimen (ifosfamide, etoposide, epirubicin/methotrexate-ASCT) provides best results with a 5-year PFS of 52% and OS of 60%. The relatively high rate of SETD2 mutations and 1q gains (harboring the anti-apoptotic MCL-1 gene) provides some mechanistic explanation of the intrinsic chemoresistance observed in EATL.

Hepatosplenic T-cell lymphoma (HSTCL)

This rare MTCL subtype constituted by gamma/delta T cells, affects predominantly young males with a median age of 35 years. There is an association with autoimmune disorders and chronic immune suppression. Genomic alterations of *SETD2*, *INO80* and *ARID1B* are frequently observed. Besides *SETD2*, whose contribution to doxorubicin resistance is well established, *ARID1B* and *INO80* are a key components of the nucleosome remodelling complexes and are directly implicated in doxorubicin resistance.

Due to the dismal outcome with reported 5-year OS rates < 10% , when feasible intensive chemotherapy regimens such as ESHAP (Etoposide, cisplatin, high-dose cytarabine, metiprednisolone) or HyperCID-DDoxil (fractionated cyclophosphamide, liposomal doxorubicin, vincristine, and dexamethasone, alternating with methotrexate and high-dose cytarabine) has been employed. First-line consolidation with allogeneic stem cell transplant should be attempted as early as possibile in this difficult-to-treat MTCL subtype.

Breast implant associated ALCL (BI-ALCL)

BI-ALCL was first described in late 1990s and is associated with specific types of textured breast implants. The overall prognosis is extremely good with 5-year OS approaching 100%. This is the only lymphoma subtype where surgery, with implant removal and capsulectomy, is usually curative. Systemic therapies have no role in the treatment of localized BI-ALCL, however BI-ALCL cases with evidence of systemic spreading are usually treated with the same regimens employed for systemic ALCL. Dysruption of epigenetic modifiers with genomic alterations of *KMT2C, KMT2D, CHD2* and alterations of the JAK/STAT signaling pathway are the main pathogenetic features of BI-ALCL. Notably epigenetic alterations involved in chemoresistance such as *DNMT3A* and *SETD2*, commonly observed in poor prognosis TFH and extranodal MTCL subtypes (primarely EATL and HSTCL) are uncommon in BI-ALCL.

MTCL therapy: future directions and conclusions

Recent advances in the definition of the molecular mechanisms of action of anthracycline-based regimens could provide the rationale for the use of specific rRNA synthesis and MDM2 inhibitors as potential chemofree alternatives to doxorubicin in *NPM1-ALK* and *DUSP22* rearranged ALCL. However, since anthracycline-based chemotherapy is effective only in these 2 ALCL subtypes, alternative molecularly-driven therapeutic options are strongly needed for all the remaining MTCL subsets. Epigenetic dysregulation is one of the main recurrent molecular alterations in many MTCL subtypes, especially in the nodal TCL with TFH

phenotype subgroup comprising AITL, TFH lymphoma and PTCL with TFH phenotype, and in PTCL-NOS, which provides the rationale for the use of HDAC inhibitors (HDACi) and hypomethylating agents (HMAs). In line with these findings HDACi plus HMA based combinations (Romidepsin + Azacitidine) demonstrated efficacy in MTCL, with CR rates exceeding 50%[2]. The fact that this HDACi+HMAs-based combo was found to be more active in MTCL than B-cell NHL, may indicate a rather specific activity in MTCL further corroborating the underlying molecular rationale. Additional recurrent alterations amenable for targeted therapeutic interventions include *RHOA* mutations in AITL and PTCL-NOS and genomic alterations of the JAK/STAT pathway found in EATL and HSTCL. In this light, the HDACi+HMA combo could be a promising novel therapeutic backbone in MTCL, which could be further optimized with the addition of different targeted agents including PI3Ki, JAKi and immunotherapy-based treatments such as immune-checkpoints inhibitors. Clinical trials with doublets and triplets combinations are currently underway.

New insights in the molecular pathogenesis of MTCL provided the rationale for novel tailored therapeutic approaches for MTCL, and led to the FDA approval of 5 novel drugs in the last decade: Pralatrexate, the histone deacetylase (HDAC) inhibitors Romidepsin and Belinostat and the antibody-drug conjugate Brentuximab Vedotin.

Despite these advances, the antibody drug conjugate BV and L-asparaginase remain at the time being the only targeted agents succesfully incorporated into first-line MTCL regimens available in real-life clinical practice. In conclusion, the rarity and extreme heterogeneity of MTCL represented major obstacles for successful drug development in the field. Results from prospective and retrospective studies (often prone to interpretation biases) generated treatment controversies regarding first-line intensification strategies and management of responding patients. However, refractoriness to standard first line induction chemotherapy remains a major challenge in everyday clinical practice and novel effective regimens are urgently needed for the majority of non-ALCL subtypes.

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HEMOPOIETIC CELL TRANSPLANTATION FOR THALASSEMIA IN YEAR 2021

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The hallmark of beta thalassemia is the imbalance in the alpha/beta globin chains production that results in variable grades of ineffective erythropoiesis for apoptosis of late-stage erythroid precursors. It follows chronic hemolytic anemia, compensatory hemopoietic expansion, hypercoagulability and increased iron absorption.

Hemopoietic cell transplantation (HCT) has proved, since almost 40 years, to be able to correct the clinical disease. The rationale for HCT in thalassemia is to replace ineffective endogenous erythropoiesis and to correct the phenotypic expression of the disease, sparing patients from lifelong transfusion treatment and long-term complications.[1] HCT is, so far, the only consolidated approach with a curative potential in this disease.

Transplantation in TDT has been executed in thousands of patients and is today implemented worldwide, with excellent results.[2–5] The latest report from the European bone marrow registry (EBMT) registry showed global overall survival (OS) and event free survival (EFS) of 88% and 81%, with best results obtained in patients £ 14 years of age (OS and EFS of 90% and 83%, respectively).[6] Current recommendations identify young TDT patients, before development of iron-related organ damage, as the ideal candidates for HCT;[7] adults can also be offered this strategy in the setting of dedicated programs, provided that they have been well-chelated since infancy. According to the afore-mentioned EBMT registry data, better outcomes are still deriving from transplants with HLA-matched siblings, with a stunning 91% of OS and 83% of EFS. More recently outstanding rsults have been published by Chinese[8] and Indian[9] studies demonstrating the wide diffusion and affordability of transplantation even in low income countries.

In the era of high resolution HLA typing, transplantation from matched unrelated donors in TDT is considered feasible and effective providing a full compatibility (i.e. 10/10 matched) between patient and unrelated donor.

Transplantation from alternative donors has resulted in conflicting results so far. Primary graft failure appears as the major complication of unrelated cord blood unit transplants while the need to abate the risk of GVHD has driven the search for effective modalities of ex-vivo or in-vivo T-cell depletion with grafts from haploidentical donors. Recent reports of alternative donors HCT are summarized in table 1. Of relevance the recent approach by Anurathapan[10] with a deeply immunosuppressive regimen in haploidentical transplantation. In this experience, that promise to extend the transplant option to almost all patients, an outstanding, 96% OS and EFS have been reported.

Last 50 years have witnessed dramatic improvements in thalassemia understanding and patients' care. These improvements have built a series of previously unimaginable therapeutic opportunities for patients with thalassemia and many others are coming. All this was made possible by a synergy between the various fields of biological and clinical research that have mutually reinforced each other leading to a common success.

Having access to many therapeutic opportunities is undoubtedly a benefit for our patients but it also leads to a problem of choice. As opportunities have grown, the costs of optimal therapies have increased dramatically and so has the demand for a better selection of the appropriate sequence of treatments in terms of best cost / benefit ratio.

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Table 1. Reports on HCT from alternative donors in transfusion dependent thalassemia patients. Abbreviations=OS Overall Survival ; TFS Thalassemia-free Survival ; TRM Transplant-related Mortality ; BU Busulfan ; TT Thiotepa ; Cy Ciclophosphamide ; Flu Fludarabine ; CsA Cyclosporin A ; MTX Methotrexate ; MAC myeloablative conditioning ; MUD matched unrelated donor ; MMUD mismatched unrelated donor ; UCB umbilical cord blood ; GF Graft Failure; GVHD Graft versus Host Disease ; MP Methylprednisolone ; MMF Mycophenolate Mofetil ; Hu Hydroxyurea ; Az azathioprine ; ATG Anti-thymocyte Globulin ; Dxm Dexamethasone ; Vel Velcade ; Rit Rituximab ; PT-Cy post transplant Cyclophosphamide NR Not reported.

Reference	Patients	Donor	Manipulation	Conditioning	GVHD prophylaxis	OS	TFS	GF	Notes
Li et al (2019)	355	MUD /MMUD	/	MAC Bu based	Various	87% MUD 78% MMUD	82% MUD 78% MMUD	6% MUD 11% MMUD	aGVHD21% cGVHD 13%
Fleischauer et al. (2006)	72	MM /MMUD	/	Bu Cy Bu Cy TT Bu Flu TT	CsA+MTX ATG+CsA+MTX. (25%)	NR	76% HLA DPB1 matched or permissive 57% HLA DPB1 non permissive	10%	aGVHD 27%
Huang et al. (2018)	50	MUD/MMUD	/	Bu Cy Flu	ATG+CsA+MTX	94%	92%	0%	Median age 4.6 years (range 2-12)
Ruggeri et al. (2011)	35/51	Unrelated UCB	/	MAC 30/35	CsA-based 27/35	65%	21%	52%	
Jaing et al. (2012)	35	Unrelated UCB	/	BU-CY	ATG + CsA+MP	88%	74%	12%	
Gaziev et al. (2018)	40	Haploidentical	CD3+/CD19+ depletion	BU-TT-CY (preceded by Flu)	ATG + CsA+MP	78%	39%	45%	
Gaziev et al. (2018)	11/14	Haploidentical	αβ/CD19+ depletion	BU-TT-CY (preceded by HuAzFlu)	ATG + CsA+MP/MMF	84%	69%	14%	
Anurathapan et al. (2020)	83	Haploidentical	/	ATG-BU-Flu (preceded by DxmFluVelRit)	PT-Cy +tacrolimus/sirolimus	96%	96%	NR	52 pts treated with the full pretransplant immunosuppression protocol engrafted uneventfully

DRUGS TARGETING INEFFECTIVE AND STRESS ERYTHROPOIESIS

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Beta-thalassemia is an inherited monogenic disorder characterized by chronic anaemia caused by the reduced or absent production of functional haemoglobin (Hb). A broad spectrum of phenotype is observed in this condition, mainly defined by the degree of anaemia: transfusion-dependent-thalassemia (TDT) is characterized by a lifelong requirement for blood transfusions, while non-transfusion-dependent-thalassemia (NTDT) may require limited transfusions for a restricted period.

The TDT patients (beta-thalassemia major and severe forms of HbE/beta-thalassemia) are those who commonly present in early childhood with severe anemia and require lifelong transfusion therapy for survival. Although the introduction of transfusions improved survival in TDT patients, it did not come without its own side-effect, systemic iron overload leading to end-organ damage and increased mortality from cardiac or hepatic disease. Advances in iron chelation therapy and the introduction of MRI techniques to detect organ-specific iron overload have led to improved management and patient outcomes. Still, TDT comes with considerable burden to the patient, clinician, and overall healthcare system owing to persistent morbidity and high healthcare utilization, poor access to optimal care and high treatment cost especially in resource-limited countries, and several unmet needs in terms of efficacy, safety and adherence to conventional therapies.

Patients with NTDT (beta-thalassemia intermedia and mild–moderate forms of HbE/beta-thalassemia) usually present later in childhood or even in adolescence with mild–moderate anemia that does not require immediate placement on a regular transfusion program. Progress made over the past few decades has indicated that the diagnosis of NTDT carries greater morbidity than previously recognized. Ineffective erythropoiesis and anemia have been linked to an array of morbidities stemming from chronic hypoxia and an established hypercoagulable state. There are currently no approved agents for the management of anemia in NTDT. Transfusions are used in settings of expected drop in Hb such as pregnancy, infection or surgery; and some physicians also elect to use short courses of regular transfusions to promote growth in childhood or prevent/treat morbidity in adulthood in view of evidence of benefit from observational studies. Even in the absence of transfusions, NTDT patients remain at risk of iron overload secondary to ineffective erythropoiesis, low hepcidin levels, and increased intestinal iron absorption.

In the past decade several promising targets and associated therapeutic options have emerged for patients with thalassemia, though primarily for those with beta-thalassemia, which is reasonable considering the patient's more complex and advanced management needs. These therapeutic options can be classified into three major categories on the basis of their attempts to address different aspects of the underlying pathophysiology of thalassemia: fetal hemoglobin inducing agents, addressing ineffective erythropoiesis, and improving how the body handles iron (Tables 1 and 2). Here, we will shortly discuss some of the emerging approaches in each of these areas.

Table 1. Key completed or ongoing clinical trials of novel therapies in β-thalassemia targeting ineffective erythropoiesis and red blood cell pathology.

Agent	Clinical trials	Design	N, population, age	Key efficacy measures
• Luspatercept (ACE-536)	•NCT01749540 • Completed	• Phase 2 • Open-label	• *n* = 64 • TDT, NTDT with Hb <10 g/dl • ≥18 years	• TDT: Transfusion reduction (≥20%) • NTDT: Hb increase ≥1.5 g/dl[g], Hb • Biomarkers of erythropoiesis, hemolysis, iron metabolism, bone metabolism
• Luspatercept (ACE-536)	•NCT02268409 • Completed	• Phase 2 extension	• n = 51 • TDT, NTDT included in phase 2	• TDT: Transfusion reduction (any, ≥20%, ≥50%), Hb • NTDT: Hb increase ≥1.5 g/dl, Hb • Reticulocytes, EPO, nRBC, sTfR, SF, TIBC, TSAT, NTBI • HR-QoL
• Luspatercept (ACE-536)	• BELIEVE • NCT02604433 • Active, not recruiting	• Phase 3 • Randomized, placebo-controlled, double-blind	• *n* = 336 • TDT • ≥18 years	• Transfusion reduction (≥33%[d], ≥50%) • Transfusion requirement • Transfusion independence • SF, LIC, MIC, ICT use • BMD • HR-QoL, healthcare resource utilization
• Luspatercept (ACE-536)	• NCT04143724 • Not yet recruiting	• Phase 2 • Open-label	• *n* = 46 • TDT • 6 years-18 years	• Transfusion reduction • Hb
• Luspatercept (ACE-536)	• BEYOND • NCT03342404 • Active, not recruiting	• Phase 2 • Randomized, placebo-controlled, double-blind	• *n* = 145 • NTDT with Hb ≤10 g/dl • ≥18 years	• Hb increase (any, ≥1 g/dl, ≥1.5 g/dl) • Transfusion requirement • PRO, HR-QoL, 6MWT • SF, LIC, ICT use
Sotatercept (ACE-011)	• NCT01571635 • Active, not recruiting	• Phase 2 • Open-label	• *n* = 46 •TDT, NTDT • ≥18 years	• Transfusion reduction (any, ≥20%)
Mitapivat (AG-348)	• NCT03692052 • Active, not recruiting	• Phase 2 • Open-label	• *n* = 20 • NTDT (including α-thalassemia) with Hb ≤10 g/dl • ≥18 years	• Hb increase ≥1 g/dl • Hb, Reticulocytes, bilirubin, LDH, haptoglobin • EPO, nRBC, sTfR
Mitapivat (AG-348)	ENERGIZE-T • NCT04770779 • Not yet recruiting	• Phase 3 • Randomized, placebo-controlled, double-blind	• *n* = 240 • TDT (including α-thalassemia) • ≥18 years	• Transfusion reduction (≥50%, ≥33%) • Transfusion independence • Transfusion requirement • SF, TSAT, TIBC
Mitapivat (AG-348)	• ENERGIZE • NCT04770753 • Not yet recruiting	• Phase 3 • Randomized, placebo-controlled, double-blind	• *n* = 171 • NTDT (including α-thalassemia) with Hb ≤10 g/dl • ≥18 years	• Hb increase ≥1 g/dl • PRO • Hb increase ≥1.5 g/dl • Reticulocytes, bilirubin, LDH, haptoglobin, EPO, • SF, TSAT
Ruxolitinib (INCB018424)	• NCT02049450 • Completed[c]	• Phase 2 • Open-label	• *n* = 30 • TDT with spleen enlargement • ≥18 years	• Transfusion requirement[d] • Spleen volume, length • Hb

Abbreviations: BMD, bone mineral density; EPO, erythropoietin;; Hb, hemoglobin; HR-QoL, health-related quality of life; ICT, iron chelation therapy; LDH, lactate dehydrogenase; LIC, liver iron concentration; MIC, myocardial iron concentration; nRBC, nucleated RBC; NTDT, non-transfusion-dependent β-thalassemia; NTBI, non-transferrin-bound iron; PRO, patient-reported outcomes; RBC, red blood cells; SF, serum ferritin; sTfR, soluble transferrin receptor; TDT, transfusion-dependent β-thalassemia; TIBC, total iron binding capacity; TSAT, transferrin saturation; 6MWT, 6-minute walk test.

Fetal hemoglobin inducing agents

There has also been a considerable effort to stimulate gamma-globin and HbF production through various pharmacological agents. In general, data in beta-thalassemia were never as encouraging as in sickle cell disease. Several agents have been evaluated mostly off-label, as monotherapy or in combination, including DNA-methylation inhibitors, cytotoxic agents, short-chain fatty acids, erythropoietic-stimulating agents, and immunomodulatory imide drugs.

Hydroxyurea (also known as hydroxycarbamide) is the first drug approved for treating sickle cell anaemia. Evidence suggested that hydroxyurea exerts a dose-dependent, bimodal effect on erythropoiesis by downregulating the expression of GATA1 and upregulating GATA2, and favours the Hb balance towards HbF by delaying RBC maturation and stimulating-globin expression. Moreover, the main globin gene repressor BCL11A is inhibited by hydroxyurea, and this promotes the reactivation of globin and induction of HbF synthesis. Hydroxyurea use has been associated with durable hematologic responses in both TDT and NTDT patients, but this was mostly observed in patients from India or Iran, especially those with homozygosity for the *Xmn*I polymorphism. Studies from Italy have conversely shown limited durability of response. A positive effect of hydroxyurea in reducing the risk of leg ulcers, pulmonary hypertension, and osteoporosis emerged from a study in a large cohort of NTDT patients. Furthermore, several case reports showed efficacy of hydroxyurea in treating masses of extramedullary haematopoiesis. However, robust and consistent data are still missing, and the usefulness of the compound in this pathology is still debated.

Thalidomide is commonly known for its immunomodulating and antiangiogenic activity. Thalidomide has also been associated with hematologic responses in both NTDT and TDT patients in observational studies and small trials from India or China. Polymorphisms in *HBG2* and *HBS1L-MYB* contributed significantly to thalidomide response in these patients.

However, these studies involved a very limited number of patients, and high variability was observed in baseline characteristics of the study populations, including extreme conditions such as baseline Hb 4.0 g/dL in 4 out of 25 patients. Larger clinical trials with thalidomide in TDT patients are ongoing in Pakistan (NCT03651102) and China.

A recent study investigated the association of thalidomide and hydroxyurea in TDT patients and showed that almost half of them maintained about Hb 9 g/dL without any transfusion for 6 months consecutively. A high rate of adverse events was reported, including sedation and liver disease. IMR-687 is a highly selective and potent small-molecule inhibitor of phosphodiesterase (PDE) 9. Although the precise mechanism needs to be fully clarified, the blockage of PDE9 acts to increase cGMP levels, which is associated with the reactivation of HbF. The effect of this compound was firstly proved in sickle-cell disease, resulting in a significant increase in HbF in phase 1 and early stage of phase 2. A phase 2, randomized, double-blind, placebo-controlled study is currently underway to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of IMR-687 administered once daily for 36 weeks in two populations of 120 adult subjects with beta-thalassemia (NCT04411082). The primary objective of this study is to assess the safety and tolerability of IMR-687 in adult subjects with TDT and NTDT. Secondary objectives in the TDT patients include reduction in transfusion burden, iron load rate, iron chelation dose requirements, and serum ferritin levels. Secondary objectives in NTDT patients include increase in Hb and HbF levels and in the absence of a transfusion.

Benserazide is a small compound that has been recently added to the candidate drugs able to induce HbF expression. It was originally approved in its racemic form for the treatment of Parkinson's disease to enhance plasma levels of L-dopa. A phase 1b sequential, open-label, dose-ranging study is currently evaluating the safety, pharmacokinetics, and preliminary activity of benserazide in 36 adult patients with NTDT and a baseline Hb of 6–10 g/dl (NCT04432623).

Targeting ineffective erythropoiesis and red blood cell pathology

Erythroid maturation agents

Luspatercept (formerly ACE-536) is the first disease-modifying drug for beta-thalassemia, currently approved by the US Food and Drug Administration (FDA) in 2019 and the European Medicines Agency (EMA) in 2020 for TDT patients (REBLOZYL, Celgene Corporation). It is a recombinant fusion protein with an adjusted extracellular domain of the activin receptor type IIB (ActRIIB) linked to the Fc domain of human IgG1. Together, the domain binds to select transforming growth factor (TGF) beta superfamily ligands, block SMAD2/3 signaling. The reduction in aberrant Smad2/3 intracellular signaling removes the erythropoiesis inhibition on promoting late-stage red blood cell precursor differentiation and maturation. A multicenter, open-label, dose-ranging phase 2 study of luspatercept in 64 adults with beta-thalassemia (NCT01749540, with 5-year extension NCT02268409) confirmed its safety and effectiveness in reducing transfusion requirement in TDT and improving Hb level in NTDT. On the basis of encouraging data in this

Table 2. Key completed or ongoing clinical trials of novel therapies in β-thalassemia targeting iron dysregulation.

Agent	Clinical trials	Design	N, population, age	Key efficacy measures
LJPC-401	• NCT03381833 • Unknown [prematurely terminated]	• Phase 2 • Randomized, open-label	• *n* = 100 • TDT with high TSAT and MIC • ≥18 years	• MIC • TSAT • Hematology, Chemistry, Endocrine labs
PTG-300	• TRANSCEND • NCT03802201 • Completed	• Phase 2 • Open-label	• *n* = 63 • TDT, NTDT with Hb <10 g/dl • 12–65 years	• NTDT: Hb •TDT: Transfusion reduction
TMPRSS6-LRx (ASO)	• NCT04059406 • Recruiting	• Phase 2 • Randomized, open-label	• *n* = 36 • NTDT with Hb 6–10 g/dl and LIC 3–20 mg/g • 18–65 years	• Hb increase (≥1 g/dl, ≥1.5 g/dl) • LIC decrease ≥1 mg/g
SLN124 (siRNA)	• NCT04718844 • Recruiting	• Phase 1 • Randomized, placebo controlled, single-blind	• *n* = 112 • NTDT (including α thalassemia) or very low/low-risk MDS with Hb 5–11 g/dl and (SF >250 ng/ml or LIC >3 mg/g or TSAT >40%) • ≥18 years	• TSAT, hepcidin • Hb
VIT-2763	• VITHAL • NCT04364269 • Recruiting	• Phase 2 • Randomized, placebo-controlled, double-blind	• *n* = 36 • NTDT with Hb ≤11 g/dl • 12–65 years	• Hb • SF, serum transferrin, TSAT

Abbreviations: ASO, anti-sense oligonucleotides; Hb, hemoglobin; LIC, liver iron concentration; MDS, myelodysplastic syndromes; MIC, myocardial iron concentration; NTDT, non-transfusion-dependent β-thalassemia; SF, serum ferritin; siRNA, small interfering ribonucleic acid; TDT, transfusion-dependent β-thalassemia; TMPRSS, transmembrane serine protease; TSAT, transferrin saturation.

phase 2 study, a recent phase 3, double-blind trial (BELIEVE, NCT02604433) involving adults with transfusion-dependent beta-thalassemia who were randomly assigned to receive subcutaneous luspatercept at a dose of 1.00 to 1.25 mg per kilogram of body weight (224 patients) or placebo (112 patients) every 3 weeks showed that luspatercept reduced the transfusion burden by at least 33% (in 21.4% of the luspatercept group vs. 4.5% of the placebo group) over a fixed 12-week period. Secondary endpoints of a ≥33% reduction in transfusion burden versus baseline from weeks 37–48 and over any 12-week or 24-week rolling periods also favored treatment with luspatercept over placebo. Adverse events, consisting of transient bone pain, arthralgia, dizziness, hypertension, and hyperuricemia, were more common with luspatercept than with placebo. Higher rates of thrombosis were noted in the luspatercept-treated patients. Although these thrombotic events occurred mainly in patients with known risk factors, monitoring patients for signs and symptoms of thrombotic events is recommended. A 5-year open-label extension phase of the BELIEVE trial is under way to provide long-term data on the safety of luspatercept and its effects on transfusion burden and iron overload outcomes. Initial data show that patients on luspatercept continue to experience reductions in transfusion burden and events over 2 years of therapy. A higher proportion of luspatercept-treated patients also shifted to lower serum ferritin, LIC, and myocardial iron levels during the first 48 weeks, with long-term luspatercept treatment leading to an increased proportion of patients with serum ferritin levels < 1000 ng/ml and decreasing trends of overall iron chelation use. Luspatercept could also have a place in the treatment of NTDT patients. The BEYOND trial (NCT03342404) is a phase 2 study to define the effectiveness and safety of luspatercept in 145 adults with NTDT. The primary objective is the increase of mean Hb without any transfusions over a 12-week period, from week 13 to 24, compared to the initial phase. The study has been completed and the results are expected. Luspatercept is now also being evaluated in pediatric TDT patients between the ages of 6 years and 18 years (NCT04143724).

Pyruvate kinase activators

The enzyme pyruvate kinase (PK) has recently become of interest in thalassemia. Preclinical studies on PK-deficient mice have indicated that the metabolic disturbance in PK deficiency alters not only the survival of RBCs but also the maturation of erythroid progenitors, resulting in ineffective erythropoiesis. Mitapivat (AG-348) is an oral, small-molecule allosteric activator of RBC pyruvate kinase (PKR), a pivotal enzyme to regulate ATP production via glycolysis. In a phase 2 study on patients with pyruvate kinase deficiency, mitapivat administration resulted in a sustained Hb increase In mouse models of beta-thalassemia, mitapivat increased ATP levels, reduced markers of ineffective erythropoiesis, and improved anemia, RBC survival, and indexes of iron overload. An ongoing phase 2, open-label, multicenter study (NCT03692052) is evaluating mitapivat in 20 NTDT (including alpha-thalassemia) adults with a Hb level ≤ 10 g/dl, and assessing safety and efficacy in achieving Hb increase ≥ 1.0 g/dl and changes in markers of hemolysis and ineffective erythropoiesis. Mitapivat showed a significant effect on Hb level and improved markers of haemolysis and ineffective erythropoiesis in almost all patients, suggesting a promising role in the treatment of the late phase of ineffective erythropoiesis. Among the reported adverse events, some could negatively affect the overall burden of the disease on thalassemia patients, such as osteoporosis or hormonal alterations. Particular caution will be necessary for addressing their relevance and causal relation to the study drug during later trials. Two phase-3 studies evaluating the efficacy and safety of mitapivat in patients with - or -TDT (ENERGIZE-T, NCT04770779) and NTDT (ENERGIZE, NCT04770753) have been recently started, but they are not yet recruiting patients.

Janus kinase 2 inhibitors

Janus kinase 2 (JAK2) is another signaling molecule that regulates proliferation, differentiation, and survival of erythroid progenitors in response to erythropoietin. Several studies have provided evidence on the role of JAK2 as a potential target to treat disorders of ineffective erythropoiesis.

Studies in mouse models of beta-thalassemia major and intermedia indicated that a short treatment with a JAK2 inhibitor can ameliorate ineffective erythropoiesis and decrease spleen size. A single-arm, phase 2A study to evaluate the efficacy and safety of the JAK2 inhibitor ruxolitinib (INCB018424; INC424) administered orally at a starting dose of 10 mg twice daily among 30 adults with TDT and splenomegaly has been conducted (NCT02049450). A decrease in spleen size from baseline was observed in ruxolitinib-treated patients. No clinically significant improvements in pre-transfusion Hb were seen, thus there was no related reduction in transfusion needs. For these reasons, the study did not proceed into phase 3.

Targeting iron dysregulation

Improving iron dysregulation could represent an effective therapeutic strategy to control ineffective erythropoiesis of thalassemia. Several molecules were proved able to restrict iron availability to the erythron and improving RBC survival in preclinical studies and a few of them are currently under clinical trial.

Hepcidin mimetics

In beta-thalassemia, ineffective erythropoiesis and hypoxia lead to decreased production of the hepatic hormone hepcidin which in turn results in increased intestinal iron absorption and its release from macrophages in the reticuloendothelial system, contributing to a state of iron overload with preferential hepatic iron storage. Erythroferrone, a hormone secreted by erythroblasts as a consequence of EPOR/JAK2/STAT5 pathway activation, has been identified as the main erythroid regulator of this process although other factors have also been proposed. Recently, pre-clinical studies have suggested that synthetic long-acting hepcidin analogues (often called minihepcidins) in combination with chronic red blood cell transfusion, ameliorated ineffective erythropoiesis, splenomegaly, and cardiac iron overload in a new model of TDT mice. Thus, although initial interest in the hepcidin pathway was to ameliorate iron dysregulation, it prompted the initiation of several clinical trials targeting both hematologic improvement and iron overload in beta-thalassemia. However, clinical trial data were not as encouraging. LJPC-401, a synthetic human hepcidin given as a subcutaneous injection, was being evaluated in a phase 2, multicenter, randomized, open-label study (NCT03381833) in adult patients with TDT and a primary endpoint of improvement in myocardial iron overload detected by MRI. The trial was prematurely terminated, as an interim analysis showed absence of efficacy thus indicating an unfavorable risk–benefit profile. The TRANSCEND study (NCT03802201) was another phase 2, open-label, single-arm, dose-escalation study evaluating another injectable hepcidin mimetic PTG-300, in adult patients with NTDT (to increase Hb level) and TDT (to decrease transfusion burden). Altogether, although theoretically favourable and technically feasible, the direct administration of hepcidin, both in a complete or truncated form, did not show relevant benefits in the clinical setting until now. Different approaches to the modulation of iron metabolism target the upstream regulation of hepcidin and are now on a clinical trial.

Stimulators of hepcidin production

Other novel therapeutic approaches to target iron dysregulation include increasing the hepatic synthesis of hepcidin. This can be achieved by suppressing a metalloprotease, transmembrane serine protease 6 (TMPRSS6) which plays a key role in hepcidin expression from the liver, and its inactivation leads to increased hepcidin levels, ameliorated iron overload, and improved ineffective erythropoiesis.

The use of second-generation antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) targeting TMPRSS6 have also been de-

scribed in mouse models of beta-thalassemia intermedia. Anti-sense oligonucleotides (ASO) and small interfering RNA (siRNA) targeting TMPRSS6 have been effectively used to stimulate hepcidin, reduce iron burden, and improve ineffective erythropoiesis and RBC survival in mouse models of beta-thalassemia intermedia.

A phase 2a study by Ionis Pharmaceuticals using TMPRSS6 inhibitors will soon be initiated in 36 adult patients with NTDT and baseline Hb ≤10 g/dl. In this study patients will be subcutaneously administered IONIS TMPRSS6-LRx every 4 weeks (NCT04059406)

A randomized, single-blind, placebo-controlled, phase 1b, single-ascending and multiple-dose study in adult patients with NTDT and very low- and low-risk myelodysplastic syndrome (MDS) is currently underway to investigate the safety, tolerability, pharmacokinetics, and pharmacodynamic response of SLN124 (NCT04176653).

Ferroportin inhibitors

A more recent approach to target ineffective erythropoiesis through the modulation of iron metabolism involves the use of ferroportin inhibitors. A newly described compound in the field is VIT-2763, a small oral molecule that acts as a ferroportin inhibitor. In beta-thalassemia intermedia mouse models, it restricted iron availability, ameliorated anemia, and reversed the dysregulated iron homeostasis. It reduced the percentages of early erythroid precursors in the bone marrow and spleen, and increased the percentage of mature erythrocytes, providing evidence of improved ineffective erythropoiesis; while extending the lifespan of RBCs, thereby improving anemia and tissue oxygenation.

In order to determine the safety, tolerability, pharmacokinetic properties, and pharmacodynamic effects of VIT-2763, a phase 1 randomized, double-blind, placebo-controlled, parallel-group, dose-escalation study, comprising a single ascending phase (SAD) and multiple ascending phases (MADs), was performed on healthy male and female volunteers aging between 18 and 65 years. There were no serious or severe AEs or discontinuations due to AEs. Following VIT-2763, a rapid, temporary decrease in serum iron levels was observed.

Based on the data from this study, an ongoing phase 2 study by Vifor Pharma has been initiated in NTDT patients, assessing the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of VIT-2763 in 36 NTDT patients aged ≥12 years with a baseline Hb ≤11 g/dl (NCT04364269).

Conclusion

The treatment landscape for beta-thalassemia is swiftly evolving, so it remains of utmost importance to pause and reflect on successes and failures to inform gradual integration of such advances into routine clinical practice over the next decade. Despite the large array of pharmacologic agents in development, the goals of therapy remain the same: transfusion reduction in TDT and improvement of Hb level in NTDT. The first lesson learned from recent trials is that observations in animal models do not always translate to similar effects in humans, as exemplified by data from ruxolitinib. This further strengthens the importance of relying on data from multicenter, large clinical trials representative of the global patient population; and more importantly, the need for continued data generation through real-world evidence.

In patients with NTDT, improvement in Hb level should also lead to short-term and long-term benefits in patient reported outcomes and morbidity risk, respectively. Luspatercept now has available clinical evidence for its ability to achieve hematologic responses in patients with NTDT, and long-term data on durability of such effects would be of merit.

To conclude, all these emerging treatment modalities require long-term experience in order to further establish their efficacy and safety. Another concern is the availability and the high cost, especially in low- and middle-income countries. All novel developments need to go in parallel with programs that ensure access to patients in these countries, since the majority of beta-thalassemia patients live in such regions.

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PROGRESS IN THE TREATMENT OF ADULT B-CELL PRECURSOR PHILADELPHIA CHROMOSOME-NEGATIVE ALL: RISK ADAPTED TREATMENTS AND NOVEL IMMUNOTHERAPIES

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Over the past 20 years, significant therapeutic progresses have occurred in the treatment of adult B-cell precursor Philadelphia-negative acute lymphoblastic leukemia (Ph-ALL). Reasons of this progress include a better definition of the cytogenetic and molecular profile of the disease at presentation, the use of pediatric-inspired chemotherapy programs and precise monitoring of minimal residual disease (MRD). All these factors have led to a modern, risk-adapted treatment strategy of this disease. More recently, novel targeted immunotherapies have further improved our ability to treat not only relapsed and refractory patients but also those with evidence of MRD. These topics will be covered during my presentation.

Monitoring and treating minimal residual disease

We have recently reported results with an updated strategy combining pediatric-based chemotherapy with a risk-oriented indication to an allogeneic hematopoietic cell transplant (HCT)[1]. Following induction–consolidation chemotherapy, responsive patients received maintenance chemotherapy or underwent an early HCT according to the risk stratification criteria and MRD status. Of the 117 study patients with B-cell precursor Ph- ALL (median age 42 years, range 17-67), 97 achieved a complete remission (CR, 82.9%); 46 patients were assigned to maintenance chemotherapy and 51 to HCT due to very high-risk characteristics (hyperleukocytosis, adverse genetics and MRD persistence). The median overall and relapse-free survival were 47% and 49% at 5 years, respectively. In an intent-to-treat analysis, no significant differences between maintenance and HCT cohorts were documented, strongly supporting the concept that only high-risk patient should be exposed to the transplant-related risks (Figure 1). MRD negativity and age ≤55 years were the most favorable independent prognostic factors.

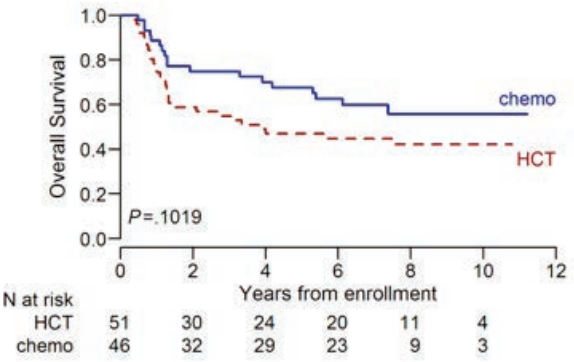

Figure 1. Intent to treat analysis of Overall Survival in adult, Ph-negative B-precursor ALL patients treated according to the NILG 10/07 protocol. Patients were allocated to allogeneic transplant if defined at high risk of leukemia relapse (hyperleukocytosis, adverse genetics and MRD persistence at week 10 of treatment).

Overall, no matter the underlying disease subset, the clinical risk profile and the treatment itself that played a primary key prognostic role, the course of MRD proved to be a fundamental, independent prognostic parameter that reflects the dynamics of chemo-sensitivity. For these reasons, our data confirm that the collection of MRD data is an essential component of modern treatment strategies for ALL at all ages. In the relapsed/refractory disease blinatumomab, the first bispecific T-cell engager (BiTE), proved more effective and better tolerated than conventional chemotherapy. For its efficacy and safety profile in the relapsed/refractory setting, blinatumomab has been extensively evaluated for the treatment of patients with molecular evidence of MRD persistence in first or later CR. Interestingly, in this setting, the clinical outcome after achieving a molecular remission was not different for patients having or not a subsequent allo-HSCT. In addition, in patients who proceeded to an allogeneic transplant, a reduced transplant-related mortality was observed, suggesting that the sequence of inducing a MRD response by blinatumomab followed by an allo-HSCT is effective and safe[2]. For these reasons, in the GIMEMA LAL2317 trial we have added two doses of the bispecific monoclonal antibody blinatumomab to the same chemotherapy backbone used for the NILG 10/07 and GIMEMA LAL1913 studies[3]. The two blinatumomab cycles were given sequentially after early consolidation cycle 3 (high-dose methotrexate and Cytarabine) and late consolidation cycle 6, to all study patients regardless of MRD being assessable. The preliminary results have been presented by Renato Bassan during the recent EHA meeting 2021[4]. A hematological CR was achieved in 131/149 patients (90.4%). After early consolidation, 73% of these patients were MRD-negative ($<10^{-4}$). MRD negativity increased to 96% after the first blinatumomab administration (P=0.018), with a conversion rate from MRD positivity to MRD negativity of 87% (20/23) patients. These results are in line with those observed in the front-line GIMEMA LAL2116 protocol for Ph+ ALL patients treated with dasatinib followed by blinatumomab[5].Blinatumomab was also capable of eradicating MRD in Ph-like patients who are usually resistant to chemotherapy programs[3]. With a median follow-up of 10 months, the 12-month overall and disease-free survival rates are 83.8% and 71.6%, respectively. However, in many Ph-like positive patients a remarkable incidence of leukemia relapse occurs despite the promising early response to blinatumomab[4].

The promise of cellular therapy

Over the last few years, chimeric antigen receptor (CAR) T-cell therapy has rapidly moved from early phase trials to registered pharmaceutical products and daily clinical practice. Results are particularly impressive in the treatment of relapsed/refractory pediatric and young adult B-lineage ALL[6] where a reproducible CR rate has been reported in more than 80% and a convincing duration of response is consistently observed even in patients not receiving a subsequent allogeneic transplant. In the adult setting results are less impressive [7–9], but the field is rapidly expanding and novel CAR-T cell products are underway[9,10]. Among these, we have developed a new product based on donor-derived CD19 CAR cytokine induced killer (CIK) cells. These cells are engineered with the Sleeping Beauty transposon and demonstrate a high expansion rate, low toxicity and lead to complete remission in relapsed/refractory B-ALL.[11]

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FROM GENOMICS TO CHEMOGEGNOMICS IN T CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Despite the significant advances in the genetic characterization of acute lymphoblastic leukemia (ALL) and the identification of putative druggable targets, treatment options in the relapsed or refractory (R/R) setting have been limited to conventional cytotoxic chemotherapies, with survival of less than 6 months.[1] T-cell ALL represents approximately 12% to 15% of all newly diagnosed ALL cases and, historically, outcomes for T-ALL were inferior to those of B-ALL. However, with modern therapeutic approaches, event-free survival (EFS) rates have been gradually improving both in adult and pediatric newly diagnosed T-ALL patients. For R/R T-ALL patients instead, little has been achieved and nelarabine, a purine nucleoside antimetabolite, remains the most recently approved drug.[2] Furthermore, genetically engineered autologous chimeric antigen receptor T (CAR T) cells approaches or immunotherapies are just in the early phase of clinical trials,[3,4] suggesting that new therapeutic modalities are much needed.

Differently from other kinase-driven leukemias, T-ALL arises from genetic alterations, often chromosomal defects, that lead to dysregulated transcriptional programs and cause the ectopic expression of transcription factors (TF). Altered TF activity initiates leukemogenesis and defines the majority of T-ALL subgroups as *TAL1*, *TLX1*, *TLX3*, *HOXA9/10*, *LMO2*, or *NKX2-1*. Similarly, hyperactive *NOTCH1* signaling, secondary to gain-of-function mutations occurring in more than 50% of the patients, plays a critical role in the pathogenesis of this disease.[5] Full leukemic transformation requires additional genetic lesions that in many cases occur within druggable pathways and serve as therapeutic vulnerabilities. This is, for example, the case of mutations in the kinase receptors signaling cascade (*e.g. IL7R*, *JAK1*, *JAK3*, and/or *STAT5*), or in the PI3K-AKT pathway, or the impairment of the *CDKN2A/2B* cell cycle regulators. However, direct and reverse targeting strategies have proven challenging and stumbled during clinical development such as the γ-secretase inhibitors in *NOTCH1* mutated T-ALL. An alternative strategy for R/R T-ALL may involve functional precision medicine approaches to repurpose available or innovative therapeutic agents based on information by drug response profiling (DRP) of leukemia cells.[6-8] For example, we designed the combination of venetoclax and bortezomib as potential salvage regimen based on recurrent patterns with DRP using a selection of 85 drugs in three in R/R Early T-cell precursor (ETP). More recently *ex-vivo* pharmacothyping of a large cohort of ALL, revealed that 41% of T-ALL cases respond to dasatinib. Furthermore, dasatinib-sensitive cases are venotoclax resistant suggesting a clinically attractive solution for mature T-ALL where leukemic cells express little BCL-2 and more abundantly BCL-XL.[9] In conclusion, drug-screens of leukemia primary T-ALL samples on panels of targeted inhibitors are a promising forward genetic approach to establish pharmacogenomics models for clinical decision making and explore biological basis controlling drug response. These approaches have the potential to improve individual patient survival by the selection of individualized sensitivity-directed regimens.

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PATHOGENIC MECHANISMS OF DIC AND THERAPEUTIC APPROACHES

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Disseminated intravascular coagulation (DIC) is a systemic syndrome secondary to several clinical conditions, particularly conditions associated with a systemic inflammatory response. DIC is characterized by the extensive and uncontrolled activation of blood clotting and is manifested by failure of several organs, caused by small thrombi of platelets and fibrin in the microcirculation, and by profuse bleeding, caused by the massive consumption of clotting factors and platelets (1). However, clinically, the severity of manifestations can vary widely, from nearly asymptomatic or paucisymptomatic clinical pictures to extremely severe conditions with multi-organ failure and severe or fatal bleeding (1,2). In any case, the progressive loss of the patient hemostatic capacity, once established, is difficult to control, as it occurs with simultaneous thrombosis and bleeding, with predominance of one over the other depending on specific cases. The clinical conditions most frequently associated with DIC are: sepsis and severe infections; extensive tissue damage, from burns or trauma; obstetric complications, eg. *abruptio placentae* or amniotic fluid embolism; neoplasms, both solid and hematological (3). Given the heterogeneity of the clinical manifestations of DIC and the lack of a simple test for diagnosis in the early stages, it is very difficult to establish the real incidence of this potentially catastrophic condition. From the available data, an incidence of about 5% is estimated in intensive care units, with peaks of up to 30-40% if only patients with severe sepsis are considered. The molecular mechanisms of inflammation play an important role in the activation of coagulation that determines DIC, particularly in sepsis- and cancer-associated DIC. Inflammatory cytokines (i.e. IL-1β, TNFα) can trigger the exposure of tissue factor (TF), the most important activator of blood coagulation in humans, by endothelial cells and circulating monocytes. The extensive activation/damage exerted by cytokines on the endothelium also causes the reduction of the anticoagulant Protein C/Protein S/thrombomodulin system and the imbalance of fibrinolytic proteins (1,3). Finally, cytokines stimulate the production of neutrophil extracellular traps (NETs), the highly procoagulant DNA filaments, from activated leukocytes, and the release of procoagulant microparticles from platelets, neutrophils and endothelial cells. Bacterial sepsis DIC may present differently from viral sepsis DIC. In patients with DIC, systemic abnormalities of hemostasis are found, such as thrombocytopenia, hypofibrinogenemia, prolongation of clotting times (PT, aPTT, and TT), increased levels of fibrin degradation products (FDPs, D- Dimer). There are non-routine specific tests detecting peptides derived from activated coagulation factors (eg F1 + 2), abnormal fibrinolytic proteins (t-PA, u-PA, PAI 1 and 2) and enzyme-inhibitor complexes (eg TAT, PAP), which may allow to detect hypercoagulability and hyperfibrinolysis at pre-clinical stages. However, the results of routine clotting tests along with thrombocytopenia and clinical symptoms are usually sufficient to establish the diagnosis of DIC. For this purpose, several diagnostic algorithms have been proposed, based on scoring systems. The two most popular ones come from the International Society of Thrombosis and Haemostasis (ISTH) and the Japanese Association of Acute Medicine (JAAM) (4-6). However, it is important to remember that the parameters can vary greatly depending on the underlying disease associated with DIC. Since DIC is a result of an acute medical illness, prognosis depends almost entirely upon the speed in handling the bleeding emergency, as well as the ability to treat the underlying disorder. The underlying disease that causes the disorder will usually predict the probable outcome. Concerning the management, the cornerstone treatment

is to treat the underlying disorder. In addition, supportive treatment directed towards the coagulation system may be essential in restoring microvascular failure and reducing organ dysfunction, *i.e.*: replacement therapy (plasma, platelet, fibrinogen), anticoagulants (heparins), other treatments (antithrombin concentrates, thrombomodulin, antifibrinolytics).

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COVID-19 RELATED COAGULOPATHY

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COVID-19, the disease caused in humans by severe acquired respiratory syndrome Corona Virus-2 (SARS-CoV-2) has been and is still today a global health emergency, with more than 214 millions of confirmed cases and more than 4 millions deaths.[1]

Besides respiratory failure, thromboembolic complications are among the most frequently reported severe clinical manifestations of severe COVID-19. Indeed, a recent meta-analysis showed an estimated overall prevalence of venous thromboembolism of 14·1% (95% CI 11·6–16·9) in COVID-19 patients, raising up to 45% in studies that applied routine screening strategies to patients admitted to ICU.[2]

Notably, even in the absence of clinically relevant macrothrombi, a pro-thrombotic derangement of the hemostatic system is often seen in COVID-19 patients, as assessed by either conventional (D-Dimer) or viscoelastic (TEG® and ROTEM®) laboratory methods.

Moreover, the severity of the derangement of coagulation parameters in COVID-19 patients has been associated with a poor prognosis, and the use of low molecular weight heparin (LMWH) at doses registered for prevention of venous thromboembolism (VTE) has been endorsed by the World Health Organization and by Several Scientific societies.[3]

Despite a rapidly growing amount of literature on this topic[4] the pathophysiological mechanisms underlying the derangement of the hemostatic system induced by SARS-Cov2 is not yet completely unravelled. As there is no evidence of a procoagulant effect directly exerted by SARS-CoV-2 virus, it is reasonable to assume that the virus activates the coagulation cascade by eliciting a large-scale inflammatory response, as already observed in any form of severe sepsis.

Several studies have already demonstrated the tight interconnection between thrombosis and inflammation, two processes mutually reinforcing each other, and named "immunothrombosis".[5]

Both coagulation factors and platelets are directly implicated in the modulation of the host immune response, displaying proinflammatory functions independent of their haemostatic effects [In turn, cytokines elicited bay the virus stimulate the expression of tissue factor on monocytes/macrophages and vascular endothelial cells, on whose surfaces the coagulation cascade is initiated. The thrombus formation at the microvascular level contributes to tissue ischemia and organ dysfunction.

Respect to such a simple pathophysiological scheme, shared with many other forms of acquired microangiopathy, the COVID-Associated Coagulopathy (CAC) shows some peculiar features, making it a distinct clinical entity respect to both Sepsis-Associated Coagulopathy (SIC) and Disseminated Intravascular Coagulation (DIC).[6]

The most striking one is the prominent involvement of the pulmonary microvascular bed respect to that observed in other diseases. Indeed, a higher incidence of in situ pulmonary arterial thrombosis has been reported in COVID-19 patients as compared to those affects by sepsis-associated acute respiratory distress syndrome. This could be linked to the mechanism of SARS-CoV-2 infection, as , the surface Spike (S) protein of SARS-CoV-2 contains a receptor-binding domain (RBD) specifically recognizing ACE2, which represents the main gate entry of SARS-CoV-2 into the cells, namely type II pneumocytes and endothelial cells.

Following attachment to ACE2, SARS-CoV-2 is internalized into susceptible cells and downregulates this receptor, thus impairing the conversion of Ang I to Ang 1-9 and AngII to Ang 1-7.

This results in an intracellular accumulation of Ang II which induces the expression of PAI-1 in endothelial cells. The increase of PAI1 triggers hypofibrinolysis, which leads to vascular microthrombosis. Moreover, increased angiotensin II stimulates vascular constriction and decreased angiotensin 1-7 suppresses nitric oxide production, which in turn triggers increased thrombogenicity because of leucocyte and platelet adhesion and vasoconstriction. Infected endothelial cells also release Von Willebrand factor (VWF), factor VIII and Angiopoietin2 from Weibel Palade bodies into the circulation. Angiopoietin2 competitively antagonizes Angiopoietin1/Tie2 signaling, thus turning the anticoagulant and anti-inflammatory features of endothelial cells to the opposite ways. This prothrombotic and pro inflammatory scenario is further enhanced by the direct infection of macrophage by SARS-CoV-2 always via binding to ACE2 receptor. Viral RNA has been shown to activate Toll-like receptors (TLR)-3 and -7 to enhance the NF-jB pathway and the interferon regulatory factors (IRFs), which consequently increases the synthesis and release of pro-inflammatory cytokines. Overall, severe COVID-19 infection leads to systemic hyperinflammation similar to macrophage activation syndrome or cytokine storms characterised by increased plasma concentrations of interferon c (IFNc), IFNc-inducible protein 10, tumour necrosis factor a (TNFa), interleukin (IL)-1b, IL2, IL6, IL7, IL8, IL10, IL17, monocyte chemoattractant protein 1 (MCP1), and macrophage inflammatory protein (MIP).

Elevated IL-1, IL-6, and TNFa could further activate endothelial cells to promote thrombosis, and elevated TNFa and angiotensin II have been implicated in the enhancement of tissue factor overexpression in platelets and macrophages. In addition, damaged alveolar endothelial cells also expose TF to promote fibrin deposition and thrombosis

In addition, the complement system and innate immunity contribute to the endothelial damage.[8] Lectin and classical pathways of Complement activation (LP and CP, respectively) are triggered by interactions of mannose binding lectin (MBL) and C1q, respectively, to pathogens and damaged cells. When associated with a mannose-rich foreign particle (such as a virus-infected cell surface), Mannose Binding Lectins triggers MBL-associated serine protease (MASP)-mediated prothrombin (FII) activation to thrombin (FIIa), which feedback amplifies its own generation via the coagulation cascade (black lines). MASP and thrombin share substrate specificity resulting in cell modulation and crosslink-stabilized clot formation.

Thrombin crosses-over into complement by cleaving C3 and C5, propagating inflammation, anaphylaxis and deposition of membrane attach complex (MAC) on infected cell surfaces. C5a and C3a receptors stimulates P-selectin (P-Sel), which can localize complement cofactors C3b and C3(H2O) on the endothelium and platelets. The MACs damage cellular membrane and activate neutrophil release of neutrophil extracellular traps (NETs), damage□associated molecular patterns (DAMPs) and chemical mediators that further hurt the vasculature. Moreover, MAC induces release of extracellular vesicles (EV), which contain Tis-

sue Factor. Thrombin and MASP directly cleave Protease Activated Receptors (PAR) leading to activation of endothelial cells, leukocytes and platelets and additional production of thrombin. The process of inflammation and coagulation activation induced by SARS-CoV-2 infection is also supported by an altered platelet activation status.[9] As the SARS-Cov-2 infection progresses, the uncontrolled overproduction of inflammatory cytokines activates platelets, with an increased exposure of P-selectin and Tissue Factor on their surface. Platelet hyperreactivity may also be a consequence of the effect of SARS-CoV-2 on megakaryocytes. Indeed, virus proliferation within lung tissue may induce activation of megakaryocyte leading to the production of platelets with a significantly altered gene expression profile. Of note, the COVID-19-associated transcriptome differs by hundreds of transcripts from that observed in influenza and sepsis suggesting a unique transcriptional footprint that characterizes SARS-COV -2 infection. The altered platelet activation also can be a direct consequence of the virus activity that, once internalized, can determine a Toll like receptor 7-mediated release of platelet granules. It is noteworthy that this platelet activation is peculiar of COVID-19, being characterized by the formation of platelet-leukocytes rather than platelet–platelet aggregates and by an increased procoagulant potential supported by elevated levels of TF positive platelets and microvesicles.

Finally, activation of endothelial cells, which is another hallmark of COVID-19 disease, may result in a NO pathway dysfunction that can promote and sustain further platelet activation.

These mechanisms, not reciprocally exclusive, are responsible for (a) an increase in circulating procoagulant platelets expressing Tissue Factor, which are therefore able to support thrombin generation, and P-selectin-positive platelets available for the formation of heteroaggregates with monocytes and neutrophils (b).

Neutrophil recruitment and activation with subsequent NETosis, endothelial cell damage and activation, and platelet activation and aggregation, together with coagulation protease activation, all participate in the complex process of immunothrombosis, especially located in the lungs.

The key components of NETs released from cell death are cell-free DNA (cfDNA) and extracellular histones, which enhance host inflammation and induce thrombosis. Histones directly bind to prothrombin fragments F1 and F2, to facilitate FXa cleavage of prothrombin to release active thrombin, even in absence of phospholipid surfaces to anchor the classical prothrombinase complex. Overall, CAC features of the resemble those of an unique thrombotic microangiopathy (TMA) syndrome that is non- identical to other TMAs but shares key features with complement- mediated TMA conditions that involve infection- induced, organ transplant- related, autoimmune- mediated or inherited disorders of the complement system.

Relevant to this, a significant alteration of the VWF-ADAMTS13 axis in COVID-19 patients, with an elevated VWF:Ag to ADAMTS13 activity ratio, strongly associated with disease severity, has been demonstrated. Such an imbalance enhances the hypercoagulable state of COVID-19 patients and their risk of microthrombosis. A deeper knowledge of the CAC pathophysiology is crucial to a more effective approach to such a compelling disease. The simplest approach is to tackle the coagulation activation by the most widely used antithrombotic agent, i.e. heparin. In addition to anticoagulation, heparin also has anti-inflammatory effects and has even shown potential antiviral effects in pre-clinical trials in COVID-19. Heparin also acts to prevent histone-mediated cytotoxicity and has been shown to improve survival in sepsis. This may have relevance to recent findings on the role of extracellular histones in severe COVID-19. The importance of pharmacologic prophylaxis for venous thromboembolism in COVID-19 hospitalized pts has repeatedly been reported. In a recent meta-analysis, Patell *et al*.[10] reported data from 35 cohort studies to compare pharmacologic dosing strategies among nearly 11,000 hospitalized COVID-19 patients and found a lower incidence of venous and arterial thromboembolism in patients who received pharmacologic prophylaxis. However, the optimal anticoagulation dose continues remains to be defined, and strong evidence in favour of more intensive anticoagulation treatments are still pending, while we are waiting for the results of several randomized controlled trials and of two planned meta-analyses. The key role played by platelets in the development and progression of CAC has led to consideration of the possible efficacy of antiplatelet therapy. Indeed, aspirin is effective in reducing replication, propagation, and infectivity of several DNA and RNA viruses, including different human coronavirus (such as the human CoV-229E and the MERS-CoV). Moreover, aspirin is able to reduce NETs' release in a sepsis model, thus limiting their potential to induce thrombin generation and drive intravascular coagulation. Also, the antiplatelet agent dipyridamole may prevent NETosis by promoting 3′,5′-cyclic adenosine monophosphate (cAMP) generation in neutrophils, as was shown in the context of antiphospholipid syndrome. Furthermore, this agent, apart from its antiplatelet function, was shown to provide broad-spectrum antiviral activity (especially against positive-stranded RNA viruses), suppress inflammation and favour mucosal healing, and prevent acute injury and fibrosis in the lungs, heart and kidney.

Overall, these data provide a strong rationale for proposing the use of antiplatelet drugs in the treatment of COVID-19, but only few observational studies on this issue have been published, and several randomized clinical trials are currently in progress to clarify whether the use of antiplatelet drugs could be potentially useful to mitigate the clinical consequence of SARS- CoV-2 infection.

Several other therapeutic approaches, based on the above described pathophysiology of COVID-19 coagulopathy, have been proposed and are currently under evaluation in clinical trials.

Given the key role played by Neutrophil-mediated activation and injury of the endothelium in the pathogenesis of COVID-19, polyanionic compounds such as the recently FDA-approved defibrotide have gained attention for their potential role in protecting the endothelium from thromboinflammation with potential implications for myriad NET- and histone-accelerated disease states. Defibrotide (DF) is a naturally derived, complex mixture of poly-deoxyribonuleotides extracted originally from bovine lung and now exclusively from porcine gut mucosa with locally acting pro-fibrinolytic, antithrombotic, anti-ischaemic and anti-inflammatory activities, which exert protective effects on small vessel endothelia. DF is currently approved for the treatment of paediatric and adult hepatic VOD/SOS with MOF, thanks to its demonstrated potential to decrease levels of pro-inflammatory proteins, such as TNF-a, IL-6, vascular endothelial growth factor (VEGF), to downregulate major histocompatibility complex (MHC) Class I and Class II molecules and to decrease interaction between leucocytes and ECs by downregulating P-selectin, ICAM- and VCAM-1. Based on such properties, the use of DF can be reasonably extended to other microangiopathies involving CRS complicating a variety of disease states and treatment modalities, such as chimeric antigen receptor (CAR) T-cell therapy, or severe COVID-19. Actively accruing, international Phase II clinical trials are now underway and should shed critical light on DF's therapeutic potential in patients with COVID-19 (examples include clinicaltrials.gov; NCT04348383, NCT04530604, NCT04335201 and NCT04652115)

Besides the several, overwhelming downsides of the COVID-19 pandemic, we are forced to look for a few upsides. In this context, we have to recognise that COVID-19 has taught us a lot about the various aspects in the field of haemostasis and thrombosis, revisiting our framework of the tight interplay between haemostasis and inflammation. Due to global warming and the rapid spread of international trade and traveling, the risk of new and revisited viral infectious diseases is real.

In this perspective, the lesson learnt from the present pandemic in terms of basic and clinical research will be crucial to effectively face future threats for the global health.

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HEMOPOIETIC STEM CELL TRANSPLANTATION: 2021

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Hemopoietic stem cell transplantation (HSCT) has been increasing in numbers from a few transplants/year in the early seventies, to almost 100.000/year in 2020, and now totlling over 1 million procedures (Figure 1). Increasing numbers indicate increasing success, expanding indications, increasing upper age limit. The Italian scientific community has made a significant contribution to the evolution of HSCT : Ruggero Ceppelini , for the discovery of HLA and first chairman of the International HLA Workshop in 1967, Alberto Marmont who started allogeneic transplants in 1976, Guido Lucarelli, pioneer of allogeneic HSCT in thalassemia, Massimo Martelli starting haploidentical transplants in the nineties, and Massimo Gianni, who mobilized autologous CD34+ cells with cyclophosphamiode (CY), before the advent of GCSF (!), just to mention a few (more in my presentation).

Autologous HSCT for multiple myeloma , remains standard of care for patients under the age of 70, despite the introduction of several effective disease modifying agents. Autologous HSCT remains an important therapeutic option for patients with severe autoimmune disease, such as multiple sclerosis (MS): randomized trials have proven autologous HSCT to be superior to monoclonal antibodies in relapsing remitting MS. High dose chemotherapy followed by gene modified autologous stem cells, is an exciting and expanding field of research, for diseases such as thalassemia major and sickle cell disease.

There are several areas of investigation in the field of allogeneic HSCT. HLA matching with high resolution typing, is now standard of care, but recent studies have focused on variations in HLA DP expression due to changes in regulatory regions, patient germline variations in DRB1, influencing peptide binding regions and the function of HLA B leading genotype. Unmanipulated HLA haploidentical mismatched grafts, were first introduced in 2006 by the Bejing group, with intensive anti-thymocyte (ATG) based, GvHD prophylaxis. In the same year the Baltimore group reported the use of post-transplant CY (PTCY) combined with a calcineurin inbitor and mycophenolate, for unmanipulated HAPLOs . The triple PTCY based GvHD parophylaxis has proven to be so effective, that unmanipulated HAPLOs have rapidly increased world wide, also in countries without a strong unrelated donor (UD) program. The triple PTCY based regimen is now being tested in HLA matched grafts. The Baltimore group has more recently reported a quadruple PTCY-ATG based GvHD prophylaxis, for patients with non malignant diseases, resulting in GvHD free survival close to 100%. T cell depletion programs have become more sophisticated with alfa/beta and CD19 depletion, or with selection and expansion of regulatory T cells.

Relapse of the original disease remains a major cause of failure: this is being addressed by assessment of minimal residual disease (MRD) at transplant, changes in the conditioning regimen, selection of donors, modulation of immunsuppressive therapy, targeted and cellular therapy post-transplant . Prospective randomized trials are underway to optimize transplant outcomes for patients with acute leukemia.

Fifty years down the line the field of HSCT is healthy and growing steadily.

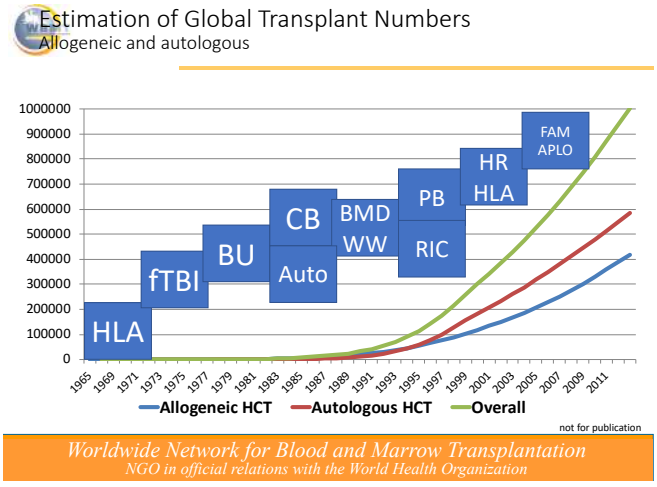

Figure 1.

SMOLDERING MULTIPLE MYELOMA: TREATMENT VERSUS OBSERVATION

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Smoldering multiple myeloma (SMM) is an asymptomatic plasma cell neoplasm consisting in an intermediate condition between monoclonal gammopathy of undetermined significance (MGUS) and active multiple myeloma (MM).[1] The rate of progression from SMM to MM is approximately 10%/year during the first 5 years and is slower thereafter. However, SMM is characterized by a deep genomic heterogeneity[2] that is reflected in a markedly variable progression risk among patients, with individual cases that may go from a true "MGUS-like" behavior, where patients will never progress during their lives, to that of an "early MM", in which transformation into symptomatic disease, based on genomic evolution, may be rapid and aggressive. This variable clinical outcome poses challenges for prognostication and management of individual patients.

In the last decade several biological and clinical advances have im-

proved the definition and the risk stratification of SMM. The 2014 International Myeloma Working Group (IMWG) diagnostic criteria were a milestone in the definition of the new borders between SMM and MM.[3] In fact, the consensus allowed to upstage a proportion of asymptomatic patients having active disease and requiring first-line treatment, since it recognized low dose whole body computer tomography (CT) as the standard tool to detect bone lytic lesions and 3 biomarkers of impending myeloma progression [marrow plasma cell infiltration ≥ 60%, free light chain ratio ≥100 and more than one focal lesion by magnetic resonance imaging (MRI) or positron emission tomography (PET)-CT scan] as MM defining-events (3). Furthermore, the risk stratification of SMM, historically based on the "PETHEMA" and the "Mayo Clinic" prognostic models, was recently implemented by the 2/20/20 IMWG system (including serum M-protein >2 g/dL, free light chain ratio >20 and marrow plasma cell infiltration >20%), integrated with the presence of at least 1 unfavorable cytogenetic abnormality [among t(4;14), t(14;16), +1q, and/or del13q)] and validated in nearly 2000 SMM patients, allowing the separation of 4 groups of patients with significantly different risk of progression at 2 years.[4] Notably, the intermediate and high categories had 46% and 63% risk of progression at 2 years, respectively, thus representing homogeneous subgroups of patients, potentially eligible for early treatment inside clinical studies.

Observation and regular monitoring have been the standard of care suggested by scientific societies and guidelines for SMM up to now days. However, several prospective studies with active treatments have been conducted in recent years in SMM and the most significant of them are summarized in Table 1. These trials are likely to pursue two different aims: low-intense treatments, to delay progression, and more intense therapies, with the goal of reaching minimal residual disease (MRD) negativity and potentially cure the patents. In particular, two independent randomized trials comparing lenalidomide ± dexamethasone vs observation have so far demonstrated a significant advantage for selected patients with SMM in terms of progression-free survival (PFS) in both studies and overall survival (OS) only in one study (OS difference in the other trial, but too short follow-up).[5-7] These studies, however, have not changed the current "no treatment" paradigm, due to several limitations: 1) both trials had a limited number of patients and started before the 2014 update criteria had been settled, therefore, a proportion of the patients enrolled were likely to be reclassified as having active disease; 2) a relevant number of patients discontinued the experimental treatment voluntarily or because of adverse effects; 3) clinical results of the studies were not presented to the regulatory agencies for the drug authorization in the market.

Monoclonal antibodies as monotherapy were also tested in SMM. Elotuzumab as single agent showed low clinical activity and failed the biological study end-point based on the demonstration of a relationship between NK cell stimulation and M protein decrease.[8] A prolonged schedule of daratumumab delayed clinical and biochemical progression more efficiently than a shorter schedule[9] and is going to be tested as subcutaneous administration in a phase 3 in comparison with observation in selected patients with SMM. A phase 2 study is exploring the efficacy of isatuximab in high-risk SMM.[10] Best responses occurred in 64% of

Table 1. Clinical results of selected clinical trials in smoldering multiple myeloma (Legend: Len: lenalidomide; Dexa: dexamethasone; KRd: carfilzomib, lenalidomide, dexamethasone; ASCT: autologous stem cell transplantation; Rd: lenalidomide, dexamethasone; C: cycle; prog: progression; obs: observation; elo: elotuzumab; dara: daratumumab; isa: isatuximab; elo: elotuzumab; ixa: ixazomib; d: day; w: week; OR: overall response; CR: complete response; MRD: minimal residual disease; NGS: next generation sequencing; NGF: next generation flow ; NA: not available; NR: not reached; m: median; y: year; TTP: time to progression; PFS: progression free survival; OS :overall survival; MM multiple myeloma; * p <0.05).

Drug/drug combination	Reference	Phase	Design	N pts	Response	Outcome
Len±dexa	[5,6]Mateos et al, Lancet Oncol 2016 and Hemasphere 2020	III	C1-9: Len 25mg d1-21 +dexa 20 d1-4 e d12-15; C1-24:len 10mg 1-21 vs obs	119	OR 90% (CR 26%) vs NA	mTTP 9y vs 2.1y* mOS NR vs 7.8 y*
	[7]Lonial et al, J Clin Oncol 2019	III	Len 25 mg d1-21 until progr vs obs	182	OR 50% (CR 0%) vs NA	3y-PFS 91% vs 66%* Deaths 6 vs 2 pts
Dara	[9]Landgren et al, Leukemia 2020	II	Dara 16mg/Kg x 8-wk Extendend intense: C1 every 1 w; C2-3 every other w;C4-7every 4 w;C8-20 every 8w	123	OR 56% (CR 5%)	2y-PFS 90%
			Interm intense :C1 every1w;C2-20 every 8 w		OR 54% (CR 10%)	2y-PFS 82%
			Short dosing: C1 every 1 w		OR 38% (CR 0%)	2y-PFS 75%
Elo	[8]Jagannath et al, Br J Haematol 2018	II	Elo 20 mg/Kg d1,8, then every 4 w Elo 10 mg/Kg d1,8,15,22, then every 2 w	31	OR 10% (cumulative)	2y-PFS 69% (cumulative)
Isa	[10]Manasanch et al, Blood 2019	II	Isa 20 mg/kg i.v. in 4 w cycle [C1] every w; [C2-6] every other w; [C7-30] every 4 w	24 (planned 61)	ORR 64%, CR 5%, with MRD negativity	NA
KRd	[11]Kazandjian et al, Blood 2020	II	C1-8: K20/36 mg d1,2,8,9,15,16+len 25 d mg1-21 + 20 mg (C1-4) or 20 mg (d 1,2,8,9,15,16 C1-24: len 25 mg d1-21	18	OR 100%, MRD 92% by NGF and 75% by NGS	4y-PFS 71% 4y-OS 100%
KRd plus ASCT	[12]Puig et al, Blood 2020	II	C1-6: K20/36 mg d1,2,8,9,15,16+len 25 d mg1-21 + 20 mg (C1-4) or 20 mg (C5-8) d 1,2,8,9,15,16 ASCT melphalan 200 mg/mq C7-8 = C1 C1-24: len 25 mg d1-21 + dexa 20 d1,8,15,22	90	OR 100%, CR 69%, MDR 55% post-consolidation and maintenance	PFS 93%
Dara-KRd	[13]Kumar et al, Blood 2020a	II	C1-6: K20/36 mg d1,2,8,9,15,16+len 25 d mg1-21 + 40 mg d 1,8,15,22+ darat16 mg/kg for 8 w, every other w for 16 w C7-12: K36 mg d1,2,8,9,15,16+len 25 d mg1-21 + 20 mg d 1,8,15,22+ dara 16 mg/kg every 4 w C8-20:len 10 mg 1-21+ dara every 4 w	46 (83 planned)	NA	NA
Ixa-Rd	[14]Mailankody et al, J Clin Oncol 2019	II	C1-94 mg: Ixa 4 mg d 1,8,15+len 25 d mg1-21 + Dexa 40 mg d 1,8,15 C10-24: Ixa 4 mg d 1,8,15+len 15 d mg1-21	26 (56 planned)	OR 89%, CR 19%	No progression to MM
Elo-Rd	[15]Liu et al, Blood 2018a	II	C1-2: Elo10 mg/Kg d1,8,15,22 +len 25 mg 1-21+dexa 40 mg1,8,15,22 C2-8: PBSC collection and Elo10 mg/Kg d1,15+len 25 mg d1-21+dexa 40 mg d1,8,15 C9-C24: Elo10 mg/Kg d1+len 25 mg d1-21	50	OR 84% (CR 6%)	No progression to MM

initially enrolled patients, including 5% MRD negativity. Health-related quality of life scores were improved by treatment.

The backbone of lenalidomide plus dexamethasone was tested in association of the proteosome inhibitor carfilzomib or ixazomib. KRd has been experimented in 2 phase II trials including small groups of patients: in the first pilot study KRd was administered for 2 years.[11] in the second trial an autologous stem cell transplantation followed the KRD induction.[12] In both these studies the "proof of principle" of the achievement of MRD negativity was obyained in the majority of the selected patients with good tolerability and prompted another ongoing phase II study testing the combination of daratumumab plus KRd.[13] Only preliminary data are available for the combination of Rd with ixazomib[14] or elotuzumab,[15] showing 19% and 6% complete responses, respectively, and no case of progression at a short follow up. At present it is difficult to reach firm conclusions on the basis of the previously reported studies testing novel combinations, since they do not include an untreated control group or compare two arms with active treatments. Results of the ongoing multi-center, open-label phase 2 trial promoted by HOVON group randomizing between KRd and Rd, followed by lenalidomide maintenance for 2 years are eagerly awaited and will provide a direct comparison of the efficacy of the regimens described above. Likewise, a phase 3, randomized, multicenter study comparing isatuximab-lenalidomide-dexamethasone versus lenalidomide-dexamethasone in higher risk SMM is ongoing.

In conclusion, we endorse the 2021 European Myeloma Network consensus on SMM for clinical practice.[16] In particular, we recommend an extensive work-up at diagnosis: beside routine serum and urine exam, marrow should be evaluated with morphological and (possibly) phenotypic quantification of clonal plasma cells, bone trephine biopsy and FISH cytogenetics, while bone disease should be detected with low dose whole body CT and with whole body MRI, if CT is negative. Axial MRI or PET-CT are reasonable alternatives. The 2/20/20 model integrated with cytogenetics is a useful tool in order to define the risk of progression, inform the patients and decide the timing of the follow-up, that, importantly, should be risk-adapted.

Regarding possible early treatments, in patients with lower risk SMM, diagnosed according to current criteria, only careful observation is recommended. On the other hand, a treatment similar to patients with active MM might be considered for selected, high-risk patients, particularly for those showing the coexistence and/or the deterioration of multiple risk factors over the time. Such an approach, however, should be still administered in the controlled setting of a clinical study, after a thorough risk/benefit discussion with the patient considering that improving OS, without negatively affecting quality of life, remains the primary objective. In the close future, it will be of great importance to identify new predictive biomarkers for further refining risk prediction and selecting SMM patients who require simply observation and those who instead warrant more stringent attention in order to establish the most appropriate moment to start an appropriate treatment. The key issue, in fact, will be to identify patients with high-risk SMM who "must" receive therapy, because they could obtain a significantly longer survival. In this case, the necessary balance between reduced risk of progression with early treatment vs short- and long-term possible adverse effects also warrants to be further investigated, particularly by choosing between intensive approaches with "curative" intent vs prolonged immunological ~~control of t~~he disease, according to "preventive" strategies.

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COMPLEMENT-MEDIATED DISEASE: FROM BASIC RESEARCH TO THERAPY

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Introduction

Complement is part of the innate immune system and plays a fundamental role in the clearance of immune-complexes and cell debris. The main effector mechanisms of complement activation are induction of inflammatory response as well as phagocytosis and cell lysis. However, complement activation is a double-edged sword and has a potential for damage self-tissues. In order to avoid self-damage, there is an absolute need for strict control by fluid-phase and membrane-bound regulatory proteins. Thus, an underperforming regulatory system (due to either genetic or acquired abnormalities) can shift the balance between regulation and activation toward the latter and lead to tissue injury in response to otherwise innocuous stimuli. Both deposition of plasma active complement fragments in glomeruli, and complement locally produced and activated in the kidney may contribute to many kidney disorders, including lupus nephritis, postinfectious glomerulonephritis, membranous nephropathy, anti-neutrophil cytoplasmic antibody-vasculitis, membranoproliferative glomerulonephritis, and anti-glomerular basement membrane glomerulonephritis. Interest in the complement system was boosted in the past 20 years by discovery that a rare devastating kidney disease, the atypical hemolytic uremic syndrome (aHUS) is caused by excessive activation of the alternative complement pathway.

Definitions

The hemolytic uremic syndrome belongs to a group of rare diseases, the thrombotic microangiopathies (TMA) that manifest with thrombocytopenia and microangiopathic hemolytic anemia accompained by renal and neurological dysfunction.[1] Thrombocytopenia is due to platelet consumption by platelet-rich thrombi in the microcirculation. Hemoglobin levels are low and the peripheral smear reveals fragmented erythrocytes, which is crucial to confirm the microangiopathic hemolytic anemia. Other indicators of intravascular hemolysis include elevated lactate dehydrogenase (LDH), increased indirect bilirubin, and low haptoglobin levels. The Coombs test is negative.

In the last twenty years different pathophysiologic mechanisms have been identified that have allowed differentiation of the different forms. Most childhood cases of HUS are caused by certain strains of E.coli bacteria that produce potent exotoxins, the Shiga-like toxins that damage microvascular endothelial cells. The other form of HUS, atypical HUS (aHUS), accounts for 10% of HUS cases.[2] The majority of patients with aHUS have genetic abnormalities in complement genes that lead to uncontrolled activation of the alternative pathway of complement. On the other hand, abnormal Von Willebrand Factor processing has been reported in TTP due to either genetic or acquired deficiency of ADAMTS13, a plasma metalloprotease that cleaves Willebrand Factor multimers. Kidney involvement is present in all patients with HUS and in about 25% of patients with TTP and manifests with hematuria, proteinuria, and kidney insufficiency of different severity.

Atypical Hemolytic Uremic Syndrome: pathogenesis and diagnosis

Currently, the term aHUS is used when a genetic or autoimmune abnormality causing complement dysregulation is strongly suspected and other causes have been excluded. Secondary HUS or secondary TMA instead includes a broad group of patients in whom TMA occurs in the context of another condition such as malignant hypertension, autoimmune disease, certain infections, malignancy, transplantation, or drugs.[3] However, this differentiation is not absolute because in more than 50% of patients with primary aHUS and genetic risk factors, a trigger is required for disease to manifest, including viral and bacterial infections, and pregnancy. Conversely, complement gene abnormalities have been identified in subgroups of patients with secondary HUS, indicating the relevance of genetic background for disease susceptibility.

Several genetic abnormalities in members of the alternative pathway of complement have been described in aHUS, which account for about 60% of cases.[2] Functional studies revealed that aHUS-associated mutations mainly result in complement activation that is restricted on the cell surface and proceeds until the formation of C5b-9. This explains why circulating complement parameters (C3, C5a, sC5b-9) are normal in about 50% of patients even during the acute phase of the disease.

In search of biomarkers of cell surface-restricted complement activation for the diagnosis of aHUS, we established an ex vivo test based on complement deposits on human microvascular endothelial cells (HMEC-1) incubated with serum collected from patients.[4] We found that serum from all patients with acute aHUS, but not serum from the same patients studied in remission (after recovery of hematological parameters), caused more C5b-9 deposition on unstimulated (resting) HMEC-1 than control serum. In the same studies, we pre-exposed HMEC-1 to ADP to mimic a condition of activated/perturbed endothelium that may trigger complement activation. Under these conditions aHUS sera induced more C5b-9 deposition than control sera independently from the phase of the disease (either taken during the acute phase or in remission out of treatment), and increased C5b-9 deposition was also observed with the sera from unaffected relatives carrying the same complement gene mutations of aHUS patients.[4] Thus, the assay based on ADP-activated endothelial cells is useful to identify subjects with genetically determined predisposition to complement dysregulation on cell surface. In an *ex vivo* thrombus formation system in which microvascular endothelial cells pre-exposed to serum were perfused in a flow-chamber with normal whole blood, massive thrombus formation occurred on cells pre-exposed to aHUS serum. Thrombus formation was significantly prevented by adding complement inhibitors to aHUS serum.

Genetic abnormalities associated with aHUS

The large majority of aHUS-associated genetic abnormalities are heterozygous and involve different genes encoding both regulatory and effector proteins of the alternative pathway of complement.[5,6] The alternative complement pathway is initiated spontaneously in plasma by C3 hydrolysis responsible for deposition of a low amount of C3b onto all plasma-exposed surfaces. On bacterial surfaces, C3b leads to phagocytosis by neutrophils and macrophages. Without regulation, a small initiating stimulus is quickly amplified to a self-harming response. On host cells, such a dangerous cascade is controlled by membrane-anchored and fluid-phase regulators that favor the inactivation of C3b by the plasma serine protease factor I (CFI, cofactor activity) and dissociate the C3 and C5 convertases (decay acceleration activity).

Complement factor H (CFH) regulates the alternative pathway both in the fluid phase and on the cell surface by exerting cofactor activity and decay acceleration activity. CFH pathogenetic or likely pathogenetic variants have been identified in about 30% of aHUS patients. These genetic abnormalities result in dysfunctional protein that cannot regulate complement on endothelial cells and platelets. A high degree of sequence identity between CFH and the genes CFHR1-5 for five factor H–related proteins (CFHR) predispose to gene conversions and genomic rearrangements. Hybrid CFH/CFHR1 and CFH/CFHR3 genes, coding abnormal FH proteins in which the carboxy-terminal domains that mediate complement regulation on cell surface are substituted for those of FHR1 or by the entire FHR3 have been identified in 3-5% of aHUS patients. Different gene conversion events between the CFH and CFHR1 genes have been reported, which convert the FHR-1 C-terminus into that of CFH. The resulting FHR1 mutant competes with CFH for cell surface binding. Anti-CFH inhibitory antibodies have been found in 5% to 10% of aHUS patients and around 25% to 50% of pediatric cases.

MCP is a transmembrane complement regulator widely expressed on all cells that serves as a cofactor for CFI. MCP gene abnormalities account for 8% to 10% aHUS cases. Expression on cell surface was reduced for about 75% of mutants. Others MCP mutants have decreased cofactor activity.

CFI genetic abnormalities affect 4% to 8% of patients. Approximately 50% of mutants are not secreted in blood; however, some mutants are secreted but have impaired proteolytic activity.

Gain-of-function mutations can affect genes encoding the alternative pathway C3 convertase components, CFB and C3. CFB variants are rare (1% to 2% of patients). Some CFB mutants have excess C3b affinity and form a hyperactive C3 convertase resistant to dissociation.

About 9% of aHUS patients carry heterozygous variants in C3, usually with low C3 levels. Most mutations reduce C3b binding to complement regulators, severely impairing its inactivation and result in increased C3 deposition on endothelial cells.

Heterozygous mutations in the gene THBD encoding thrombomodulin, an endothelial surface anticoagulant protein that also modulates complement on cell surfaces, have been found in 3% to 4% of patients with aHUS.

Penetrance of aHUS in carriers of complement gene abnormalities is incomplete and ranges from 20 to 50%. A further mutation in one of the above genes occurs in about 10% of aHUS patients and increases the risk for developing the disease. Common genetic risk variants (single-nucleotide polymorphisms and haplotype blocks) in CFH, MCP, and CFHR1 have been shown to act as susceptibility factors for the development of aHUS.[7] Full analysis of disease-associated genes and testing for anti-CFH antibodies is recommended since the nature of the underlying complement defect influences disease progression, the risk of relapses after kidney transplantation and responses to therapies**.**

Disease recurred in 60% to 80% of transplanted patients with mutations in complement circulating proteins (CFH, CFI, CFB, and C3). Lowest incidence of recurrence was observed in patients with MCP mu-

tations. MCP is highly expressed in the kidney, and a graft that brings normal proteins corrects the defect. However, about 20% of patients with MCP mutations also carry a mutation in another complement gene and have a worse graft outcome, with higher incidence of recurrences than patients with an isolated MCP genetic abnormality.

Testing affected patients for mutations on all disease-associated genes should allow patients and clinicians to make informed decisions regarding listing for transplantation based on risk for recurrence.

Complement C5 inhibition in aHUS

The humanized anti-C5 monoclonal antibody eculizumab induces remission of acute episodes of aHUS and maintains long-term remission, both in native kidneys and in the kidney grafts.[8] The drug is now widely used as a first-line therapy for aHUS, provided that other causes of TMA are excluded. aHUS serum-induced C5b-9 deposition on resting and ADP-activated HMEC-1 fully normalized during treatment with eculizumab. Eculizumab prophylaxis is used to prevent aHUS relapses after kidney transplantation however controlled prospective studies are required to evaluate the advantage of eculizumab prophylaxis vs. eculizumab treatment at the time of aHUS recurrence in kidney transplant patients.

The main concern with eculizumab is increased susceptibility to infection with encapsulated organisms, particularly Neisseria infections. For this reason, patients must receive vaccination against meningococcus. It is not clear how long anti-C5 therapy should be extended, a relevant issue because of the very high cost of the drug, and the risk of infections. In a prospective multicenter study of eculizumab discontinuation in 55 children and adults with aHUS, 13 patients experienced a relapse and this was predicted by the presence of a rare complement gene variant. Reliable biomarkers of early relapse are strongly needed. In this regard, abnormally elevated serum-induced C5b-9 deposition on cultured endothelial cells highlighted aHUS relapses during eculizumab tapering/discontinuation.[4]

Thrombotic microangiopathy associated with allogeneic bone marrow/hematopoietic stem cell transplantation

Among secondary forms, TMA associated with hematopoietic stem cell transplantation (HSCT) or bone marrow transplantation (BMT) is of great relevance for hematologists. It is a severe complication, which may present in 20-30% of recipients usually between 20 days and 100 days after transplantation. The clinical presentation ranges from mild (laboratory test changes only) to severe life-threatening disease. Multiorgan involvement typically manifests as pulmonary hypertension, polyserositis, gastrointestinal symptoms, central nervous system injury, and renal impairment.[9]

There is increasing evidence that complement is involved in the pathophysiology of HSCT/BMT-associated TMA and ensuing renal injury, similar to what occurs in aHUS. Pre-transplant screening of 17 complement genes in 77 HSCT recipients revealed variants with minor allele frequency (MAF) <1% in 65% of patients who developed TMA compared with 9% of patients without TMA.[10] Reports showing remission of HSCT-associated TMA after administration of eculizumab further indicated a central role of the complement cascade in the pathogenesis of this HSCT/BMT complication.

In preliminary experiments, we found that serum from 6 patients with acute TMA associated with HSCT or BMT, induced higher than normal C5b-9 deposits both on resting and on ADP-activated HMEC-1. In addition, 3 patients who underwent remission of HSCT-associated TMA by eculizumab treatment showed normal serum-induced C5b-9 deposits both on resting and on ADP-activated endothelial cells (4). The above preliminary results indicate that complement activation at endothelial cell level occurs in HSCT/BMT-associated TMA.

Conclusions

In summary in aHUS a complex set of genetic abnormalities causes complement dysregulation mainly restricted on cell surfaces rather than in fluid phase. The *ex vivo* test with serum and cultured endothelial cells may reproduce this feature in vitro and is useful for a rapid diagnosis and for personalized therapy in aHUS but the test can also to pick up other secondary forms of the disease associated with complement activation.

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NOVEL THERAPIES IN THE TREATMENT OF DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of Non-Hodgkin lymphoma (NHL), accounting for 30% to 40% of all newly diagnosed NHL worldwide. About 55% of DLBCL patients are cured with initial standard chemo-immunotherapy (R-CHOP). However, it is estimated that 35% to 45% of DLBCL patients treated with R-CHOP will have primary refractory disease or will experience disease relapse after attaining a complete response.[1] Salvage therapy, including high-dose chemotherapy and autologous stem cell transplant (ASCT), can effectively treat DLBCL with chemotherapy-sensitive relapse. However, over half of the patients will not have long-term disease control, and a significant proportion of patients are not eligible for aggressive treatment.[2] Therapy failure is common for patients who are refractory to first-line therapy or exhibiting unfavorable characteristics, such as MYC and BCL2 and/or BCL6 translocations ('double/triple hit' lymphoma), MYC and BCL2 and/or BCL6 protein overexpression ('double/triple expressor' lymphoma) or harboring TP53 mutations/deletions. For numerous years, patients failing conventional second-line therapy remained with no valid therapeutical alternatives and had a dismal short-term outcome. Likewise, no specific agents or combinations have been identified for high-risk patients. On the contrary, a wide variety of novel im-

munotherapies, targeted therapies, and cellular therapies have flourished in recent years, revolutionizing treatment for DLBCL and promising to have a significant impact in future years (Table 1).

Genetically modified autologous T cells targeting CD19 (CAR T-cells) have had the highest resonance and have been granted rapid approval by the FDA and EMA for use in r/r DLBCL. CAR T-cells induce durable remissions in 30-40% of patients and are currently the preferred third-line therapy in r/r DLBCL.[3-5] Specific toxicities, such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome (ICANS), have become more manageable and preventable. Compared to the current standard, CAR-T cells have also been tested as second-line therapy; results from such trials are greatly awaited.

Other CD19-targeting agents have shown considerable efficacy. Among these, the combination of Tafasitamab and Lenalidomide reported an ORR of 60% (43% CR rate) in phase II registrative trial, including DLBCL patients ineligible for ASCT.[6] Despite several limitations, such as the lack of chemorefractory and biological high-risk patients, such combination was granted approval from the FDA and EMA for the treatment of r/r DLBCL not otherwise specified, including DLBCL arising from low-grade lymphoma or who are not eligible for ASCT. Updated follow-up studies have reported an impressive median overall survival of 31.6 months. More recently, Loncastuximab tesirine, a humanized CD19-targeting antibody-drug conjugate (ADC), was also approved in monotherapy by the FDA to treat r/r DLBCL after at least two lines of the therapy. The phase II clinical trial reported an ORR of 48% (24% CR rate), with an acceptable safety profile, and included many heavily pre-treated patients. Some of them were already failing CAR T-cell therapy.[7] Currently, Loncastuximab is under investigation in combination with other agents as well as in the second-line setting.

Other recent additions to the therapeutic armamentarium include the anti-CD79 ADC Polatuzumab, which has been approved for clinical use in combination with Bendamustine/Rituximab after two or more lines of therapy based on promising results from a phase II clinical trial (ORR 45%, CR 40%),[8] and Selinexor, an inhibitor of the nuclear export receptor XPO1, which has also been approved for the same indication.[9] Reported response rates to Selinexor are significantly inferior compared to other novel therapies (ORR 28%, CR 12%); however, the drug was well tolerated and may represent a reasonable alternative for more fragile patients ineligible for CAR T-cells. Among future therapies still under investigation, bispecific agents targeting CD20 and CD3 are of primary interest. They are designed to bring T cells close to tumor cells to trigger T-cell-mediated cytotoxicity and have shown highly promising activity. Specifically, Glofitamab (RO7082859) exhibits a longer half-life and superior in vitro cytotoxicity when compared to other CD20xCD3 bispecific antibodies. Co-administration with Obinotuzumab and implementation of a step-up dosing schedule resulted in a complete metabolic response rate of 71.4% in aggressive lymphoma, most of which was ongoing at 18 months. No safety issues have emerged, with CRS being mild (mainly grade 1 and 2) and manageable.[10] Other promising bispecifics currently being tested in phase I/II clinical trials include Mosunetuzumab, Odronextamab, and Epcoritamab. With such an increasing availability of novel therapeutic agents, the current challenge is to identify the correct sequence of such therapies, the patient population that may best benefit from each type of therapy, and, possibly, the addition of such treatments to standard treatment to achieve higher rates of frontline cure.

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Table 1. Novel FDA/EMA approved therapies for the treatment of r/r DLBCL.

Agent	Mechanism of action	Indication	Efficacy	Reference
Axicabtagene Ciloleucel	Anti-CD19 CAR T-cell, CD28 costimulatory domain	r/r DLBCL after ≥2 lines of therapy	ORR 83% CR 59%	(3)
Tisagenlecleucel	Anti-CD19 CAR T-cell, 4-1BB costimulatory domain	r/r DLBCL after ≥2 lines of therapy	ORR 52% CR 40%	(4)
Lisocabtagene Maraleucel	Anti-CD19 CAR T-cell, 4-1BB costimulatory domain	r/r DLBCL after ≥2 lines of therapy	ORR 73% CR 53%	(5)
Tafasitamab + Lenalidomide	Fc-enhanced CD19 targeting agent + immunomodulatory drug	r/r DLBCL after ≥1 lines of therapy	ORR 60% CR 43%	(6)
Loncastuximab tesirine	Anti-CD19 PBD-ADC	r/r DLBCL after ≥2 lines of therapy	ORR 48.3% CR 24.1%	(7)
Polatuzomab Vedotin + Bendamustine/Rituximab	Anti-CD79b ADC	r/r DLBCL after ≥2 lines of therapy	ORR 43% CR 40%	(8)
Selinexor	XPO1 inhibitor	r/r DLBCL after ≥2 lines of therapy	ORR 28% CR 12%	(9)

CLINICAL IMPACT OF MEASURABLE RESIDUAL DISEASE (MRD) IN ACUTE MYELOID LEUKEMIA (AML)

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MRD denotes the presence of leukemia cells that survives chemotherapy and persist in the bone marrow (BM) below the threshold of morphologic complete remission (mCR). Increasing evidence indicates that MRD is a very powerful, independent prognostic factor associated with an increased risk of relapse and a shorter overall survival (OS), in patients with AML. In a large meta-analysis of 81 publications, including 11151 patients with AML, the estimated 5-year disease-free survival (DFS) was 64% for patients without MRD and 25%for those with MRD. The estimated OS was 68% for patients without MRD and 34% for those with MRD. Although based on the analysis of retrospective or non-MRD directed trials, these finding confirm that, in patients with AML, eradication

of MRD and the achievement of a MRD negative mCR is associated with a significantly longer survival estimate. Indeed, the ELN2017 recommendations have acknowledged the prognostic role of MRD including, among the criteria of response, the one of mCR without MRD. According to the recommendations, MRD negativity should be demonstrated by flow cytometry or RT-q-PCR. The whole body of this evidence suggests that MRD determination warrants consideration as a clinical trial end point that may allow for a more accurate evaluation of the quality of response, after chemotherapy. Numerous trials that are MRD-directed or that have MRD as a primary end-point are ongoing (Tables 1 and 2); at the same time, the results of some pivotal trials have already been published. The HOVON-SAKK 132 trial randomized patients with de novo AML, aged 18-65, between conventional chemotherapy and conventional chemotherapy plus lenalidomide. After induction and consolidation, patients belonging to the ELN2017 favorable risk category were submitted to autologous stem cell transplant (AuSCT) whereas those in the ELN2017 adverse risk category to allogeneic stem cell transplant (ASCT). Patients belonging to the intermediate risk category received AuSCT if MRD negative after consolidation or ASCT if MRD positive. After AuSCT the patients randomized in the lenalidomide arm were to continue lenalidomide as a maintenance. No lenalidomide maintenance was allowed after ASCT. Besides showing the lack of any additive effect by the addition of lenalidomide to chemotherapy, the authors demonstrated an equivalent duration of relapse free survival (RFS) of the MRD positive and MRD negative patients, within the intermediate risk category. By applying a MRD directed, risk adapted strategy they were able to prolong RFS of MRD positive patients, to equalize that of MRD negative ones. Such a strategy relies on the use of the greatest intensity (ASCT) for patients at higher risk of relapse (MRD positive) and on avoiding over treatment (AuSCT rather than ASCT) for those with lower risk of disease recurrence. Similar results were reported by "Gruppo Italiano Malattie Ematologiche dell'Adulto" (GIMEMA) in the AML1310 trial. This was a trial of risk-adapted, MRD-directed therapy for young adults with newly diagnosed AML. Similarly to the HOVON-SAKK trial, in the AML1310 trial patients belonging to the intermediate risk category were to receive AuSCT or ASCT according to the level of MRD, as assessed after the first consolidation course. Again, by applying such a MRD driven approach, the investigators demonstrated that the OS and DFS of MRD positive patients can be prolonged to match the one of MRD negative subjects. Once established that patients belonging to the adverse risk category should receive ASCT regardless of the level of MRD, the next critical question is whether such a MRD directed therapy represents an option also for patients within the favorable risk category. Ivey investigated, by RT-q-PCR, MRD in 2569 peripheral blood (PB) samples obtained from 346 patients with NPM1-mutated AML who had undergone intensive treatment in the National Cancer Research Institute (NCRI) AML17 trial. The authors demonstrated that the persistence of MRD in the PB after 2 courses of chemotherapy was significantly associated with a shorter duration of OS and higher cumulative incidence of relapse (CIR) (Ivey A et al, NEJM 2016;374:422-433). A Chinese study has demonstrated that patients with RUNX1/RUNX1T1 positive AML benefit from the delivery of ASCT if MRD reduction, after 2 courses of chemotherapy, is lower than 3 log. Indeed, patients receiving ASCT had a lower CIR, longer duration of OS and DFS than those receiving chemotherapy alone. These results were replicated in a retrospective French study analyzing patients with NPM1 mutated AML. Those with a MRD reduction < 4 log after induction, if addressed to ASCT, had a longer duration of OS and DFS than those receiving an AuSCT. On the other hand, in situation of deep MRD clearance (> 4 log), patients treated with AuSCT had a longer duration of OS and DFS than those treated with ASCT. Once again, such an obser-

Table 1. Ongoing clinical trials of MRD-directed therapy for patients with acute myeloid leukemia (by Ngai LL *et al.*, Front.Oncol. 2021;10:1-14).

	Clinicaltrials.gov	n	Terms used	Age	Group	Technique
Induction						
MRD use in choosing targeted therapy	NCT03537560	300	MRD directed	>20	*De novo*	PCR, MFC
MRD use in intensifying treatment at induction	NCT03769532	28	MRD guided	>18	*NPM1*	PCR *NPM1*
MRD use in choosing extra therapy	NCT02349178	6	NA	<39	MRD +	MFC, molecular
	NCT03989713	80	MRD triggered	18–75	Relapse/refractory	MFC
Before transplant						
MRD use in risk stratification and choice consolidation	NCT02870777	743	MRD directed	18–60	Low/intermediate	Unknown
	NCT01041040	200	Risk adapted	All	All	MFC
	NCT03846362	100	MRD based	<18	Intermediate/high	PCR, MFC
	NCT04168502	414	MRD driven	18–60	Favorable/intermediate	Unknown
	NCT03515707	30	NA	18–69	Favorable/intermediate MRD negative	MFC, cytogenetics, FISH, molecular
	NCT03620955	1000	Risk stratified	14–60	*De novo*	MFC
	NCT04174612	172	NA	18–65	*FLT3*	MFC
	NCT02272478	1600	NA	>60	*De novo*	MFC
	NCT01723657	862	Risk adapted	18–70	*De novo*	MFC
	NCT03417427	100	NA	14–60	Intermediate	MFC
Post-transplant						
MRD use in post-transplant intervention	NCT02458235	67	Risk adapted	<29	Post-transplant	MFC, gene expression profiling
	NCT03121079	29	NA	18–60	Standard	Flow and RQ-PCR *WT1*
MRD use in tapering treatment	NCT02458235	67	Risk adapted	<29	Post-transplant	MFC, gene expression profiling
	NCT03466294	42	NA	>60	*De novo*/elderly	Unknown

vation emphasizes the favorable impact of ASCT in patients who are MRD positive and the excess of non relapse mortality in those MRD negative and with lower risk of relapse. Based on this, the GIMEMA has recently activated the AML1819 trial (Figure 1), to explore the role of MRD detection in patients with an ELN2017 favorable/intermediate risk profile.

Similarly to the AML1310 trial, the post remission therapeutic decision (AuSCT or ASCT) is made based on the level of MRD after the first consolidation course. Major differences with AML1310 trial consist in the addition of gentuzumab ozogamicin (GO) to intensive chemotherapy, to explore its impact on the level of MRD, and the addition of glasdegib as a maintenance after AuSCT or ASCT. Indeed, in the AML1819 trial, MRD assessment is used not only for therapeutic purposes, but it is a co-primary end-point, together with DFS. There are data representing a strong clinicobiologic background for the GIMEMA AML1819 trial. In a post hoc analysis of the ALFA 0701 trial (Castaigne S *et al.*, The Lancet 2012;9825:1508-1516), it was demonstrated that patients randomized in the GO arm achieved more frequently a status of BM MRD negativity than those randomized in the no GO (chemotherapy alone) arm. In this post hoc analysis, MRD was assessed by RT-q-PCR at the end of induction and at the end of treatment in patients with NPM1 mutated AML (Lambert J *et al.*, Oncotarget 2014;5:6280-6288). In the phase 3, 09-09 trial from the German AML Study Group, patients with *de novo* NPM1 mutated AML were randomized between chemotherapy plus ATRA and chemotherapy plus ATRA plus GO (Kapp-Schwoerer S, *et al.* Blood 2020;136(26):3041-3050). At any time point of evaluation (post induction 1 through post consolidation 3), patients receiving GO had significantly lower BM MRD levels than those randomized in the control arm and such a difference translated into a lower CIR. A further piece of information comes from the results of the NCRI AML18 trial presented at the EHA meeting in 2020 (Russell N, *et al.* EHA Library. 06/12/20; 294955; S135). Older patients with AML, after 1 cycle of GO containing induction and a second no GO induction were addressed to conventional chemotherapy or intensified regimens based on the level of MRD after induction 2. The addition of GO to chemotherapy and the intensification strategy in MRD positive patients resulted in an equivalent duration of OS of MRD negative and MRD positive patients (3 year OS 46.6% *versus* 51.1%).

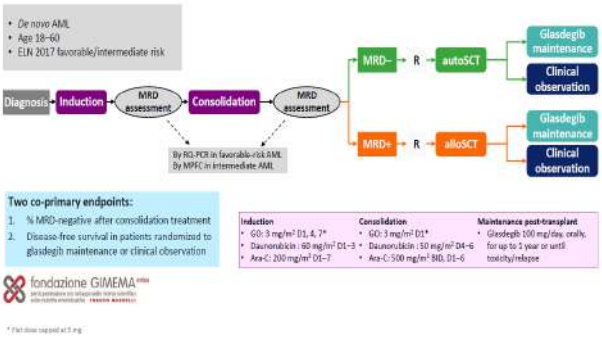

Figure 1. GIMEMA AML1819 Trial design.

Table 2. Ongoing clinical trials, which include MRD determination as a primary end-point, for patients with acute myeloid leukemia (by Ngai LL *et al.*, Front.Oncol. 2021;10:1-14).

	Clinicaltrials.gov	Phase	n	Age	Treatment	Group	Technique
Groups primary endpoints							
CR_{mrd}	NCT04284787	II	76	>60	Pembrolizumab, Azacitidine, venetoclax	Unfit	Duplex sequencing, MFC
	NCT03150004	II	90	>18	CLAG-M	R/R secondary AML	MFC
	NCT04476199	II	100	60–75	Venetoclax, decitabine	*De novo*, alloSCT	MFC, cytogenetics, RT-qPCR
	NCT03573024	II	36	18–59	Venetoclax, azacitidine	*De novo*	MFC
	NCT03701295	I/II	36	>18	Pinometostat, azacitidine	11q23	Unknown
	NCT03654703	II	100	3–18	Cyclophasphamide regimens	Pediatric R/R	MFC
	NCT01831232	NA	24	18–74	Idarubicin, cytarabine, pravastatin sodium	*De novo* AML MDS	MFC
	NCT04196010	I	45	>18	CI-CLAM	AML r/r or other high-grade myeloid neoplasms	Unknown
	NCT04214249	II	124	>18	Pembrolizumab + intensive chemotherapy	*De novo*	MFC
Proportion MRD negativity/ positivity	NCT04168502	III	414	18–60	Gemtuzumab, glasdegib	*De novo*, favorable intermediate risk	Unknown
	NCT04093505	III	252	>60	GO, glasdegib	*De novo*, post remission	MFC
	NCT04000698	I/II	25	<25	Different targeted therapies	Pediatric R/R	Unknown
	NCT03699384	I/II	0	>18	Azacitidine Avelumab	MRD positive	MFC
	NCT02614560	I/II	14	18–75	Vadastuximab Talirine	R/R AML	Unknown
	NCT04347616	I/II	24	>18	NK cell therapy	R/R AML	MFC/PCR
MRD change/ conversion	NCT03737955	II	36	>2	GO	MRD positive + prior treatment	MFC/PCR
	NCT01677949	II	0	<60	Clofarabine, cyclophosphamide, etoposide	ALL, AML	MFC/PCR
	NCT00863434	II	2	18–75	Clofarabine, Cytarabine	MRD positive	MFC
	NCT03697707	II	20	>18	Dendritic cell therapy	R/R AML persistent MRD	MFC
	NCT03021395	I/II	300	14–55	Decitabine	After consolidation	Unknown
MRD not specified	NCT04209712	Early phase I	6	1–80	NK infusion	MRD positive, after two cycles chemotherapy and no SCT	MFC
	NCT01828489	III	300	0–80	Cytarabine/fludarabine, DaunoXome, etoposide/ cytarabine	Children/adolescents	MFC
	NCT00965224	II	50	>18	Dendritic cell therapy	Myeloid leukemia and Myeloma	WT1 PCR
	NCT04086264	I/II	212	18–120	IMGN632, venetoclax, Azacitidine	CD123 positive AML	MFC
	NCT01347996	IV	84	>18	Histamine, IL-2	AML in CR1	RQ-PCR
	NCT03665480	II/III	122	14–65	G-CSF	*De novo*	Unknown

This represented a promising change as compared to the previous NCRI AML16 trial in which the absence of an intensified arm resulted in a 3 year OS of 46% *versus* 24% in MRD negative and MRD positive patients, respectively. All together, these results indicate that GO may have a role in reducing the level of MRD as compared to chemotherapy alone, therefore generating a superior quality of the mCR. A final issue pertains the role of MRD positivity prior to the ASCT. Publications of some retrospective analysis demonstrated that patients with a MRD positive status prior to the ASCT had, after the transplant, an OS superimposable to the one of subjects with active disease. (Araki D *et al*, JCO 2016;34:329- 336 – Hourigan C et al, JCO 2016;34:2557-2558). Based on this, the scientific community may derive the erroneous indication that patients who are MRD positive pre-ASCT should not be transplanted. Indeed, these publications suffer from biases that should induce caution and that deserve interpretations. These were retrospective analysis, including a heterogeneous population of patients (< and > 60 years) and heterogeneous conditioning regimens (reduced intensity or myeloablative). Moreover, there was a higher proportion of adverse karyotype and secondary AML in the group of MRD positive patients. At variance with the conclusions of these publications, a HOVON-SAKK retrospective analysis of 547 patients demonstrates that, even though all categories benefit from the ASCT, the absolute benefit was greater in the pre-ASCT MRD positive patients than pre-ASCT MRD negative. Although MRD assessment appears as a valuable prognostic tool to include in clinical trials, some issues are still awaiting solution: the role of leukemic stem cell, the role of MRD in older patients, the role of MRD in the era of new agents. Most of our knowledges in terms of MRD detection have been generated in the context of trials of intensive chemotherapy. Therefore, there is a need to understand the kinetic of MRD and its prognostic implications when new agents are delivered. In strict correlation with this, there is need to understand the role of MRD during maintenance therapy. The advent of new agents has revived such an approach and MRD assessment may have a critical role also in this context. Finally, the role of MRD assessment as a surrogate end point should be pointed out. The scientific community, the regulatory agencies and the pharmaceutical companies are hugely interested in such a topic in the attempt to accelerate the approval process of new drugs.

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CURRENT TREATMENT OF PATIENTS WITH RELAPSED/ REFRACTORY ACUTE MYELOID LEUKEMIA

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Introduction

In the recent years, research in the underlying pathogenic mechanisms of acute myeloid leukaemia (AML) has led to remarkable advances in our understanding of the disease. In addition, different newly approved treatment options, with the majority of new drugs targeting specific gene mutations and/or pivotal cell survival pathways have become available. This has expanded the access of treatment for patients with high risk AML, including those with relapsed/refractory disease who are not eligible to receive traditional salvage chemotherapy. Noticeably, these newer treatments have the potential to outperform traditional chemotherapy as well. Currently, approximately 60% to 80 % of young/adults with AML are expected to attain complete remission (CR) after appropriate induction therapy based on intensive chemotherapy; comparable CR rates have been more recently reported in older patients with new approaches based on venetoclax combination with hypomethylating agents or low dose cytarabine (ARA-C). Notwithstanding, more than half of patients still relapse and 10-40 % fail to achieve CR following two induction courses (primary refractory AML according to European Leukemia Net definition). A general agreement exists concerning the administration of aggressive salvage therapy in young adults followed by allogeneic stem cell transplantation; on the contrary, different therapeutic approaches varying in intensity, from conventional salvage chemotherapy based on intermediate-high-dose ARA-C to best supportive care, are currently considered in the relapsed older AML patient population. In general, treatment of relapsed and refractory AML is very challenging, with poor response rates and low chance for cure. This is especially true when treating patients who are elderly, have clinically relevant comorbidities and therefore unfit for traditional salvage chemotherapy regimens. Additionally, these patients are often not candidates for allogeneic stem cell transplant (allo-SCT) given comorbid conditions and lack of suitable donors. Notwithstanding, while relapsed AML remains a challenge for both patients and clinicians, knowledge of the molecular pathogenesis of the disease is fast in progress, potentially leading to further improvement of therapeutic results with potentially personalized approaches in most patients.

Pathogenesis of AML relapse

AML is an extremely heterogenous disease caused by mutations occurring during the process of myeloid differentiation and proliferation. Recent multiomic-studies, including analysis of genetic alterations at the single-cell resolution, have revealed a high heterogeneity of lesions in over 200 recurrently mutated genes affecting disease initiation, clonal evolution and clinical outcome. Following induction chemotherapy, whatever its intensity, residual AML cells survive in an altered chemoresistant state and result in disease relapse. Leukemia cells with stem cell characteristics, commonly defined as leukemic stem cells (LSCs), are thought to be at the origin of relapse initiation.. In an individual patient, the underlying model of clonal evolution can be assessed by comparing the extent of any single mutation at multiple time points. Clonal evolution during disease progression and therapy occurs in both linear and branched models, with a clear order of mutational events. In linear evolution, mutations of the major clone present at diagnosis are also found at relapse, accompanied by additional mutations and are unlikely to get lost; in particular, mutations reverting a mutated allele back to its wild type configuration are very rare events. In contrast, the loss of a mutation is typical of branching evolution. The dominant clone at diagnosis disappears after treatment and a new clone that is resistant to the therapy is found at relapse. A further possibility is represented by additional mutations which are detected at relapse; in these cases , the

clone detected at relapse is the result of an evolution from a common ancestor that was found at diagnosis. In general, in neither of the two models, the relapse has evolved from the dominant clone at diagnosis itself. In conclusion, during the disease progression, individual AML populations may follow distinct models of clonal evolution and the presence and the extent of mutations at different time points define distinct dynamic changes. Linear evolution is chracterized by stepwise acquisition of single mutations, whereas the eradication of the dominant clone, followed by outgrowth of a subclone define the branching evolution. Given that the molecular profile of AML is changing during the disease, any patient in relapse must be reinvestgated at molecular level for the possibility of detection of druggable mutation.

Prognostic factors in relapsed AML

Different prognostic factors, in the context of an extremely disappointing overall survival (OS), have been demostrated as determinant in affecting OS in relapsed patients with AML (Table 1); in particular, age at relapse, the duration of the first CR, cytogenetic risk at diagnosis and previous allo-SCT in CR1 were found in most studies as particularly relevant. These four easily appliable clinical parameters were integrated in a prognostic index, which was effective for estimating the outcome of AML patients in first relapse.A simplified prognostic score based on the multivariate analysis of 138 refractory/relapsed AML patients considered three subgroups with striking different outcomes at 2 years: no adverse factor (favourable, N=36): OS 58%, EFS 45%; one adverse factor (intermediate, N=54): OS 37%, EFS 31%; two or three adverse factors (poor, N=43): OS 12%, EFS 12% (P<10(-4), P=0.001). This Prognostic Scoring System was then validated on an independent cohort of 111 refractory/relapsed AML patients and used three clinical and biological parameters routinely applied, i.e. disease status (relapse <12 months, including refractory patients), FLT3-ITD-positive status and high-risk cytogenetics, It allow to discriminate around two third of the patients who should benefit from a salvage intensive regimen in the setting of refractory/relapsed AML patients. The other one third of the patients should receive investigational therapy.[4] In a Japanese experience, Kurosawa et al. found that both achieving second complete remission and salvage bone marrow transplantation in second complete remission were crucial for improving the prognosis of AML patients after first relapse.

Table 1. More relevant prognostic factors in relapsed/refractory AML.

Factor	Comment
Age	Comorbidity, unfavorable cytogenetic at diagnosis, difficulties in accrual into clinical trials
Duration of first CR	Lower CR2 rate, more frequent refractory relapse
Cytogenetics at diagnosis	Lower CR2 rate, more frequently refractory to induction treatment, need for experimental tretment,
Previous allo-SCT	Lower CR2 rate, more frequent refractory relapse
FLT3/ITD status	Lower CR2 rate, in most cases relapse occurs after allo-SCT
Relapse after treatment with hypomethylating agents +/- venetoclax	Poor response to conventional salvage therapy, need for experimental approaches

Treatment of relapsed/refractory AML

For many years, intensive salvage chemotherapy having intermediate/high dose ARA-C as backbone has been the standard of care for R/R patients with AML. In absence of a standard regimen which can be universally reccomended, we prefer IDA-FLAG for patients managed with three plus seven regimens in induction and MEC for those pretreated with fludarabine based regimens. Expected CR rates with these regimens are around 29%–66%, depending on CR1 duration, age of patients, cytogenetic and molecular findings at diagnosis and previous allo-SCT. None of these regimens have shown superiority over the others, highlighting negligible progress over the years. The situation is more complex now, in that new options are available. In particular, it is fundamental that any patient is reinvestigated at relapse for cytogenetic and molecular profiling, given the possibility of druggable mutations. In general, medical assessment, cytogenetic and mutational analysis and potential eligibility in a clinical trial (preferred) must be considered. Allogeneic HSCT is the treatment of choice for AML patients relapsing after salvage chemotherapy provided that CR2 or substantial reduction of bone marrow blast precentage (at least less than 10%) is achieved. Transplant eligibility depends on patients' age as well as comorbidities and this is particuarly relevant on a clinical ground in a disease whose median age is 65 yeras. A subgroup of patients with particularly unfavorable genetic charcteristics and therefore unlikely to benefit from conventional salvage chemotherapy (early relapse, complex Karyotype, tp53 mutations) could benefit from direct allogeneic . Ideally, all poor-risk AML patients, particularly older ones with refractory/relapsed diseas would be enrolled in clinical trials based on the use of new agents. However, in daily practice, different factors represent common obstacles, including patient frailty and comorbidities, caregiver availability, and social support dynamics. In addition, protocol eligibility criteria are often stringent *a*nd account for further exclusion.

An emerging clinical challenge concerns the treatment of older AML patients who relapse after CR or progress after any response following initial therapy with HMAs. In this category, intensive chemotherapy in most cases was already excluded at the time of diagnosis and therefore it should be even more so at the time of relapse. Moreover, low CR rate and high treatment-related mortality further discourage any intensive approach. In the absence of a clinical trial based on the use of experimental drugs, best supportive care and/or hydroxyurea for the control of leukocytosis still represent the best option for this subset of patients, with the only aim of improving quality of life in an outpatient setting. The use of venetoclax in combination with azacitidine or decitabne (depending on the drug used at diagnosis) could be considered in the context of clinical trials. Therapeutic options are summarized in Table 2.

Table 2. Therapeutic options for refractory/relapsed AML

Factor	Comment
Conventional chemotherapy based on intermediate/high dose ARA-C	CR2 rate ranging from 25 to 60 % depending on prognostic factors indicated in table 1
Allogeneic stem cell transplant	Only curative option. Better results in CR2 or low bone marrow blast count (< 10 %). Difficult to perform in older patients
Gilteritinib	Tretament of choice for relapsed FLT3 positive patients (either ITD or TKD mutation); oral administration
Quizartinib	Approved in Japan for relapsed FLT3 positive patients
Ivosidenib	Approved in US for relapsed AML patients harboring IDH2 mutation; oral administration. Differentiation syndrome can occurr.
Enasidenib	Approved in US for relapsed AML patients harboring IDH1 mutation; oral administration. Differentiation syndrome can occurr.
Venetoclax + HMA	The combination should be limited to very high risk patients in the context of clinical trial.
Clinical trial	Preferred option for most patients

Hypomethylating agents (HMAs)

Several retrospective study has investigated the efficacy of HMAs in R/R AMl. Recently, the efficacy of HMAs in R/R AML was investigated in a large international patient cohort. Using an international multicenter retrospective database, the authors investigated the effectiveness of HMAs in R/R AML and evaluated for predictors of response and OS.

655 patients (median age: 65 years) received azacitidine (57%) or decitabine (43%), including 290 refractory patients (44%) and 365 relapsed patients (56%). The best response to HMAs was CR (11%) or CR with incomplete haematological recovery (CRi; 5.3%), with an addi-

tional 8.5% of patients showing hematologic improvement. The median OS was 6.7 months, with significant differences based on best response: patients who achieved CR and CRi had a median OS of 25.3 and 14.6 months, respectively, compared to 6.7 months for the overall population. In multivariate analysis, the presence of ≤5% circulating blasts and 10-day decitabine therapy were associated with improved response rates, whereas a shorter OS was reported for patients with >5% circulating blasts and >20% bone marrow blasts. Given the paucity of patients achieving CR with HMAs, it is easy to understand that such therapy should not be considered the best options for these patients.

Novel agents

in case of relapse, the first evaluation to be done concerns the identification of patients with molecular alterations, which can be treated with targeted therapies. Molecularly targeted therapies have been shown to be more effective and less toxic than chemotherapy, and therefore should be preferred both in the young fit and in the elderly unfit population, at relapse.

FLT-3 inhibitors

FLT-3 mutation is the most frequently identified genetic mutation in

Table 3. Ongoing clinical trials for R/R AML.

ClinicalTrials.gov Identifier	Study	Drugs	Phase, patients
NCT04196010	Continuous Infusion Chemotherapy (CI-CLAM) for the Treatment of Relapsed or Refractory Acute Myeloid Leukemia or Other High-Grade Myeloid Neoplasms	Cladribine and cytarabine via CIV on days 1-2, 1-3, 1-4, 1-5, or 1-6 depending on dose level assignment, and mitoxantrone via CIV on days 1-2 or 1-3 depending on dose level assignment.	Phase I, 45 pts
NCT03067571	Daratumumab in Treating Patients With Relapsed or Refractory Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome	Daratumumab IV 1, 8, 15 and 22 of cycles 1-2, on days 1 and 15 of cycles 3-6, and on day 1 of subsequent cycles.	Phase II, 36 pts
NCT04477291	A Study of CG-806 in Patients With Relapsed or Refractory Acute Myeloid Leukemia	CG-806 will be given orally in ascending doses starting at 450 mg PO BID until the maximum tolerated dose or candidate recommended Phase 2 dose is reached.	Phase I a/b, 80 pts
NCT04173585	TEAM-Trial: Targeting Epigenetic Therapy Resistance in AML With Bortezomib (TEAM)	Bortezomib (1.3 mg/m2) sc on day 1 and 3. Cytarabine (1g/m² twice daily) iv over 3 hours on day 1, 2 and 3 Gemtuzumab Ozogamicin (3 mg/m², up to a maximum of one 5 mg vial) iv over 2 hours on day 1 and day 4	Phase II, 50 pts
NCT03132454	Palbociclib and Sorafenib, Decitabine, or Dexamethasone in Treating Patients With Recurrent or Refractory Leukemia	Experimental: Arm I (palbociclib, sorafenib) palbociclib PO QD on days 1-28 plus sorafenib PO QD on days 1-28 beginning on cycle 2, every 28 days for up to 8 cycles Experimental: Arm II (palbociclib, decitabine)palbociclib as in Arm I. Beginning cycle 2, patients receive palbociclib PO QD on days 1-7 and decitabine IV QD over 1 hour on days 8-17 of cycle 2 and days 8-12 of cycles 3-8, every 28 days for up to 8 cycles. Experimental: Arm III (palbociclib, dexamethasone) palbociclib as in Arm I, dexamethasone PO QD or IV over 15-30 minutes on days 1-4 and 15-18 beginning on cycle 2, every 28 days for up to 8 cycles.	Phase I, 54 pts
NCT04989335	Bisantrene Combination for Resistant AML	Fludarabine IV 10 mg/m2 days 1-5 Clofarabine IV 30 mg/m2 days 1-5 Bisantrene IV 250 mg/m2 days 1-5.	Phase II, 29 pts
NCT03904251	CPX-351 and Gemtuzumab Ozogamicin in Treating Patients With Relapsed Acute Myeloid Leukemia	Induction: CPX 351 (44mg/m2 - 100mg/m2) IV on days 1, 3, and 5, and gemtuzumab ozogamicin 3 mg/m2 (max 4.5 mg) IV on day 7, or days 4 and 7, or days 1, 4, and 7 in the absence of disease progression or unacceptable toxicity. Consolidation: Patients who achieve CR/CRi receive consolidation therapy at the discretion of the treating physician and/or proceed to allogeneic HSCT.	Phase I, 33 patients
NCT04774393	Decitabine/Cedazuridine and Venetoclax in Combination With Ivosidenib or Enasidenib for the Treatment of Relapsed or Refractory Acute Myeloid Leukemia	Experimental Arm A decitabine/cedazuridine PO daily on days 1-5, venetoclax PO daily on days 1-14, and ivosidenib PO daily on days 1-28, every 28 days for up to 12 cycles. Experimental Arm B decitabine/cedazuridine PO daily on days 1-5, venetoclax PO daily on days 1-14, and enasidenib PO daily on days 1-28, every 28 days for up to 12 cycles.	Phase Ib/II, 84 pts
NCT03683433	Enasidenib and Azacitidine in Treating Patients With Recurrent or Refractory Acute Myeloid Leukemia and IDH2 Gene Mutation	Azacitidine SC or IV over 30 minutes on days 1-7 and enasidenib mesylate PO QD beginning on day 1. Cycles repeat every 4-6 weeks in the absence of disease progression or unacceptable toxicity.	Phase II, 50 pts

AML, and it is frequently associated with unfavorable outcome. Currently, FLT3 inhibitors, namely gilteritinib, quizartinib, sorafenib, midostaurin and crenolanib which have shown promising activity as single agent, are under evaluation in clinical trials, in combination with HMAs or chemotherapy. At present, gilteritinib is the only FLT-3 inhibitors approved by regulatory authorities and available on the market for R/R AML. In the phase III ADMIRAL study, 371 adult patients with R/R AML were randomly assigned in a 2: 1 ratio to receive gilteritinib (120 mg per day) or salvage chemotherapy. The two primary endpoints were OS and the percentage of patients in CR with complete or partial hematological recovery (CRh). The drug was generally well tolerated. In a challenging scenario, characterized by low response rates and median survival in the 4-6 months range with conventional chemotherapy, single agent gilteritinib doubled the composite response rate (cCR), with a significant improvement in median OS (9.3 versus 5.6 months for standard chemotherapy). Furthermore, a reduction in transfusion requirements was also observed for most patients, in absence of CR achievement, thus resulting in a clinical benefit, and survival benefit was observed in most subgroups.

The data from the Admiral trial showed a significant advantage for gilteritinib, if compared to chemotherapy, but remain not entirely satisfactory. For this reason, novel combinations are under evaluation. Among others, venetoclax plus gilteritinib combination was able to induce a modified composite complete remission (mCRc) rate of 83.8% in a cohort of AML patients with FLT3 mutation, heavily pretreated, most of whom had previous exposure to FLT3 inhibitors. Cytopenias were evident but manageable with dose interruption/modification in subsequent cycles. Non-hematological toxicities were modest and the combination was well tolerated.

IDH1/IDH2 inhibitors

Isocitrate dehydrogenases (IDH) 1 and 2 mutations affect approximately 20% of AML patients. Targeting IDH1 and 2 mutations has recently led to the development of an individualized treatment strategy, by promoting differentiation and maturation of the malignant clone. Ivosidenib is the first-in-class, selective, allosteric IDH1R132 inhibitor approved by the U.S. Food and Drug Administration FDA for patients with R/R IDH1-mutated AML.

Ivosidenib 500 mg daily was administered to 174 adults with IDH1-mutated R/R AML in a single-arm trial, resulting in CR + CRh rate of 33%, with a median follow-up of 8.3 months. Median duration of response was 8.2 months, and an additional 37% of patients became transfusion independent (20). A subsequent trial with 125 R/R AML patients showed ORR, cCR, and CR rates of 41%, 30%, and 22%, respectively. After a median follow-up of 14.8 months, the median OS was 8.8 months, with 18-month survival for cCR patients of 50%. Molecular remission was observed in 21% of patients with cCR and was associated with longer OS. Enasidenib is the first-in-class selective inhibitor of mIDH2. Enasidenib at 100 mg daily in 28-days cycles was initially tested in 119 R/R AML patients (median age 67 years), including 32% refractory to initial induction, 23% who had relapsed within 1 year of treatment, and 11% who relapsed after prior allo-SCT). ORR was 40.3% (95% CI, 33-48%), and CR rate 19.3%. Median time to first response was 1.9 months (range, 0.5-9.4 months). The median duration of response was 5.8 months and median OS in patients with R/R AML was 9.3 months (8.2–10.9 months) with an estimated one-year survival of 39%. In patients who achieved a CR, the median OS was 19.7 months.

Both ivosidenib and enasidenib showed a better clinical activity in comparison to intensive chemotherapy, with a significant decrease in side effect and a dramatic increase in quality of life. Accordingly, these drugs should be preferred to intensive chemotherapy in R/R AML patients bearing IDH1 or IDH2 mutations.

Venetoclax +/-HMAs

Quite a few clinical trials with venetoclax alone, venetoclax + HMA or venetoclax + low dose cytarabine plus a third novel agent are currently underway in patients with R/R AML. As the large majority of these studies are not randomized, it is difficult to assess the weight of venetoclax, alone or in combination, in the R/R-AML setting.

Recently, a systematic review and a meta-analysis was performed to evaluate the efficacy of venetoclax in R/R AML. Seven studies enrolling in total 224 R/R AML patients (median age 68.9 years), treated with venetoclax alone in 2 studies and with venetoclax plus HMA/LDAC in 5, were analyzed. The primary outcome was a combined CR/CRi rate. A total of 156 patients (69.6%) had previously received HMA and 48 patients (21.4%) had a previous allo-SCT. The ORR was 31.1% (20.7% venetoclax monotherapy, 38.7% combinations), with a CR/CRi rate of 26.7% (20.7% monotherapy, 32.8% combinations). The median duration of follow-up was 7.3 months (range: 1.8-15.8). There was significant heterogeneity between studies examining venetoclax + HMA/LDAC for both ORR ($p= 0.02$) and CR/CRi ($p = 0.004$). Although response rates were encouraging, median OS was disappointing, ranging from 1.8 to 7.8 months for Venetoclax monotherapy and from 3.0 to 6.6 months for combinations. Among responding patients, the median OS was higher, and approached 1 year, for both patients receiving venetoclax alone and in combination. As a whole, these data do not support the routinely use of venetoclax in the R/R AML setting.

Immunotherapy

Immunotherapy is an emerging, promising strategy in AML that will be further investigated in ongoing trials. Currently, it is still unclear its ideal setting, and are still lacking biomarkers predictive for response. Immune-based therapeutic modalities comprise monoclonal antibodies, T cell engager antibodies, alloreactive NK cell, checkpoint blockade via blockade of PD-1/PD-L1, CTLA4, TIM3 and macrophage checkpoint blockade via the CD47/SIRPa axis. Several agents, such as Flotetuzumab, Magrolimab, Sabatoliomab and many others are now being tested in monotherapy or in combination with chemotherapy, venetoclax or HMA. Phase II trials demonstrated that immunotherapy could provide long-term disease control and may contribute to improving quality of life of R/R AML patients. Expansion cohorts phase 3 trials of agents are ongoing or planned in a near future. Immunotherapies seems to be particularly effective in high-risk patients with TP53 mutations.

Future Directions

Progress in the treatment of AML is strictly related to a more precise understanding of the pathobiology of the disease. Not by chance, after more than twenty years of substantial lack of therapeutic innovation, in the last 5 years the FDA approved several different new drugs. Among these, three were specifically approved for patients with R/R disease and represent a paradigm of precision medicine, even though results of either gilteritinib or IDH inhibitors are still unsatisfactory and must be improved. Of interest, targeted therapy can also represent and ideal bridge to allogenic transplantation. For high-risk patients such as older, early relapsing and primary refractory ones as well as those relapsing after allo-SCT, it is clear that conventional salvage chemotherapy has a very limited or absent role and, in most cases, should be avoided. These patients represent an ideal subset for experimental trials, based on novel agents possibly addressing specific leukemia pathways and/or targeting the immune system.

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TREATMENT OF HIGH-RISK TRANSPLANT-ELIGIBLE MULTIPLE MYELOMA PATIENTS

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Identifying high-risk multiple myeloma at diagnosis

Criteria allowing the identification of high-risk multiple myeloma (MM) are standardized in the Revised International Staging System (R-ISS), that include markers of tumour burden and replication (beta-2 microglobulin, LDH and albumin serum level) and biological prognostic factors [represented by 3 FISH karyotype abnormalities del(17p), t(4;14) and t(14:20)].[1] The outcome impairment of R-ISS 2 and R-ISS 3 stages patients appeared to be independent from the treatment administered. However, several other clinical features have been recognized to compromise the outcome, including age, renal failure, extramedullary localizations, circulating plasma cells and IgD M protein. Recently, the biological high-risk factors have became more and more important and are represented by further FISH cytogenetic abnormalities such as t(14;16), gain1q and del1p and alterations of groups of genes (genetic signatures) recognized through gene expression profile (GEP) by different research groups (e.g. UAMS, IFM, HOVON). Ultra high-risk MM and double or triple hit MM are new definitions to identify patients with a very poor outcome and an expected OS inferior to 2-3 years, mostly characterized by the combination of 2 or more unfavourable genetic abnormalities.[2]

A risk-adapted strategy or a "one fit all" treatment approach

Although there is a scientific consensus about the heterogeneity of the biology and the clinical outcome of MM patients, a risk-adapted strategy is not the standardized policy yet due to the following factors: 1) the identification of high-risk patients is still somewhat uncertain, since the previously mentioned R-ISS staging is just "the tip of the iceberg" of a complex biology; 2) very few clinical studies have been dedicated to high-risk patients, therefore responses to different classes of drugs, single compounds or drug combinations should be deduced by comparisons among subgroups of patients, that are not the specific study objectives or have no adequate statistical power or are not uniformly identified, explaining the frequently conflicting clinical results; 3) the regulatory agencies, especially in Europe and in Italy, are late to authorize new drugs in comparison with USA, so that there are no enough drugs in the market to construct risk-adapted strategies in the real-word population. While in USA the policy of the Majo Clinic has been orientated to differentiated courses from induction to maintenance between standard and high-risk patients for several years, either in the setting of transplant-eligible candidates or in elderly patients (2), the majority of the current clinical guidelines of the scientific society such as the European society of Medical Oncology (ESMO) or the Società Italiana di Ematologica (SIE) recommended a uniform first-line treatment approach, suggesting less evidence-based modifications for high-risk patients in a few steps of the front-line therapy.

Induction

The standard first-line treatment for fit patients younger than 70 years consists of 4-6 cycles of bortezomib-based induction, one or two autologous stem cell transplantations (ASCT) and lenalidomide maintenance until progression. Triplets containing bortezomib before ASCT demonstrated superiority in terms of responses and long-term outcome in comparison with duplets both in standard and high-risk patients in prospective studies and in meta-analysis.[3-4] Recently, quadruplets combining monoclonal antibodies with triplets have demonstrated higher and deeper responses in comparison to standard triplets, but data on subgroups of high-risk patients are still conflicting and immature (Table1). In the phase 3 CASSIOPEIA trial the benefit of Daratumumab-VTD in comparison to VTD alone as a pre-ASCT and post-ASCT regimen was not statistically significant in the high-risk group [defined as del(17p) or t(4;14) or ISS 3] in reaching stringent complete response (sCR) (HR, 0.83; 95% CI, 0.42–1.66) and in the risk of progression or death at a median follow-up of 18 months (HR, 0.67; 95% CI, 0.35–1.3) (5). Moreover, in the phase 2 randomized GRIFFIN the subgroup analysis on high-risk patients did not favor Daratumumab-VRD versus VRD arm, neither in achieving sCR (HR, 0.52; 95% CI, 0.09–2.90) nor in achieving minimal residual disease (MRD)-negativity (HR, 1.5; 95% CI, 0.32–6.99).[6] Two recent prospective studies enrolled exclusively patients with high-risk clinical and/or cytogenetical features. The GMMG-CONCEPT trial consists of 6 cycles of Isatuximab-KRD induction, 4 cycles of Isatuximab-KRd consolidation followed by Isatuximab-Kd maintenance: preliminary data showed deep responses (46%CR, 44%VGPR) after induction, adequate peripheral blood stem cell collection and low rates of non-hematological toxicity.[7] The second study specifically addressed to high-risk disease was the phase 2 SWOG-1211 trial, randomizing patients to receive VRd induction and maintenance, with or without Elotuzumab and deferring ASCT until progression. Even though the addition of Elotuzumab to VRd backbone did not improve patient outcome, in both arms PFS and OS exceeded the original statistical assumption (PFS >30 months, OS >60 months), which supports the notion that continuous triplet therapy might be favorable in this group of patients.[8] The current practical approach to high-risk patients is to administer a four-drug induction combining daratumumab to VTD or VRD, since these associations showed to achieve the highest rates of complete remission, waiting for mature clinical results of longterm outcome and subgroups comparison. In patients with primary plasma-cell leukemia or significant extramedullary disease, multi-agent combination chemotherapy such as bortezomib/dexamethasone/ thalidomide/cisplatin/doxorubicin/ cyclophosphamide/ etoposide (VDT-PACE) may be preferred initially to achieve rapid disease control.

Autologous stem cell transplantation: single versus double

It is a standard of care to proceed to ASCT in eligible patients as a consolidation of the first-line treatment, including high-risk patients, even in the era of novel drugs. The long-term results of 2 recently published randomized studies highlighted the benefit of a second ASCT performed 2-3 months after the first procedure in the high-risk population. In the EMN02/H095 double ASCT significantly improved 5-year OS in comparison with single ASCT and the HR favoring tandem ASCT was higher in patients carrying one or more cytogenetic abnormalities and particularly in those with del(17p). In fact, in this latter subgroup double SCT was likely to overcome the adverse prognostic factor of del(17p) on OS and PFS.[9] The update of the STAMINA study comparing lenalidomide maintenance or RVd consolidation followed by lenalidomide maintenance or second transplant reported a significantly higher 6-year PFS when high-risk patients received tandem ASCT in comparison to single ASCT (43.6% and 26%, respectively; p = .03).[10] Moreover, tandem ASCT was associated with longer OS in comparison to single ASCT and to ASCT followed by a reduced-intensity allogeneic stem cell transplantation (RIC Allo-SCT) in 488 patients with extramedullary disease (100%) and unfavourable cytogenetics (41%) in a EBMT retrospective study.[11]

Consolidation and maintenance

Consolidation consists of the administration after ASCT of 2-4 cycles of the same drug combination given at induction. Generally, a short 3-4 cycles induction has been followed by the same combination at attenuated doses. In clinical trials consolidation has showed to increase rate and deepness of responses and has continued to be planned in several recent study designs incorporating monoclonal antibodies and aiming to improve MRD (mentioned in the above sections). In real word, consolidation lacks formal authorization (at least in Italy). Moreover, it not clear if the same response improvement demonstrated by consolidation can be achieved also with a longer induction (from 4 to 6 cycles)

Lenalidomide maintenance until progression has been shown to be associated with a significant improvement of PFS and OS following ASCT in comparison with placebo or no therapy. The impact of lenalidomide maintenance in patients with high-risk MM is unclear. In a meta-analysis, no significant OS benefit was seen in these subsets of high-risk patients.[12] However, in the Myeloma XI trial that was not part of the meta-analysis, PFS and OS were prolonged across all subgroups, either standard risk patients (no cytogenetical abnormality), or high-risk patients (one cytogenetical abnormality), or ultra-high risk patients (two or more cytogenetical abnormalities). However, these subgroup analyses should be interpreted with caution since results were not powered to detect significant differences and could be influenced by the fact that most patients did not received a standard bortezomib-based induction.[13]

The suggestion that high-risk patients may benefit from a maintenance including a proteosome inhibitor came from the long-term follow-up of the HOVON-65/GMMG-HD4 trial study, where patients with del(17p) (but not the subsets with other adverse cytogenetic abnormalities) treated with a bortezomib-based regimen before and after ASCT exhibited better PFS and OS than patients in the VAD arm followed by thalidomide maintenance (60-month PFS 22% *vs* 5%, respectively, and 60-month OS 65% vs 18%, respectively) (14). Maintenance with the oral proteosome inhibitor ixazomib for 2 years after ASCT improved PFS in patients with poorer prognosis, such as ISS stage 3 or presence of high risk cytogenetic; however, we should acknowledge that the study was not powered for these subgroups and the median PFS after ixazomib in the whole population was much shorter than that reported after lenalidomide (16). Collecting these few clinical data on maintenance and the larger evidence about activity of proteosome inhibitors in transplant ineligible and relapsed/refractory patients, we can hypothesize that bortezomib alone given every other week or low intensity VRd for a limited period of time after ASCT is preferable for high-risk patients, even if an evidence based recommendation cannot be made and bortezomib maintenance has not been authorized in clinical practice yet.

Although the standard maintenance is recommended until progression on the basis of the design and the clinical results of the studies leading to lenalidomide approval, several factors such as cost, toxicity, patient compliance have been raised the challenging issue of the optimal duration of maintenance . Maintenance is now evolving towards the administration of a combination of drugs including monoclonal antibodies, a limited period of treatment (up to 2-3 years) and MRD monitoring as potential tool for modulating maintenance. The results of the ongoing prospective clinical trials are eagerly waited in standard and high risk MM patients.

Allogeneic stem cell transplantation (allo-SCT)

Evidence that the graft versus myeloma effect can overcome high-risk clinical and biological features is scarce. Large randomized studies comparing upfront ASCT versus ASCT followed by a RIC allo-SCT were conducted in the last 2 decades, but they were not informative for the current clinical practice, since high-risk patients were selected with obsolete prognostic factors and novel drugs were not integrated in the treatment plans. One exception was the long-term follow-up of the German study, which suggested some survival benefit in patients with del(17p) treated with ASCT/allo-SCT in comparison with tandem ASCT (median OS 61 vs 23 months), although in small subsets of patients.[15] However, the risk of non relapse mortality non inferior to 15%, the significant morbidity associated to chronic graft versus host disease and the low rate of tumour eradication have substantially limited the application of up-front allo-SCT and shifted it at the time of relapse, in selected and motivated fit patients, with early relapse after ASCT, clinical or cytogenetical poor features and HLA matched donors. It can be hypothesized that the availability of new cellular immunotherapies such as bispecific monoclonal antibodies and CAR-T in the clinical practice and in earlier phases of disease will make the option of allo-SCT increasingly rare.

Table 1. Clinical results of monoclonal antibodies in the first-line treatment of high-risk patients (sCR: stringent complete remission; MRD: Minimal residual disease)

Author and reference number	study	design	High-risk patients/all patients (%)	response	outcome
Moreau et al,[5]	Phase 3 CASSIOPEIA	Daratumumab-VTd vs VTd x4 plus ASCT plus VTdx2	166/995 (17%)	No advantage in term of sCR and MRD for the experimental arm in high-risk patients	No advantage in term of 18-months PFS for the experimental arm in high-risk patients
Voorhees et al,[6]	Phase 2 randomized GRIFFIN	Daratumumab-VRd vs VRd x4 plus ASCT plus VRdx2	30/207 (14%)	No advantage in term of sCR and MRD for the experimental arm in high-risk patients	No advantage in term of 24-months PFS for the experimental arm in high-risk patients
Weisel K et al,[7]	Phase 2 GMMG-CONCEPT study	6 Isatuximab-KRD+-ASCT+4 Isa-KRD+ IsaKR maintainance	153/153 (100%)	CR 46% ≥VGPR 44%	Not reached
Usmani et al, [8]	Phase 2 randomized SWOG-1211 study	Elotuzumab-VRd vs VRd plus ASCT at relapse	103/103 (100%)	OR 85% vs 88% ≥VGPR 23% vs 26%	median PFS 31 vs 34 months median OS 86 months vs not reached

Conclusions

High-risk patients should be enrolled in prospective studies, if possible. An algorithm of a risk-adapted strategy in clinical practice is proposed in Figure 1. Results of ongoing clinical trials are warranted in order to improve the long-term treatment after ASCT .

FRONT-LINE TREATMENT OF HIGH-RISK MM PATIENTS

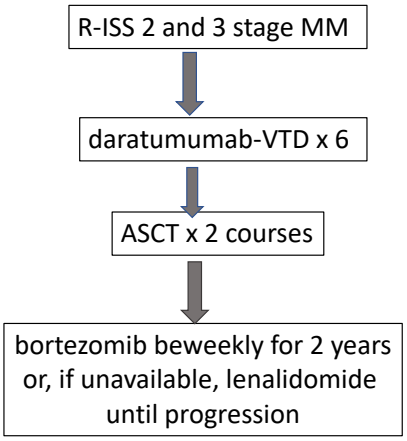

Figure 1. Proposed algorithm of first-line treatment in high-risk patients.

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TREATMENT OF ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: NEW THERAPEUTIC INDICATIONS

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Multiple myeloma (MM) is a disease of the elderly, with a median age at diagnosis of approximatively 70 years and more than one third of patients being older than 75 years. Historically, elderly patients would include those older than 65 years, usually assigned to non-intensive treatments, without autologous transplantation (ASCT), and therefore defined as transplant ineligible (NTE). Nevertheless, elderly MM patients represent a very heterogeneous population, including individuals in good physical conditions and without comorbidities, as well as frail ones with compromised health and functional status. Consequently, choosing and modulating treatment intensity in this population according to patient characteristics represent a big challenge for clinicians. The International Myeloma Working Group (IMWG) frailty score stratifies patients ≥65 years old into fit, intermediate fit and frail according to age, functional impairment and comorbidities, and it is a valid tool to guide clinicians in the choice of the most appropriate treatment.[1] Recommendations about the management of elderly patients according to their frailty status were published in the past years.[2] For example not all elderly patients are unsuitable for ASCT, and the age cut-off for ASCT eligibility has been moved to 70 years in clinical practice, as well as in ongoing clinical trials. Up to just two years ago, standard first-line treatment options for NTE MM patients outside clinical trials included lenalidomide-dexamethasone (Rd), bortezomib-melphalan-prednisone (VMP) and bortezomib-lenalidomide-dexamethasone (VRD). These combinations proved to be effective, albeit progression-free survival (PFS) and overall survival (OS) were inferior compared to ASCT-containing regimens. The addition of anti CD38 monoclonal antibody daratumumab (dara) to standard VMP and Rd induced unprecedented results in two pivotal phase III clinical trials, completely revolutionizing the treatment algorithm for elderly MM patients (Table 1).

The ALCYONE trial compared standard VMP for 9 cycles to dara-VMP for 9 cycles followed by dara maintenance until disease progression in patients ≥ 65 years of age or considered NTE. Patients receiving dara-VMP had a significant PFS benefit (median PFS 36.4 vs 19.3 months, HR 0.42, $p < 0.001$), and a 40% reduction in the risk of death (HR 0.60, p=0.003) compared to those receiving VMP. Rates of minimal residual disease negativity (MRD, by next generation sequencing [NGS], sensitivity 10^{-5}) were significantly higher in the dara-VMP arm (28% vs 7%, $p < 0.001$), and MRD negativity was sustained at one year in 14% vs 3% of patients, likely also due to the effect of dara maintenance rather than fixed duration treatment. Dara-VMP was well tolerated, with similar rates of treatment discontinuation (7% vs 9%) and grade (G) ≥3 adverse events compared to VMP, besides a slightly higher incidence of G≥3 in-

fections (22% vs 15%) during cycle 1-9, that were also the most common toxicity during dara maintenance (11%).[3]

The MAIA trial compared standard continuous Rd to dara-Rd in a similar patient population. Again, the PFS advantage of dara-Rd was striking, with a median PFS that has not yet been reached after 48 months follow-up vs 34 months in the Rd arm (HR 0.54, p < 0.001). Rates of MRD negativity (NGS, sensitivity 10^{-5}) and sustained MRD negativity at one year were higher in the dara-Rd arm (31% vs 10%, p < 0.001; and 16% vs 3%, p < 0.001 respectively). Concerning safety, patients in the dara-Rd arm experienced higher rates of G ≥3 neutropenia (53% vs 37%) and infections (40% vs 29%), although treatment discontinuations due to toxicity were lower compared to Rd (11% vs 22%).[4]

The impressive results obtained in these trials led to the approval of dara-VMP in 2018 and dara-Rd in 2019 by FDA and EMA. Both regimens are currently used in clinical practice in several countries, including Italy, with more and more patients receiving these combinations. Nevertheless, given the heterogeneity of the elderly population, particularly in the real-life setting - which is very different compared with the selected patient population of clinical trials - some challenges still remain in everyday clinical practice.

How to treat elderly fit patients

According to the IMWG frailty score, fit patients are those under 75 years of age, without significant comorbidities (Charlson Comorbidity Index [CCI] ≤1) and with preserved functional status (ADL score > 4 and IADL score >5).[1] In the presence of good organ function, ASCT can be a valid option for fit patients and in most countries, including Italy, ASCT is the first choice of treatment in patients ≤ 70 years old (Figure 1). Ongoing clinical trials in transplant eligible patients evaluating the addition of antiCD38 monoclonal antibodies to induction and consolidation/maintenance regimens (eg EMN17/Perseus trial: daratumumab-VRD vs VRD; EMN24/Iskia trial: isatuximab-KRD vs KRD) enroll MM patients up to 70 years old. In patients over 70 years the advantage of ASCT is debatable. In the United States, ASCT is offered to virtually all patients with adequate renal, hepatic, pulmonary and cardiac function independently of age, with data about feasibility also in selected patients aged ≥ 75 years.[5] This approach is supported by data showing comparable outcome in terms of toxicity (non-relapse mortality 1% vs 0) and PFS (2-years PFS 66% vs 68%, p 0.4) in patients ≥ 70 years vs 60-69 years undergoing ASCT. In Europe, this approach is rarely pursued, and patients aged > 70 years are usually offered a non-transplant option, although ASCT can be considered for fit patients older than 70 years, possibly with a reduced intensity conditioning (eg melphalan 100-140 mg/m2). Nevertheless, considering the safety and efficacy of dara-VMP and dara-Rd regimens, the advantage of ASCT in those patients is questionable. Dara-based combinations in > 70 years old patients offer similar outcome in comparison with ASCT, with the advantage of sparing patients from the burden of high-dose therapy requiring hospitalization and longer recovery. No data about ASCT with dara-containing induction/consolidation regimens in elderly patients are available yet, and it is still to be defined whether this strategy could further improve the outcome. Data from prospective comparisons in a selected population of fit elderly patients > 70 years old receiving dara-based regimens with/without ASCT are needed to shed light on this issue. Currently, in Europe dara-containing combinations without ASCT represent the first choice of treatment for fit patients aged > 70 years.

How to treat frail older patients

Several definitions of frailty have been proposed over the past few years to define reliable parameters to characterize vulnerable MM patients in whom the main goal is to preserve quality of life by modulating treatment intensity to prevent toxicity. According to the IMWG frailty

Table 1. Efficacy and safety from regulatory trials on approved treatment regimens for elderly newly diagnosed myeloma patients.

Regimen	Median PFS (months)	Median OS (months)	Toxicity (G ≥ 3 AEs)	Discontinuation for AEs Toxic deaths
Rd vs MPT	26 vs 21.9	59 vs 49	Neutropenia 30% vs 45%	23% vs 27%
	HR 0.69, p < 0.001	HR 0.78, p 0.002	Infection 32% vs 17%	not reported
			VTE 8% vs 5%	
VMP vs MP	24 vs 16.6	56.4 vs 43	Neutropenia 40% vs 38%	15% vs 14%
	HR 0.48, p < 0.001	HR 0.69, p < 0.001	Thrombocytopenia 37% vs 30%	not reported
			Gastrointestinal 19% vs 5%	
			PNP 15% vs 0	
Dara-VMP vs VMP	36.4 vs 19.3	NR*	Neutropenia 40% vs 39%	7% vs 9%
	HR 0.49, p < 0.001	HR 0.60, p < 0.001	Thrombocytopenia 34% vs 38%	4% vs 4.5%
		(*median f-up 40 months)	Infection 22% vs 15%	
			PNP 1.4% vs 4%	
Dara-Rd vs Rd	NR* vs 34.4	NR*	Neutropenia 53% vs 37%	11% vs 22%
	HR 0.54, p < 0.001	(*median f-up 48 months)	Infection 40% vs 29%	7% vs 6%
	(*median f-up 48 months)		Diarrhea 8% vs 6%	
			Fatigue 9% vs 4%	
VRD vs Rd	41 vs 29	NR* vs 69	Hematologic 49% vs 49%	Not reported
	HR 0.74, p 0.003	HR 0.71, p 0.01	Infection 18% vs 14%	<1% in both arms
		(*median f-up 84 months)	Neurologic 35% vs 11%	

Abbreviations: PFS, progression-free survival; OS, overall survival; G, grade; AE, adverse event; Rd, lenalidomide-dexamethasone; MPT, melphalan-prednisone-thalidomide; VMP, bortezomib-melphalanprednisone; Dara, daratumumab; VRD, bortezomib-lenalidomide-dexamethasone; VTE, venous thromboembolism; PNP, peripheral neuropathy; NR, not reached; HR, Hazard Ratio.

score, "frail" patients can be ≥ 80 years of age or younger but with significant comorbidities or functional impairments. These patients represent an unmet clinical need, since they are excluded from most clinical trials. Indeed, the feasibility of daratumumab-based triplets/quadruplets in frail patients is debatable. A retrospective analysis of the ALCYONE trial demonstrated that the advantage of dara-VMP over VMP was maintained in frail patients (according to age, ECOG PS and CCI; median PFS 32.9 *vs* 19.5 months, respectively, HR 0.51, p <0.001), with non-frail patients showing longer PFS with dara-VMP but not with VMP (median PFS 45.7 *vs* 19.1 months, HR 0.36, p < 0.001) compared to frail ones.[6] Frail patients experienced higher rates of G 3-4 adverse events and toxic deaths occurred in 10.9% of frail patients (dara-VMP vs VMP 13% *vs* 8.6%). In the MAIA trial, the PFS advantage of dara-RD over Rd was maintained in frail patients (HR 0.62, p=0.003), and toxic deaths occurred in 12% of frail patients, with no differences between the two arms.[7] These data suggest that dara-based triplet/quadruplets might be suitable for frail patients. Nevertheless, one can argue that a real-life frail population was not included in the two studies. Indeed, in that study, exclusion criteria were Eastern Cooperative Oncology Group (ECOG) performance status > 2, impaired renal function with creatinine clearance < 30/40 mL/min, active cardiovascular disease or elevated hepatic enzymes.[6,7] A prospective study evaluating the combination of daratumumab, ixazomib and dexamethasone in frail patients according to the IMWG frailty score showed a median PFS of 14 months, and 51% rate of premature treatment discontinuation, due to toxic deaths in 9% of patients.[8] To date, no prospective real-life analysis on frail patients receiving daratumumab-Rd/VMP is available and further data are needed to confirm the feasibility of these regimens in frail patients. Possible strategies to make these regimens safer include reducing treatment intensity over time (eg discontinuing steroids), or adjusting the doses of the delivered drugs (eg reduced doses of lenalidomide or weekly bortezomib). A recent study demonstrated that in patients receiving Rd, reducing lenalidomide dose and discontinuing steroids after 9 cycles did not impair treatment efficacy while ameliorating safety.[9] The IFM2017-03 study will evaluate daratumumab plus lenalidomide vs. standard Rd in frail patients as a similar steroid-sparing strategy. Frail patients have been traditionally treated with doublets (eg low-dose Rd or Vd); nevertheless, the addition of daratumumab, especially to Rd, with dose-adjustment strategies to avoid toxicities, might be beneficial also for frail patients and could spare a proportion of them from the need of second line treatment, by inducing longer first line remissions (Figure 1).

How to choose between dara-VMP/Rd in real life

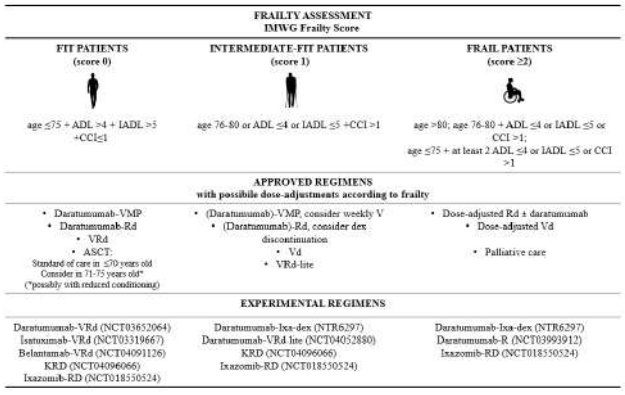
Although caution is needed when making cross-trial comparisons, the addition of daratumumab to Rd seems to induce a greater benefit compared to dara-VMP (median PFS not reached after 48 months of follow-up with dara-Rd; 36.4 months with dara-VMP). Nevertheless, to date no prospective comparison between dara-VMP and dara-Rd is available. Similarly, no prospective data evaluating VMP vs Rd are currently available. Yet, the ongoing REAL-MM study, comparing the two regimens in a real-life population, will provide some answers. Retrospective data showed no benefit of one regimen over the other (HR for PFS 0.96).[10] Consequently, to date, treatment choice between dara-VMP vs dara-Rd relies on their safety profiles and patient preference. Patients with pre-existing neuropathy might benefit more from dara-Rd, given the higher risk of peripheral neuropathy related to bortezomib, which might result in dose reductions and discontinuations. In the ALCYONE trial, peripheral neuropathy of any grade was reported in 29% of patients (1.4% G ≥ 3). For patients with severe renal

Table 2. Advantages and limitations of treatment strategies for elderly myeloma patients.

Regimen	Advantages	Disadvantages
ASCT	Survival benefit	Long hospital stay and recovery
	Low TRM (<2%) also in selected patients > 70 years	Require adequate organ function
	Possibility do adapt the dose of melphala conditioning (100/140 mg/m2)	Require adequate stem cell harvest
Daratumumab-Rd	Survival benefit	Higher risk of infection
	Subcutaneous daratumumab formulation (lower IRRs, faster drug delivery)	Suboptimal if advanced renal failure
		Risk of daratumumab IRRs
		Lack of real-life safety data
Daratumumab-VMP	Survival benefit	Risk of daratumumab IRRs
	Subcutaneous daratumumab formulation (lower IRRs, faster drug delivery)	Suboptimal if pre-existing neuropathy
		Lack of real-life safety data
VRD	Survival benefit	Suboptimal if pre-existing neuropathy/renal failure
	Possible benefit in high-risk disease	
	Possibility of dose-adjustments (VRD-lite)	
Rd	Fully oral administration, fewer hospital visits	Suboptimal if advanced renal failure
	High experience with the combination	Slower efficacy
	Suitable aslo for frail patients	
VMP	Rapid efficacy	Suboptimal if pre-existing neuropathy
	Fixed duration therapy	Similar toxicity of dara-VMP but lower efficacy
	High experience with the combination	
Ixazomib-Rd	Fully oral adminisitration, fewer hospital visits	Not approved frontline
	Suitable also for frail patients	
Carfilzomib-Rd	Possible benefit in high-risk disease	Risk of cardiotoxicity
	Rapid efficacy	Frequent intravenous administration
		Not approved frontline

Abbreviations: ASCT, autologous-stem cell transplantation; Rd, lenalidomide-dexamethasone; VMP, bortezomib-melphalan-prednisone; VRD, bortezomib-lenalidomide-dexamethasone; TRM, treatment-related mortality; IRR, infusion-related reactions.

impairment, dara-VMP (with dose-adjusted melphalan) could potentially be less nephrotoxic compared to dara-Rd. The incidence of G ≥ 3 neutropenia was similar with dara-VMP and dara-Rd (50% and 54%), but a higher incidence of infections was reported in the MAIA trial for patients receiving dara-Rd in comparison to that reported in the ALCYONE trial with dara-VMP (40% and 22%). Because of the high rate of infections with both regimens, and particularly with dara-Rd, antibiotic prophylaxis for the first months of treatment (eg levofloxacin 500 mg once daily for 12 weeks) should be considered, especially for intermediate-fit and frail patients, considering the benefit reported in the TEAMM trial (HR 0.66 for febrile infections or deaths in patients receiving vs not receiving prophylactic levofloxacin, p=0.001).[11] Finally, patient preference should be taken into account as well: some patients might prefer a more intensive regimen for the first year of treatment followed by a lighter maintenance with daratumumab (dara-VMP) compared to a continuous triplet (dara-Rd).



Abbreviations: IMWG, International Myeloma Working Group; ADL, Activities of Daily Living; IADL, Instrumental Activities of Daily Living; CCI, Charlson Comorbidity Index; VMP, bortezomib, melphalan, prednisone; Rd, lenalidomide, dexamethasone; VRd, bortezomib, lenalidomide, dexamethasone; ASCT, autologous stem-cell transplantation; KRD, carfilzomib, lenalidomide, dexamethasone; pts, patients.

Figure 1. Frailty-tailored treatment options for elderly newly diagnosed myeloma patients. Abbreviations: IMWG, International Myeloma Working Group; ADL, Activities of Daily Living; IADL, Instrumental Activities of Daily Living; CCI, Charlson Comorbidity Index; VMP, bortezomib, melphalan, prednisone; Rd, lenalidomide, dexamethasone; VRd, bortezomib, lenalidomide, dexamethasone; ASCT, autologous stem-cell transplantation; KRD, carfilzomib, lenalidomide, dexamethasone; pts, patients.

Is there space for other triplets in first line treatment for NTE patients?

The addition of daratumumab to both VMP and Rd induced impressive results in terms of efficacy and did not add excessive toxicity to the regimens. Daratumumab can be administered to virtually all patients, except in the case of severely compromised pulmonary function, due to the increased risk of severe respiratory infusion reactions. Consequently, to date, no other regimen can compete with the efficacy and safety of these dara-based combinations (Table 2). The triplet VRD for 8 cycles followed by Rd maintenance until progression - approved both in the United States and in Europe - can be considered as an alternative option. In the SWOG S0777 trial, VRD reduced the risk of disease progression/death by 30% compared to standard Rd (median PFS 41 vs 29 months, p=0.003 in the overall population), a benefit that was maintained also in ≥ 65 years old patients.[12] Again, no direct comparison between VRD and dara-Rd/VMP is available; prospective data are needed to guide physicians, particularly focusing on high-risk patients, in whom the advantage of dara-based combinations is less striking. The ongoing IMROZ and CEPEHUS trials are evaluating safety and efficacy of the addition of either isatuximab or daratumumab to VRD in NTE myeloma patients. The ongoing EMN20 trial is comparing carfilzomib-Rd (KRD) to Rd in fit and intermediate fit patients, though the use of carfilzomib in elderly patients is haunted by its risk of cardiotoxicity, that might limit its use as a first choice. In the ENDURANCE trial comparing KRD to VRD in newly diagnosed NTE MM patients, no benefit of KRD over VRD was noted (median PFS 34.6 vs 34.4 months, HR 1.04, p=0.74), with median PFS similar to that observed with dara-VMP in the ALCYONE trial.[13] High-risk patients were excluded from the trial, therefore the outcome of KRD over VRD in these patients cannot be determined. KRD was associated with higher toxicity compared to VRd, especially in terms of cardio-pulmonary and renal adverse events and toxic deaths. To date, this triplet is not approved as first line treatment for NTE patients.

An interesting treatment option for intermediate-fit/frail patients willing to receive an oral treatment (eg with impaired performance status or unable to undergo frequent hospital accesses for drug delivery due to lack of care-giver or distance from Health Care facilities) is the fully oral triplet ixazomib-Rd. In the TOURMALINE-MM2 trial, the triplet ixazomib-Rd, with dexamethasone discontinuation and lenalidomide and ixazomib dose reductions beyond cycle 18, resulted in longer – yet not statistically significant - PFS compared to Rd (median PFS 35 vs 22 months, HR 0.8, p=0.07). The triplet was well tolerated with no particular safety concerns compared to Rd.[14] To date, ixazomib-Rd is available outside of clinical trials only at relapse, and whether it will be introduced into clinical practice in front-line is yet uncertain.

Conclusions

Moving daratumumab to the frontline setting completely revolutionized the treatment landscape for newly diagnosed, elderly MM patients, with dara-VMP and dara-Rd becoming new standards of care for most fit and intermediate fit patients. The approval of subcutaneous daratumumab, which will soon enter the clinical practice also in Italy, further improved the accessibility of these regimens, sparing long in-hospital stays for intravenous drug delivery. Moreover, subcutaneous daratumumab seems to induce lower rate of infusion related reactions, which represent the most common daratumumab-related toxicity. Alternative options for elderly patients include ASCT for fit individuals up to 70 years of age or VRD; while other triplets, such as KRD and the fully oral ixazomib-Rd, are still under investigation. The doublets Rd or Vd are mainly used for frail patients ineligible for triplets/quadruplets, despite dose-adjusted dara-Rd might be considered. Special attention is needed in managing and preventing toxicities, since elderly patients are at higher risk of treatment related adverse events compared to younger ones. This is particularly true for infections, and standardized prophylactic and supportive measures should be developed and implemented. A personalized frailty-driven treatment approach is the core of the management of elderly MM patients. More real-life data are needed to better assess the feasibility and safety of the available regimens in different frailty subgroups.

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Table 1. Treatment selection at the time of first relapse based on lenalidomide refractoriness.

Lenalidomide-refractory pts			
Non frail (triplets)			Frail (doublets)
Bortezomib-based	**Carfilzomib-based**	**Pomalidomide-based**	
dara-Vd	dara-Kd	PVd	Vd
pano-Vd	isa-Kd	isa-Pd	Kd
SVd		dara-Pd	

Not lenalidomide-refractory pts	
Non frail (lenalidomide-based triplets)	**Frail (doublets)**
dara-Rd	Rd
elo-Rd	
KRd	
IRd	

Abbreviations: dara=daratumumab; elo=elotuzumab; I=isatuximab; isa=isatuximab; Kd=carfilzomib-dexamethasone; pano=panobinostat; Pd= pomalidomide-dexamethasone; pts=patients; Rd=lenalidomide-dexamethasone; S=selinexor; Vd=bortezomib-dexamethasone.

TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA

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The treatment of multiple myeloma (MM) has changed drastically in the past decade with the incorporation of novel agents into therapeutic strategies. These new drugs, in various combinations, have been added to national and international clinical guidelines and have transformed the approach to the treatment of patients with MM, resulting in substantial improvements in overall survival (OS).[1,2]

With the availability of at least seven different classes of approved agents, which can be combined in doublet, triplet, or even quadruplet regimens, or in some cases as continuous treatment, the choice of the optimal strategy at diagnosis and at relapse yet represents a challenge for physicians. Novel options include alkylators, steroids, proteasome inhibitors (PIs), immunomodulatory agents (IMiDs), histone deacetylase inhibitors, monoclonal antibodies (MoAbs), and selective inhibitors of nuclear export, with or without high-dose therapy and autologous stem-cell transplantation (ASCT), Moreover, next-generation immunotherapies or targeted agents will soon improve the therapeutic armamentarium. Thus far, several phase 3 trials have shown improved survival outcomes (progression-free survival, PFS, OS or both) with the use of triplet combinations, suggesting that at least two active drugs should be combined with steroids, if patients can safely tolerate this therapeutic regimen. At the time of relapse, the treatment choice is affected by many patient- and disease-related factors, such as patient preference, age, cytogenetic profile, pre-existing toxicities, comorbidities, and aggressiveness of the relapse, but mostly by the type of, and the response to, previous therapies.[4] At first relapse, treatment is at the moment mainly tailored on the presence or absence of refractoriness to lenalidomide (len); however, from the second relapse the scenario is far more complex, and mainly influenced by previous therapies.

Treatment of relapsed and refractory MM (RRMM) in patients who have received one previous line of therapy (Table 1)

The most important question for most cases of MM relapse, or therapy-resistant disease, is whether a patient has a len-refractory disease or not. A second consideration that will be increasingly important in the up-coming years is whether the disease is progressing on front-line therapies that include daratumumab.

On the basis of the OS benefits seen in randomised trials and meta-analyses, len is used as part of the front-line therapy for newly diagnosed MM. In patients treated with upfront ASCT, len- monotherapy at a low dose is approved as a maintenance therapy until disease progression.[3] In patients with previously untreated, newly diagnosed MM who are not eligible for ASCT, len is also approved in combination with low-dose dexamethasone until disease progression, on the basis of several randomized trials. As a consequence, a high number of patients are progressing while taking continuous treatment with len.[3]

First relapse in patients with lenalidomide-refractory disease

Patients with len-refractory disease were rightly excluded from randomised phase 3 trials testing len plus dexamethasone versus len plus dexamethasone plus a third agent. The precise effect of len-based triplet combinations in patients with len-refractory disease is unknown, but it would most likely lead to suboptimal results, and these regimens are therefore rarely used in this setting; only few restrospective small studies showed a modest effect of adding a third agent to a len-refractory disease. Moreover, despite not formally proven, no significant difference, in terms of response to subsequent len-triplet combinations, seems to exhist after the development of refractoriness to len at different doses (full versus maintenance dose).[1,2]

For a patient who incurs disease progression while taking len as part of front-line therapy, a reasonable approach would be to switch the class of agent, from an IMiD to a PI. Several phase 3 trials have evaluated PIs-based combinations using bortezomib plus dexamethasone as the control regimen in RRMM, but few patients with true len-refractory disease were included in most of those trials.[4,5]

In the randomised, phase 3 ENDEAVOR trial, bortezomib plus dexamethasone was prospectively compared with carfilzomib plus dexamethasone (Kd) in patients with relapse after one to three previous lines of therapy, until disease progression occurred; PFS and OS were significantly extended with the second generation PI carfilzomib. However, in patients with len-refractory disease after any line of therapy PFS of Kd treated patients was quite insatisfactory (median 8·6 months) and OS non significantly superior to bortezomib-dex, suggesting that patients with len-refractory disease might not benefit as much from Kd combination therapy as those with a previous response to len.

In the CASTOR trial, bortezomib plus dexamethasone was compared with daratumumab plus bortezomib plus dexamethasone in patients with RRMM who had received at least one previous line of therapy. The triplet combination was associated with significantly longer PFS in all patients; however, as in the ENDEAVOR study, the total number of patients whose disease had progressed during front-line len treatment was not specified. The only information available is based on a subgroup analysis

showing that, in patients with len-refractory disease (regardless of the number of previous lines of therapy), similarly to the ENDEAVOR study, median PFS was 7.8 months, suggesting that daratumumab plus bortezomib plus dexamethasone is suboptimal for this patient population. OS data for this subgroup of patients in the CASTOR trial are not yet available.

The phase 3 PANORAMA 1 study, comparing bortezomib plus dexamethasone with bortezomib plus dexamethasone plus panobinostat, enrolled a subgroup of patients progressing on len as front-line therapy, but the number of patients in this setting was very small and previous treatment with lenalidomide was not a stratification factor. Overall, the study showed that the combination of bortezomib plus dexamethasone plus panobinostat improved PFS, without OS benefit; however, the toxicity associated with panobinostat does not support the use of this triplet combination.

In the phase 3 OPTIMISMM trial, the combination of pomalidomide plus bortezomib plus dexamethasone was prospectively compared with bortezomib plus dexamethasone in patients with RRMM who had received one to three previous lines of therapy that included len. More than 70% of the patients had len-refractory disease. The triplet combination resulted in an improved median PFS in the whole population and in patients with len-refractory disease, either after 1 or any prior line of therapy. OS are not yet available.

Combinations of Kd plus anti-CD38 antibodies have been evaluated in 2 phase 3 studies. In the CANDOR trial, Kd was compared with Kd plus daratumumab in patients with RRMM who had received one to three previous lines of therapy; 33% had len-refractory disease. Daratumumab plus Kd was superior in terms of high quality response, minimal residula disease (MRD) negativity and PFS, both in patients with previous len exposure and in len-refractory patients. The phase 3 IKEMA trial compared Kd to Kd plus Isatuximab in patients with RRMM and one to three previous lines of therapy. Isatuximab-Kd was superior in terms of response and PFS, both in patients with previous len exposure and in len-refractory patients (30% of the population). Anti-CD38 MoAbs plus Kd are considered important treatment options for first relapse in patients with len-refractory disease.

Other combinations were tesetd in len-exposed/refractory patients in phase 1-2 studies. The combination of daratumumab plus pomalidomide plus dexamethasone was investigated in the POM MM 014 phase 2 trial, which included patients who had disease progression after len-based therapy (median two previous lines of treatment), 75% of which had len-refractory disease; the 9 month PFS was 86·3%. Pomalidomide was also combined with Kd in the prospective phase2 EMN011/HO114 trial, designed for patients with refractory disease or first progression after front-line therapy as part of the EMN02 trial, in which patients were randomly assigned to front-line ASCT versus no front-line ASCT, followed by consolidation and lenalidomide maintenance until progression. After reinduction with carfilzomib plus pomalidomide plus dexamethasone, patients were offered either salvage ASCT, if they had not received it as front-line intensive therapy. The analysis of the first 60 patients, 95% of whom had progressed on len maintenance, showed that responses to carfilzomib plus pomalidomide plus dexamethasone were rapid, with a median time to best response of 2 months and the median PFS was 18 months.

First relapse in patients with disease not refractory to lenalidomide

In patients who have received bortezomib-based front-line therapy without len maintenance, or patients treated with a fixed duration of len with progression occurring more than 6 months after cessation of therapy, second-line therapy should be based on len and dexamethasone regimens, such as carfilzomib plus len plus dexamethasone, daratumumab plus len plus dexamethasone, ixazomib plus len plus dexamethasone, or elotuzumab plus len plus dexamethasone.[4,5] In pivotal phase 3 trials with PFS as the primary endpoint, all of these combinations were found to be superior to lenalidomide plus dexamethasone. Carfilzomib plus lenalidomide plus dexamethasone and elotuzumab plus lenalidomide plus dexamethasone, investigated in the two trials with the longest follow-up also showed an OS benefit. As far as the hazard ratio and PFS is concerned, the most effective combination available in the setting of first relapse of myeloma not refractory to len is daratumumab plus len plus dexamethasone (POLLUX trial). With a longer follow-up, these results are expected to translate into an OS benefit. The daratumumab plus len plus dexamethasone triplet combination is well tolerated, and the forthcoming availability of a subcutaneous mode of administration of daratumumab will increase convenience.

After front-line therapy based on combinations including a PI, a retreatment including a PI can also be considered. Four trials have shown a PFS benefit of other regimens versus bortezomib plus dexamethasone alone, also in patient previously receiving, but not refractory to bortezomib: ENDEAVOR, CASTOR, BOSTON (evaluating selinexor plus bortezomib plus dexamethasone), and BELLINI (evaluating venetoclax plus bortezomib plus dexamethasone).[1,2] The phase 3 BOSTON trial compared bortezomib plus dexamethasone versus selinexor plus bortezomib plus dexamethasone in patients who had received one to three previous lines of therapy. Selinexor plus bortezomib plus dexamethasone significantly prolonged median PFS versus bortezomib plus dexamethasone, but this benefit was less apparent in patients previously exposed to a PI. The phase 3 BELLINI trial has compared bortezomib plus dexamethasone versus bortezomib plus dexamethasone plus venetoclax, a selective BCL2 inhibitor, in patients who had received one to three previous lines of therapy. A significant PFS benefit was reported with bortezomib plus dexamethasone plus venetoclax in patients with a t(11;14) translocation and those with high BCL2 expression. By contrast, in patients without t(11;14) and with low BCL2 expression, median PFS did not differ significantly between the two treatment groups, and increased mortality was seen in the bortezomib plus dexamethasone plus venetoclax group, mostly because of a higher rate of fatal infections (septic shock and pneumonia). Finally, the in CANDOR trial an improved PFS of the triplet over the doublet combination was confirmed in patients with previous proteasome inhibitor exposure; results are less clear in the IKEMA trial.

First relapse in patients progressing on front-line daratumumab-based combinations

The approval of daratumumab-based regimens as standard of care for front-line therapy in newly diagnosed MM patients, eligible or not for transplantation, is making treatment decisions challenging.[1,2] So far, no data exist to support daratumumab retreatment at second line, and salvage therapy with isatuximab in patients progressing on daratumumab is unlikely to be a suitable option because both antibodies target the same antigen (CD38). A suitable option for patients relapsing after daratumumab-bortezomib-melphalan-prednisone (ALCYONE trial) would be carfilzomib plus lenalidomide plus dexamethasone for fit patients, but for frail patients, dexamethasone in combination with ixazomib or elotuzumab might be the best approaches. For patients relapsing after having received up-front daratumumab plus lenalidomide plus dexamethasone until disease progression (MAIA trial), a PI-based combination without daratumumab is the logical approach. In this setting, carfilzomib plus dexamethasone, bortezomib plus cyclophosphamide plus dexamethasone, pomalidomide plus bortezomib plus dexamethasone, bortezomib plus melphalan plus prednisone, or carfilzomib plus pomalidomide plus dexamethasone are reasonable options. Alternatively, elotuzumab plus bortezomib plus dexamethasone, selinexor plus bortezomib plus dexamethasone, or ixazomib plus pomalidomide plus dexamethasone could be considered. The use of daratumumab up-front in transplant eligible patients does not represent an obstacle to the use at relapse, as the current approval is for fixed duration as induction/consolidation, and not maintenance.

Salvage ASCT

Front-line ASCT is the standard of care for fit patients younger than 70 years of age in many countries. Nevertheless, given the absence of an OS benefit of front-line ASCT in patients with standard-risk disease, compared with bortezomib plus lenalidomide plus dexamethasone followed by lena-

lidomide maintenance, for example, some investigators and patients prefer to delay ASCT to the time of the first relapse, after harvesting and storing stem cells during induction.[3] In this setting, salvage ASCT should be systematically considered in patients who have never previously received a transplant. One issue is the selection of the optimal reinduction regimen before salvage ASCT, especially for patients progressing on front-line, long-term len therapy. Few data are available regarding reinduction regimens. Salvage ASCT can also be considered in patients progressing after front-line ASCT.[6] The most important prognostic factor for PFS after salvage ASCT is the duration of remission after the first ASCT procedure. Since front-line ASCT followed by len maintenance is associated with a median duration of response of 50 months, salvage ASCT should not be recommended for patients with a response duration of less than 3 years after the first ASCT, but this cutoff is arbitrary and could be reduced to 2 years if the patient has not received maintenance therapy.

Treatment of relapsed and refractory disease after two or more previous lines of therapy (Table 2)

The treatment of patients with RRMM who have received two or more previous lines of therapy is becoming particularly challenging. Lenalidomide and bortezomib are often used as part of front-line therapy or at first relapse. MoAbs (eg, daratumumab and elotuzumab) and carfilzomib are also being increasingly used during the first two lines of treatment. Therefore, at the time of the second relapse, all agents considered but not used for first relapse can be considered again. Enrolling the patient in a clinical trial, when available, should always be considered.[1,2]

Table 2. Treatment of patients with relapsed and refractory disease who have received two or more previous lines of therapy.

Second or higher relapse		
Preferred options	**Alternatives (approved)**	**Other options (investigational)**
Isa-Pd*	Selinexor	Melflufen
DKd*	PI plus panobinostat	BCMA-targeting agents (i.e. CAR-T or bispecific Abs)
Dpd*	VdT-PACE	Venetoclax§
Isa-Kd*	Belantamab mafodotin	
Elo-Pd°		
KPd°		
PCd#		
Pd#		

*based on phase 3 trials data (Grade of recommendation: 1A)
°based on phase 2 trials data (Grade of recommendation: 1B)
#when daratumumab, carfilzomib, or elotuzumab are not available
§in patients with t(11;14) or BCL2 high expression

Abbreviations: BCMA=B-cell maturation antigen; CAR=chimeric antigen receptor; DKd=daratumumab plus carfilzomib plus dexamethasone; DPd=daratumumab plus pomalidomide plus dexamethasone; Elo-Pd=elotuzumab plus pomalidomide plus dexamethasone; Isa-Kd=isatuximab plus carfilzomib plus dexamethasone; Isa-Pd=isatuximab plus pomalidomide plus dexamethasone; KPd=carfilzomib plus pomalidomide plus dexamethasone; PCd=pomalidomide plus cyclophosphamide plus dexamethasone; Pd=pomalidomide plus dexamethasone; VdT-PACE=bortezomib plus dexamethasone plus thalidomide plus cisplatin plus doxorubicin plus cyclophosphamide plus etoposide.

Few phase 3 trials have focused on patients who have received two or more previous lines of therapy. In patients whose disease has progressed after treatment with bortezomib and lenalidomide, pomalidomide plus dexamethasone has been considered as standard of care. This combination has been compared with isatuximab plus pomalidomide plus dexamethasone in the ICARIA trial in patients previously treated with two or more lines of therapy including lenalidomide (92% refractory) and a proteasome inhibitor. Isatuximab-pomalidomide-dexamethasone significantly extended PFS in comparison with the doublet. Two other antibody-based combinations can be considered for patients with advanced disease. In the randomised phase 2 ELOQUENT-3 trial, patients who had received at least two previous lines of therapy were randomly assigned to receive either elotuzumab plus pomalidomide plus dexamethasone or pomalidomide plus dexamethasone; the triplet combination showed significantly prolonged PFS. The phase 3 APOLLO study (EMN14) compared pomalidomide plus dexamethasone versus daratumumab plus pomalidomide plus dexamethasone in patients refractory to lenalidomide and proteasome inhibitors. 11% of the patients had received at least one previous line of therapy (median 2), and 80% were refractory to lenalidomide. Again, the triplet combination significantly prolonged PFS. A simple and inexpensive option to improve the results of pomalidomide plus dexamethasone when other agents are not available is the addition of cyclophosphamide to this treatment combination. Although no direct comparisons are available from phase 3 studies, several phase 2 trials have shown that the median PFS of pomalidomide plus cyclophosphamide plus dexamethasone is approximately 7–9 months, compared with 4–6 months for the same subgroup of patients treated with pomalidomide plus dexamethasone alone.

Additional options for patients with relapsed and refractory disease after two or more previous lines of therapy

The outcome is very poor for patients whose MM has become refractory to PIs, IMiDs, and anti-CD38 Abs, with one study showing that these patients have a median overall survival of only 5·6 months.[7] In this setting, intensive chemotherapeutic combinations, such as bortezomib plus dexamethasone plus thalidomide plus cisplatin plus doxorubicin plus cyclophosphamide plus etoposide, can be used, although prospective data are not available for these combinations.

Selinexor, a selective inhibitor of nuclear export compound that blocks exportin 1 and forces nuclear accumulation and activation of tumour suppressor proteins, has been evaluated in combination with dexamethasone in patients previously exposed to (individually or in combination) bortezomib, carfilzomib, lenalidomide, pomalidomide, daratumumab, or an alkylating agent and had disease refractory to at least one proteasome inhibitor, one immunomodulatory agent, and daratumumab (triple-class refractory) in the phase 2 STORM study, showing an overall response rate of 30% and PFS of 3.7 months. One problem with selinexor is its safety profile: about 25% of the patients experienced grade 3 fatigue, gastrointestinal toxicity, and thrombocytopenia, but these side-effects are more manageable with less frequent doses and supportive care.

Melflufen (melphalan flufenamide) is a first-in-class anti-cancer peptide-drug conjugate that rapidly delivers an alkylating payload into tumour cells. This agent has been tested in combination with dexamethasone in patients with RRMM who had received two or more previous lines of therapy (including lenalidomide and bortezomib) and were refractory to their last line of therapy. The overall response rate is approximately 30%, median PFS 4.2 months, and median OS was 11.6 months.

B-cell maturation antigen (BCMA; also known as TNFSRS17) promotes multiple myeloma pathogenesis in the bone marrow microenvironment and is a very specific multiple myeloma target antigen. Immunologically based therapies targeting BCMA show promise in phase I/II studies, independent of genetic heterogeneity and genetic risk, even in patients with multiple myeloma with no other treatment options. These agents include antibody–drug conjugates, autologous chimeric antigen receptor engineered T cells (CAR T cells), and bispecific T cell or NK engagers. Little data are yet available for bispecific agents, and early clinical trials are ongoing.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION ACTIVITY DURING COVID-19 PANDEMIA

F. Ciceri

Presidente GITMO

Gitmo centers conducted a retrospective survey on HSC transplantation activity at time of Covid-19 pandemia.

The period March 2020- July 2020 was comopared to same period in 2019.

Overall, 2020 allogeneic HSCT activity was 2.4% reduced as compared to 2019. Interestingly, HSCT in acute leukemias was + 5.7% increased.

HSCT source was preferentially peripheral blood in 2020 (+10%) and overall 97.4% of transplant products were cryopreserved after collection for safety reasons, given the uncertainty of transportation during pandemia.

Gitmo centers, Italian Bone Marrow Donor registry and Centro Nazionale Trapianti overall guaranteed the continuity of transplant activity during Covid-19 pandemia.

CORD BLOOD TRANSPLANTATION: IS STILL AN OPTION?

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Hematopoietic stem cell transplantation (HSCT) has significantly improved and changed over time and nowadays, when an HLA-matched sibling is lacking, matched or mismatched unrelated donors (MUD, MMUD), umbilical cord blood (UCB) units and full-haplotype mismatched family members (haploidentical donors) are largely used.[1] Cord blood has been widely adopted for the treatment of both non-malignant and malignant hematological diseases[2]. Due to the immaturity of the immune system at birth, less alloreactive T cells are present in the graft. Consequently, after UCBT the incidence and severity of acute and chronic graft-versus host disease (GVHD) is decreased in comparison to other graft sources[3] with, on the other hand, a delayed immune-recovery and an increased risk of infections. UCB allows for less stringent matching criteria for HLA-donor recipient selection and extends the access to transplantation to patients for whom a MUD cannot be identified, especially in racial and ethnic minorities, still underrepresented in international registries.[4] To date, the global inventory of UCB units available for transplant in public cord blood banks (CBB) is more than 750 000 and more than 35 000 umbilical cord blood transplantation (UCBT) have been performed worldwide (www.wmda.info). New application of cord blood derived stem cells, also for immunotherapy using chimeric antigen receptors, are currently under investigation in clinical trials, opening new horizons for the use of UCB units.

Criteria for cord blood unit selection in patients: cell dose and HLA matching

TNC, colony-forming units, and CD34+ cells are the most important prognostic factors for outcomes of UCBT, mainly engraftment, mortality and overall survival, as demonstrated over the last years[5].

The minimum of 2.5-3×10e7/kg of TNC at cryopreservation should be obtained in a single UCB unit for transplantation in patients with malignant diseases[6]. The threshold for TNC should be higher reaching 5x10e7/Kg at cryopreservation in non-malignant diseases, to overcome the higher risk of associated graft failure[7]. When selecting the UCB unit, TNC is the standard requirement for the selection of the UCB unit in the CBB, in association with the CD34+ cell dose, whose dose is not clearly standardized across the different cell therapy laboratories. However, the mainly recommended threshold for CD34+ cell dose is 1-1.5x10e5/Kg at cryopreservation, especially when more than one unit meeting the required TNC criteria are available.

Low resolution HLA matching for UCB units is generally based on 3 loci (HLA-A, -B at antigenic level, and -DRB1 at allelic level), with a maximum of 2 out of 6 HLA mismatches being considered acceptable, as a higher incidence of NRM is associated with greater mismatches. More recently, in a study analyzing the effect of the HLA C on UCBT, Eurocord and NMDP/CIBMTR[8] reported higher NRM in patients receiving an UCB unit with a mismatch at HLA locus-C. In addition, concomitant mismatching at HLA-C and -DRB1 was associated with a highest risk of mortality. Later, a collaborative study from the same group[9] analyzed the effect of full allelic typing for HLA-A, -B; -C; and –DRB1 on UCBT outcomes, reporting significant reduction in mortality for 8/8 and 7/8. The advantage of allelic level matching was also recently confirmed in children with non-malignant disease[10]. These important findings helped in reassessing the strategy for UCB unit selection and supported the need for public CBB to expand the UCB unit inventory including the typing at locus C and the allele level matching.

The current criteria for donor selection recommend considering allele-level HLA matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1 both for malignant and non-malignant diseases and to select UCB unit with no more than 2 HLA mismatches.

Ex-vivo expansion of cord blood stem cells

Currently, multiple strategies are under investigation mainly aiming to increase the progenitor cells of a cord blood graft. Delaney et al. showed that a rapid myeloid reconstitution after UCBT was possible with a Notch-mediated ex-vivo expansion of human cord blood progenitor cells and infusion of a non-manipulated cord blood unit along with another unit expanded ex-vivo. The same group is currently assessing the use of an "off-the-shelf"' expanded UCB product in a phase II study that is currently ongoing (NCT01690520).

A different platform for progenitor cell expansion was reported by De Lima and colleagues using mesenchymal stromal cell co-culture (mesoblast) allowing shorter time to engraftment than the historical control. The use of a single cord blood unit expanded ex vivo with nicotinamide, as "stand alone graft", was recently reported by Horwitz et al on patients with hematological diseases.

Other strategies of UCB *ex vivo* expansion, or the use of agents to enhance UCB homing to the marrow have also been described. In addition, some groups have also reported encouraging results using the direct intra-bone marrow injection of the UCB unit, or co-infusion of a cord blood unit with a haploidentical T cell depleted graft. Promising results have been reported with the above-mentioned strategies, however they remain experimental and definitive conclusions cannot yet be drawn on their reproducibility, cost-efficiency, and long-term outcomes.

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HLA-HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOR NON-MALIGNANT DISORDERS

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has completely revolutionized the natural history of several life-threatening or invalidating non-malignant disorders (NMDs), including primary immune deficiencies (PIDs), bone marrow failure syndromes and hemoglobinopathies. Over the last decades, the advent of reduced toxicity conditioning regimens, progress in high-resolution HLA-typing techniques and advances in supportive care have enormously enhanced the safety and efficacy of HSCT. As the outcome of transplantation improved, the number of non-malignant conditions amenable to definitive treatment by HSCT has continued to grow, placing ever-increasing demands on the pool of stem cell donors. However, the probability of identifying a fully compatible, non-affected sibling is theoretically less than 25%; in western countries it has been calculated that, mainly because of low birth rate, this probability drops to less than 20% in the first 5 years of life. For those patients lacking an HLA-identical sibling, the likelihood of identifying a fully-matched unrelated donor (MUD) depends mainly on the ethnicity of the patient, this reflecting the number of donors present in the international databases. Caucasian patients of European descent have the highest probability of finding such a donor (around 75%), while blacks of South or Central American descent have the lowest (16%). Furthermore, the search for an HLA-matched volunteer donor may result in unacceptable delay in certain diseases, such as severe combined immunodeficiency (SCID), for which the goal is to proceed to transplantation as early as possible after diagnosis. In the absence of an HLA-matched donor, HLA-haploidentical relatives are being increasingly used to offer the chance of an allograft to any patient in need of transplantation. Indeed, the majority of patients has a family member, identical for one HLA haplotype and fully mismatched for the other (i.e., HLA-haploidentical), who can immediately serve as hematopoietic stem cell (HSC) donor. Besides availability for almost all patients, transplantation from an HLA-haplotype-mismatched family member offers other several advantages, among which no delay in graft procurement, the possibility to select the best donor from a panel of candidate members, and easy access to donor-derived cellular therapies whenever required after transplantation. The significant growth in the use of haploidentical donors is primarily the result of the successful development of several novel methods to overcome the alloreactivity generated by major donor–recipient human leukocyte antigen (HLA)-disparity, and improvements in prevention and treatment of post-transplant complications, such as primary graft failure, delayed immunologic recovery or graft-versus-host disease (GvHD) (Figure 1).

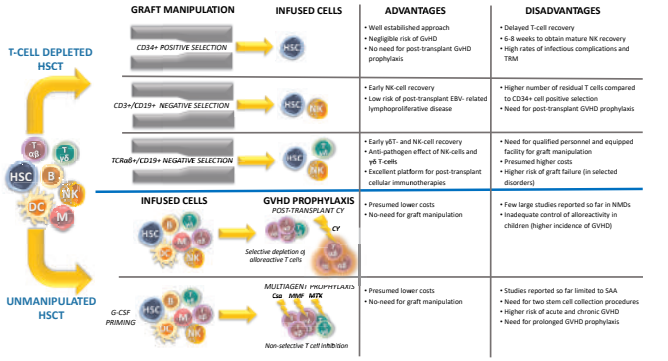

Figure 1. T cell-depleted and T cell-replete haploidentical HSCT strategies employed in non-malignant diseases.

History of haploidentical HSCT in non-malignant disorders

In the context of NMDs, the great majority of studies regarding haplo-HSCT have historically focused on T-cell depleted (TCD) platforms. Since donor-derived T lymphocytes contained in the graft are the major mediators of severe alloreactions in haploidentical HSCT (haplo-HSCT), various attempts have been made to overcome the risk of GvHD by depleting T cells from the graft prior to infusion. In 1983, Reisner and colleagues reported the first successful correction of severe combined immunodeficiency (SCID) by T-cell depleted haplo-HSCT using differential agglutination with soybean agglutinin (SBA) and subsequent E-rosette depletion (SBA-E). In the Stem Cell Transplant for primary Immune Deficiencies in Europe (SCETIDE) report on children with SCID transplanted from 1983–1995, the majority of HLA haplo–HSCT performed in European centers using marrow as graft source were depleted of T-cells by using SBA-E. The overall survival (OS) following such grafts was 52%. However, subsequent studies revealed that, outside of the SCID setting, graft failure (GF) represented a non-negligible problem in TCD transplantation from donors other than HLA-matched siblings. The introduction of more effective and standardized approaches for TCD based on immune-adsorption to antibody□coated paramagnetic beads, allowed rapid purification of CD34+ progenitor from granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC). Thanks to this strategy, it was possible to obtain high doses of CD34+ progenitors (>10 × 10^6/ kg) to be infused, while limiting T-cell doses to <10^4 CD3+ T cells/kg. The use of such "megadoses" of hematopoietic progenitors represented another milestone in the field of haplo-HSCT, allowing to overcome the barrier of HLA incompatibility in the donor/recipient pair and to elude the residual anti-donor cytotoxic T-lymphocyte activity of the recipient. This approach has been widely employed in SCID patients, offering the opportunity to improve the outcome of HSCT from mismatched donors, as documented by both SCETIDE and Primary Immune Deficiency Treatment Consortium (PDTIC) reports. The feasibility of TCD haplo-HSCT using CD34+ stem cell selection, after a reduced-intensity, fludarabine-based conditioning regimen, has been also demonstrated in pediatric patients affected by Fanconi anemia (FA). In addition, the Pesaro group reported in 2010 the outcomes of 22 children given a TCD HSCT from a haploidentical relative after a busulfan-based conditioning regimen. The majority of patients (n=14) received CD34+-mobilized peripheral blood and bone marrow progenitor cells. The pretransplant protocol consisted of an intensive hypertransfusion regimen combined with a 'preconditioning' regimen with hydroxyurea and azathioprine. Two patients died (cerebral

Epstein-Barr virus lymphoma or cytomegalovirus pneumonia), 6 patients rejected their grafts, and 14 showed full chimerism with functioning grafts at a median follow-up of 40 months.

Evolution of T Cell Depletion Strategies: From Positive Selection to Negative Depletion

The main limitation of haplo-HSCT platform based on positive selection of CD34+ cells is represented by delayed immune reconstitution consequent to the elimination of T cells from the graft, essential for preventing GvHD occurrence. In fact, with this procedure, recipients cannot benefit from the adoptive transfer of donor memory T lymphocytes, which, through peripheral expansion, are mainly responsible for protection from infections in the first months after transplantation. A profound immune deficiency has been documented to last for at least 4–6 months after CD34+ TCD haplo-HSCT and to translate into an increased risk of transplant-related mortality (TRM), mainly attributable to severe infections. In the last decade, technical improvements in immunomagnetic cell selection have enabled further refinements in graft manipulation strategies. In particular, the introduction of negative depletion of T-cell receptor (TCR) αβ+ T lymphocytes and CD19+ B-cells has allowed to retain in the graft not only donor hematopoietic stem cells, but also committed hematopoietic progenitors, as well as mature natural killer (NK) and TCRγδ+ T cells. These two latter subsets may exert a first line of defense against pathogens and it has been hypothesized that they may also facilitate engraftment¸ without increasing the risk of both acute and chronic GVHD. In 2014, Bertaina et al published the first report on 23 children affected by life-threatening NMDs given an HLA-haploidentical graft manipulated through this innovative approach. No patient received any post-transplant pharmacologic GVHD prophylaxis. In view of the high OS and DFS (91.1%) coupled with the low incidence of acute GVHD (13.1%) and the absence of chronic GVHD, these pilot study suggested that TCRαβ+/CD19 TCD haplo-HSCT could represent a suitable treatment option for children with life-threatening diseases lacking an HLA-identical sibling. More recently, the same group reported the results of 70 consecutive children affected by primary immunodeficiencies, inherited/acquired bone marrow failure syndromes, red blood cell disorders or metabolic diseases, lacking a fully-matched donor or requiring urgent transplantation, and given a TCRαβ/CD19 TCD haploidentical HSCT from an HLA-partially matched relative. Nineteen patients were already reported in 2014. Median age at transplant was 3.5 years (range 0.3-16.1); median time from diagnosis to transplant was 10.5 months (2.7 for SCID patients). Primary engraftment was obtained in 51 patients. Median time to neutrophil and platelet recovery was 14.5 (range 9-33) and 10 days (range 7-51), respectively. Nineteen and 2 patients experienced either primary or secondary GF, the overall incidence of this complication being 30.4%. Most GF cases were observed in children with disease at risk for this complication (e.g., aplastic anemia, thalassemia). All but 5 patients experiencing GF were successfully retransplanted. Six patients died of infectious complications (4 had active/recent infections at time of HSCT), the cumulative incidence of TRM being 8.5%. Cumulative incidence of grade I-II acute GVHD was 14.4% (no patient developed grade III-IV acute GVHD). Only one patient at risk developed mild chronic GVHD. With a median follow-up of 3.5 years, the 5-year probability of overall and disease-free survival was 91.4% and 86.8%, respectively. These results have been paralleled by other groups, which focused mainly on primary immunodeficiencies (PIDs). In addition, promising results were observed in patients with advanced stage sickle cell disease and given an TCRαβ/CD19 TCD haplo-HSCT after a treosulfan-based condition regimen. All studies confirmed the low incidence of both acute and chronic GVHD, with encouraging survival results, infections being the main cause of TRM. Since viral infections in particular still represent one of the major limitations of TCRαβ/CD19 TCD haplo-HSCT, carrying significant morbidity and mortality, several strategies have been investigated in order to facilitate the recovery of adaptive immunity in this setting. A recent randomized trial testing the efficacy of repeated infusions of naïve-depleted (CD45RA-depleted) DLI after TCRαβ+/CD19-depleted haplo-HSCT failed to demonstrate a clinical effect in the study population. On the contrary, Roy and colleagues demonstrated a reduction of TRM in TCD haplo-HSCT setting with the use of allo-depleted DLI as compared to TCD-HSCT alone. Our group recently tested the use of genetically-modified donor T-lymphocytes (transduced with the inducible Caspase-9 safety switch, which can be activated in case of uncontrolled GVHD), showing feasibility and encouraging results in terms of survival and immune-recovery.

T Cell-Replete Haploidentical Transplantation platforms

Literature has been largely silent on the use of unmanipulated haplo-HSCT for the treatment of non-malignant disorders. The main reason for this lack of interest must be found in the unacceptably high rates of graft rejection and severe GVHD documented by first attempts with this approach in both the malignant and non-malignant setting. However, the situation has dramatically changed in the last decade, thanks to the impressive results obtained with unmanipulated haplo-HSCT strategies in adult patients affected by malignant diseases. On the basis of these data, T-cell repleted haplo-HSCT is increasingly considered as alternative therapeutic strategy for patients with selected non-malignant diseases who do not have a matched sibling or a MUD.

The first unmanipulated approach, pioneered by the Johns Hopkins group, relies on the use of post-transplantation cyclophosphamide (PTCY). In the non-malignant setting, T-cell repleted haploidentical HSCT followed by PTCY was employed for the first time for the treatment of 2 adult patients with hemolytic paroxysmal nocturnal hemoglobinuria (PNH) and 1 with both PNH and SCD, following a nonmyeloablative conditioning regimen. Rapid and sustained engraftment without GVHD occurred in two patients, including the one with SCD, while one died of fungal sepsis. In 2012, the same group reported the outcome of 14 adult patients with SCD who underwent HSCT from related haploidentical donors. These patients were conditioned with a nonmyeloablative conditioning regimen including 2 Gy total body irradiation (TBI), while GVHD prophylaxis consisted of PTCY, mycophenolate mofetil (MMF) , and tacrolimus or sirolimus. Although this cohort had an OS of 100% at almost 2 years post transplantation, with no documented cases of GVHD, graft rejection was a major problem, being observed in 6 patients. With the aim of reducing the rate of GF, de la Fuente and colleagues evaluated the addition of thiotepa to the Johns Hopkins nonmyeloablative conditioning platform followed by infusion of marrow allografts and PTCy-based GVHD prophylaxis regimen. Among the 15 patients who were treated with this approach, no cases of GF were detected and greater than 95% myeloid engraftment was noted after at least 6 months of follow-up in 93% (14/15) patients. Another strategy to improve the rate of engraftment is based on the increase of the TBI dose in the nonmyeloablative conditioning regimen to 400 cGy from 200 cGy. Recently, a group from Thailand investigated the use of PCTY-based haplo-HSCT in 83 children and young adults affected by severe TM and β-thalassemia/hemoglobin E. In this study, all patients received intensive pre-transplant immunosuppressive therapy and two courses of fludarabine and dexamethasone, followed by a busulfan-based conditioning regimen. T-cell-replete progenitor cells were collected from peripheral blood after G-CSF administration. The 3-year OS and EFS were 96%, without cases of secondary graft failures. Six (7%) of 83 patients developed severe GVHD. In addition, several groups reported encouraging results with the use of PCTY-based haplo-HSCT in patients affected by SAA. Despite that, until very recently there were only isolated reports on the successful use of PCTY-based haplo-HSCT in benign disorders other than SAA and hemoglobinopathies. Results from the 2 largest studies published so far, including 27 and 73 patients, respectively (most of

whom affected by PIDs), showed that haplo-HSCT followed by PCTY is feasible and characterized by high engraftment rate, but at the price of high incidence of both acute and chronic GVHD (ranging between 33-46% and 16-24%, respectively). Indeed, suboptimal control alloreactivity, resulting from variable Cy metabolism in children, may be one of the major drawbacks of this approach in the treatment of NMDs.

Another unmaninuplated haplo-HSCT strategy, pioneered by the Beijing group, combines myeloablative conditioning, T-cell modulation with G-CSF-primed BM and PBSC grafts, ATG and intensive multiagent GVHD prophylaxis with cyclosporine, MMF and methotrexate. In the non-malignant setting, this approach has been almost exclusively investigated, with some modifications, for the treatment of refractory SAA. Although results obtained with this protocol in SAA are promising, available data also suggest that it carries a higher incidence of both acute and chronic GVHD when compared with PCTY-based or TCD haplo-HSCT.

Conclusions

Available data suggest that haplo-HSCT is a suitable option for the definitive treatment of an ever-widening spectrum of non-malignant disorders, in the absence of an HLA-identical donor. Moreover, while the use of haploidentical donors can extend safe transplantation to virtually all patients in need, the immediate availability of the haploidentical donor allows performing such procedure without undue delay, anticipating the development of life-threatening infections or severe disease-specific organ complications. In recent years, gene therapy, either through gene addition or genome-editing is increasingly being tested in advanced clinical trials for several NMDs and has already received regulatory approval for the treatment of ADA-SCID and transfusion-dependent Beta-Thalassemia. However, since specific vectors or genome-editing targets have to be identified, developed and studied individually for each disease-causing gene, HSCT remains the sole curative option for several different conditions.

The excellent results obtained with TCR αβ+/CD19+ TCD haplo-HSCT, for which success rates exceeding 90% have been reported, could challenge, in the near future, the current hierarchical algorithm in which MUD and UCBT are preferred to haploidentical donors. Moreover, the platform of TCRαβ+/CD19+ TCD haplo-HSCT is amenable of further refinements by the adoptive transfer of donor T lymphocytes transduced with suicide genes, thereby paving the way for even better results.

Alternatively, an unmanipulated haploidentical graft could represent an option in selected, life-threatening conditions. However, with respect to TCD haplo-HSCT, T-cell replete approaches have been so far characterized by a higher incidence of both acute and chronic GVHD, complication that are particularly detrimental in children with NMDs. Indeed, in the comparison between TCD and T-cell replete strategies, it is critical to consider that any risk of GVHD is unacceptable in NMDs, since it cannot be balanced by a stronger graft-versus-leukemia effect as seen in malignant diseases and may severely impair the quality of life in subjects with a long life expectancy. On the other hand, the risk of GF is the most challenging obstacles to be overcome with TCRαβ+/CD19+ TCD haplo-HSCT, particularly in those sub-groups of patients at known risk for this type of complication (such as HLH, thalassemia, SAA or osteopetrosis). It may be argued that T-cell depletion in conjunction with adoptive transfer of selected T-cell populations for accelerating immune reconstitution requires adequate graft manipulation facilities and specialized personnel, and might therefore be more costly. However, expenses related to T-cell depletion may be counterbalanced by the fact that this approach does not require GVHD prophylaxis and, nonetheless, has a reduced GVHD incidence compared to T-cell replete transplants. In addition, a recent pharmaco-economic analysis suggested that TCRαβ+/CD19+ TCD haplo-HSCT may be cost-effective as compared to HSCT from MUD. It is important to note that randomized studies have never been conducted to compare T-replete and T-depleted haplo-HSCT, and the majority of clinical data currently gathered for haploidentical transplants come from non-randomized trials with retrospective analysis, making difficult to prove the superiority of one specific method. Despite that, given the impressive results observed in newer approaches of TCR αβ+/CD19+ TCD haplo-HSCT, it would not be unwise to postulate that, in the near future, this strategy might become the preferred alternative option for patients with benign disorders without an HLA-identical sibling. Prospective studies comparing TCD haplo-HSCT to other alternative donor sources, including MUD and UCB, are warranted in the next few years to support more definitive recommendations.

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IMPAIRED MEGACARYOPOIESIS IN IMMUNE THROMBOCYTOPENIA (ITP)

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Megacaryocytes (MKs) are polyploid specialized myeloid cells localized primarily in the bone marrow which give rise to circulating blood platelets by a complex process called thrombopoiesis allowing to generate 10^{11} platelets per day in adult humans.[1] MKs differentiate from the hematopoietic stem cell (HSC) through a complex and finely regulated process called megakaryocytopoiesis that includes several steps synthesized below:

- the commitment of hematopoietic stem cell (HSC) toward the MK lineage
- the proliferation of committed progenitors
- megakaryocyte maturation.

During the process of MK maturation from hematopoietic stem cell, MKs undergo endomitosis, i.e. the replication of DNA without cell division, cytoplasm maturation, cytoskeletal re-organization and demarcation membrane system (DMS) formation and expansion.[1] DMS is a complex network of intracytoplasmic membranes that serves as a membrane reservoir for platelet production. DMS polarization is crucial for transendothelial proplatelet formation, and must take place exclusively in the direction of the bone marrow sinusoids to avoid ectopic platelet production.[2] Ectopic platelet production in the bone marrow has been shown to contribute to thrombocytopenia in disorders, like MYH9-RD, PT-VWD and ADAP deficiency.[3,4] The migration of MKs from the osteoblastic to the vascular niche, where they release platelets

in bone marrow sinusoids, is an essential step in thrombopoiesis and its defect may lead to ectopic platelet production in the bone marrow and thus to thrombocytopenia. MKs interaction with extracellular matrix proteins of the bone marrow microenvironment finely regulate MK maturation and platelet release within bone marrow. Interactions with matrices of the vascular niche, such as fibrinogen (FBG) or von Willebrand factor (vWF), trigger pro-platelet release in blood, while the interaction with type I collagen totally suppresses platelet release in the osteoblastic niche thus preventing ectopic platelet production.[1] Immune thrombocytopenia (ITP) is a complex, multifactorial and heterogeneous disorder in terms of clinical manifestations and response to therapy, with a not completely elucidated pathogenesis. It is characterized by isolated thrombocytopenia and bleeding. It is an autoimmune disorder caused by the generation of autoantibodies directed against some platelet and megakaryocyte surface glycoproteins. To date, the glycoproteins against which autoantibodies have been found are $\alpha IIb\beta_3$-GPIIb/IIIa, $\alpha_2\beta_1$-GPIa/IIa, GPIb/IX/V, GPIV, GPVI, $\alpha_v\beta_3$[5]. Thrombocytopenia in ITP is caused by increased platelet destruction in peripheral blood with shortened life span but also by impaired platelet production. Increased peripheral platelet clearance is caused by phagocytosis of autoantibodies opsonized platelets platelet recognized by phagocytes bearing Fcγ-receptors (FcγRs). Moreover, autoantibody-independent mechanisms, such as T cell-mediated cytotoxicity, are also involved in platelet destruction5. However, platelet autoantibodies were also detected in the bone marrow aspirate samples of more than half (56%) of patients with ITP, that together with the reported higher levels of immunoglobulin G (IgG)-coated megakaryocytes in some ITP patients, suggest that the bone marrow may be a pathologically relevant site where autoimmune reactions occur.[6] In support of the pathogenetic role of antiplatelet autoantodies on megakaryopoiesis and thrombopoiesis in ITP, some observations have been reported evaluating the effect of these autoantibodies on megakaryocyte differentiation, maturation and survival; megakaryocyte migration and adhesion on extracellular matrix proteins present in bone marrow microenvironment and proplatelet formation. Conflicting results regarding the link between a defective megakaryocyte differentiation, maturation and survival and thrombocytopenia in ITP patients are present in literature. McMillan R. *et al.* demonstrated that MK differentiation and maturation were impaired in the presence of some of the tested plasma from patients with chronic ITP.[7] While Yang L. *et al.* supposed that abnormal megakaryocyte apoptosis and maturation observed in the presence of ITP plasmas might be responsible for persistent thrombocytopenia in ITP[8], Lev P.R. *et al* reported that MK maturation was not influenced by the presence of ITP plasma, while MK apoptosis, was significantly increased in the presence of ITP plasma, but this reduction of viable MKs did not correlate with the observed reduction in proplatelet formation.[9] $\alpha_v\beta_3$ autoantibody seems do not have a role in MK maturation and survival.[10] An impaired MK adhesion and spreading on type I collagen, fibrinogen and VWF in the presence of ITP serum bearing autoantibodies against anti-$\alpha_2\beta_1$, anti-$\alpha IIb\beta_3$ and anti-GPIb/IX/V, respectively[11], has been reported, pointing out the role of these autoantibodies in altering the interaction between megakaryocyte glycoproteins and their corresponding extracellular matrix ligands and thus, megakaryocytic behavior within the BM environment.[11] Autoantibodies against integrin αvβ3 impair MK adhesion to fibrinogen and to vascular endothelial cells, as well as MK migration towards SDF-1α probably through suppression of cellular signals mediated by AKT, SRC and FAK[10]. Proplatelet formation evaluated in MKs cultured on surface coated with VWF was reduced in the presence of ITP plasmas bearing anti-GPIb/IX/V autoantibodies[11] and on fibrinogen in the presence of anti-$\alpha IIb\beta_3$ and anti-$\alpha_v\beta_3$ autoantibodies.[9–11] Recalcified plasma from ITP patients bearing anti-$\alpha_2\beta1$ autoantibodies interfere with the normal inhibition of proplatelet formation exerted by type I collagen.[9] However, apart from the effect of anti-αvβ3 autoantibodies on MK migration, there is a lack of studies on the effect of ITP autoantibodies on MK migration and no studies at all on the impact of ITP autoantibodies on MK polarization and DMS formation. Thus the effect of antiplatelet autoantibodies on these crucial steps of platelet production should be the next step to further investigate the pathogenesis of ITP. In fact, it has been hypothesized that the heterogeneity among ITP patients, with regard to both clinical features and response to treatment, could be the results of the multiple mechanisms contributing to ITP immunopathogenesis.[11] Moreover, given the growing importance given to the bone marrow as a pathologically relevant site where autoimmune reactions occur, further studies, perhaps standardizing the experimental conditions used to evaluate all the steps of megakaryocytopoiesis and thrombopoiesis, should be conducted. In addition, a larger and perhaps multicentric study including a huge number of ITP serum samples, thus trying to resolve the heterogeneity of the epitope, titer and avidity of antiplatelet autoantibodies often highlighted as a limitation of the studies reported in literature, seems to be necessary to further shed light on the pathogenetic mechanisms of thrombocytopenia in ITP patients that remains still unclear.

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PATHOGENETIC MECHANISMS OF THROMBOCYTOPENIA IN MICROANGIOPATHIC THROMBOTIC SYNDROMES

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The pathogenetic mechanisms of thrombocytopenia in thrombotic microangiopathies (TMAs) are complex and involve different factors with a wide range of clinical severity. Thrombotic thrombocytopenic purpura (TTP) is an acquired rare thrombotic microangiopathy characterized by microangiopathic hemolytic anemia, severe thrombocytopenia, and ischemic end-organ injury due to microvascular platelet-rich thrombi. TTP results from a severe deficiency of the von Willebrand factor (VWF)-cleaving protease, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type. The ADAMTS-13 deficiency leads to an accumulation of the ultra-large VWF multimers that are the most efficient VWF forms able to interact and activate platelets. The increased interaction between these ultra-large VWF multimers and platelets (via the GpIb receptor) activates and aggregates these cells in the microcirculation, causing a severe thrombocytopenia with ischemic lesions occurring downstream of the platelet-rich thrombi. Other forms of TMAs are characterized by different pathogenesis. Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy characterized by intravascular hemolysis, thrombocytopenia, and acute kidney failure. HUS is usually categorized as typical, caused by Shiga toxin-producing Escherichia coli (STEC) infection, as atypical HUS (aHUS), usually caused by uncontrolled complement activation, or as secondary HUS with a coexisting disease. In recent years, a general understanding of the pathogenetic mechanisms driving HUS has increased. The common pathogenetic features in STEC-HUS, aHUS, and secondary

HUS are simultaneous damage to endothelial cells, intravascular hemolysis, and activation of platelets leading to a pro-coagulative status, formation of microthrombi, and tissue damage. Common for the pathogenesis in STEC-HUS, aHUS, and secondary HUS seems to be the vicious cycle of complement activation, endothelial cell damage, platelet activation, and thrombosis. The knowledge of the above pathogenetic mechanisms of TMAs has recently allowed identifying successfully tailored therapies with recently developed drugs, proved helpful in clinical practice.

DIAGNOSTIC VALUE OF MORPHOLOGY IN MYELOPROLIFERATIVE NEOPLASMS

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Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the three myeloid lineage. According to the revised WHO 2017 classification, the entities included into MPN are Chronic Myeloid leukaemia BCR-ABL1 positive (CML), Chronic neutrophilic leukaemia (CNL), Polycythaemia vera (PV) Primary myelofibrosis (PMF), Essential thrombocythaemia (ET), Chronic eosinophilic leukaemia, not otherwise specified (CEL-NOS), MPN unclassifiable (MPN-U). All these forms are characterized at onset by an increased cell proliferation associated with an effective maturation that determines an increase in the peripheral blood (PB) of red blood cells, granulocytes and / or platelets, depending on the type and the number of involved lineage(s). The natural course of these forms is chronic and is characterized, depending on the entity, by progressive clonal evolution towards bone marrow failure (due to ineffective hematopoiesis or myelofibrosis) or towards acute leukemia (due to the loss of maturation capacity with progressive increase of blasts). The current knowledge of onco-genetic mechanisms and the availability of specific therapeutic treatments has profoundly modified the survival and natural course of these haematological neoplasms. An early diagnosis is essential to start specific therapeutic treatments as soon as possible to improve the quality of life and survival of patients.The WHO 2017 classification provides specific diagnostic criteria for each entity both at the time of diagnosis and in the follow-up for the identification of disease progression phases. A careful evaluation of quantitative and qualitative aspects of peripheral blood and bone marrow aspirate allows an immediate diagnostic predictivity. In Table 1 are listed the characteristics of the PB and of the bone marrow aspirate (BMA) that can predict the final diagnosis and/or the progression phases. In Table 2 are summarized the distribution among subgroups of specific molecular abnormalities. Final diagnosis must necessarily include histological evaluation of the bone marrow in the most of the cases and always the search for specific genetic and molecular alterations.

Table 1. Peripheral blood and bone marrow aspirate features that can predict the final diagnosis and/or the progression phases in myeloproliferative neoplasms.

Chronic Myeloid Leukaemia BCR-ABL1 positive: chronic phase (CML-CP)
Peripheral blood
leukocytosis neutrophilic (12-1000x10^9/L); platelets normal/increased <1000x10^9/L; hiatus leukemic with two peaks of myelocytes and segmented; blasts< 2% (every lineage and combination is possible); no dysplasia
absolute basophilia; absolute eosinophilia common; monocytes <3% (except for cases with p190 BCR-ABL1 isoform mimicking chronic myelomonocytic leukaemia)
Bone marrow aspirate
hypercellular with marked granulocytic proliferation (expansion of myelocytic stage)
normal maturation, absence of myelodysplasia, highly increased M/E ratio; blasts < 5%
megakaryocytes usually increased: mean size usually reduced (dwarf), often with nuclear hypolobulation
pseudo-Gaucher cells usually increased
Chronic Myeloid Leukaemia BCR-ABL1 positive: accelerated phase (CML-AP)
Peripheral blood
persistent/increased leukocytosis (> 10x10^9/L); persistent/increased thrombocytosis (> 1000x10^9/L) or thrombocythemia (<100x10^9/L); blasts 10-19%; basophils ≥20%
Bone marrow aspirate/biopsy
hypercellular, blasts 10-19%, appearence of dysplastic features including detection of micromegakaryocytes Diffuse fibrosis can be seen.
Chronic Myeloid Leukaemia BCR-ABL1 positive: blast phase (CML-BP)
Peripheral blood/ Bone marrow aspirate
blasts ≥ 20%
Chronic Neutrophilic Leukaemia (CNL)
Peripheral blood
leukocytosis neutrophilic ≥25x10^9/L; monocytes <1x10^9L; increased of band forms; immature precursors usually <5% with only very rare blasts allowed; no neutrophilic dysplasia
Bone marrow aspirate
hypercellular; increased myelocytes and neutrophilic percentage and number with normal maturation; M/E ratio ≥20:1; blasts < 5%
Polycythaemia Vera (PV)
Peripheral blood
usually increased Hb value associated with erythrocytosis; possible neutrophilia; rarely observed basophilia, immature granulocytes and dysplastic features: blasts never reported
Bone marrow aspirate/biopsy
panmyelosis with effective proliferation and morphologically normal maturation (erythroid and megakaryocytic expansion usually most prominent); myeloblasts are not increased
Post-PV Myelofibrosis (post PV-MF)
Peripheral blood
Leukoerythroblastostic picture
Bone marrow aspirate/biopsy
blasts ≥10%; detection of trilineage myelodysplastic features, if there is a superimposed accelerate phase; blasts ≥ 20%: blast phase. Diffuse fibrosis and osteosclerosis, the latter in advanced stage.
Primary Myelofibrosis (PMF)
Peripheral blood
leukoerythroblastosis, anysopoikilocyosis tipically with presence of dacrocytes in the fibrotic stage
Bone marrow aspirate/biopsy
dry in up to> 50% of patients; bulbous/hypolobulated megakaryocytes in all stages. Hypocellular with diffuse fibrosis and osteosclerosis in the fibrotic stage
blasts ≥10%; detection of trilineage myelodysplastic features, if there is a superimposed accelerate phase; blasts ≥ 20%: blast phase.
Essential Thrombocythaemia (ET)
Peripheral blood
usually normal WBC except for platelets(≥450x10^9/L) presenting with marked anisopoikylocytosis
Bone marrow aspirate/biopsy
usually normocellular; increased megakaryoytes with characteristic hyperlobulated, giant, cauliflower shaped nuclei without other significant abnormalities. Post-ET myelofibrosis (post ET-MF) may occur but is very rare.
Chronic Eosinophilic Leukaemia, not otherwise specified (CNL, NOS)
Peripheral blood
eosinophilia ≥ 1.5x10^9/L; mainly mature dismorphic/dysplastic eosinophils, small number of immature precursors; neutrophilia, monocytosis and mild basophilia can occur; blasts may be present but <20%
Bone marrow aspirate/biopsy
hypercellular due to eosinophilic proliferation; myelodysplasia, if present, supports the diagnosis; blast < 20%. Finbrosis can be seen.
Myeloproliferative Neoplasm, Unclassifiable (MPN-U)
Peripheral blood
variable, from mild leukocytosis to moderate or marked thrombocytosis, with or without anemia
Bone marrow aspirate/biopsy
variable, depending on clinical presentation and presence/absence of fibrosis

Table 2. Incidence of specific molecular abnormalities in the NPMs subtypes at diagnosis.

	CML	CNL	PV	PMF	ET	CEL, NOS	MPN-U
BCR-ABL1	90-95%	None	None ^	None ^	None ^	None	None
JAK2V617F	None	None/Rare	>95%	50-60%	50-60%	Rare	Variable
JAK2 Exon 12	None	None/Rare	3%*	None/Rare	None/Rare	None	None
MPL Exon 10	None	None/Rare	Rare	8%	3%	None	Variable
CALR Exon 9	None	None/Rare	Rare	30%	30%	None	Variable
Triple negative	n/a	n/a	Rare	12%	12%	n/a	Variable
CSF3R 2	None	90-100%	None	None	None	None	None

Legenda: *CML*: Chronic Myeloid leukaemia BCR-ABL1 positive. *PV*: Polycythemia vera. *ET*: Essential thrombocythaemia. *PM*: Primary myelofibrosis. *CNL*: Chronic neutrophilic leukaemia, *CEL,NOS*: Chronic eosinophilic leukaemia, not otherwise specified. *MPN-U* unclassifiable (MPN-U). *Triple negative*: absence of JAK2, MPL or CALR mutations. *n/a*: not applied.
^ rarely acquired
* associated with predominat erythroid haematopoiesis

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PRIMARY PROPHYLAXIS OF VENOUS THROMBOEMBOLISM IN PATIENTS AFFECTED BY HAEMATOLOGICAL MALIGNANCIES (LYMPHOMA, MULTIPLE MYELOMA, ACUTE LEUKAEMIA): GUIDELINES FORM THE ITALIAN SOCIETY OF HAEMATOLOGY

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Several risk factors are involved in the pathogenesis of venous thromboembolism (VTE) in patients affected by cancer, these include cancer type, central venous catheters placement, chemotherapy, radiotherapy, immunomodulatory drugs. The management of VTE may be quite complex in patients with cancer, this is mainly due to an increased risk of recurrent VTE, haemorrhages, morbidity, and hospital admissions. With reference to haematological malignancies, a high intrinsic risk of VTE is related to several disease-specific factors (high blood viscosity, JAK-2 hyper activation, nephrotic syndrome and elevated cytokines levels) and treatment related factors (immunomodulatory drugs and asparaginase). VTE occurrence may negatively affect treatment of the underlying blood cancer and expose patients to a higher risk of death. Furthermore, patients affected by haematological malignancies have a particularly higher risk of bleeding because of thrombocytopenia and hyperfibrinolysis. Defining the clinical indication to prophylaxis of VTE is quite challenging, based on the varying risks of VTE and bleeding complications across different haematological malignancies and their specific treatments. Options for the treatment and prevention of cancer associated VTE, previously mainly based on low-molecular-weight heparin (LMWH), have recently been enriched by direct oral anticoagulants (DOACs). These agents have shown a good risk/benefit profile, however they require an accurate patient-based evaluation for their potential interactions with other treatments and bleeding risks. Up-to-date, studies on the treatment of VTE in patients affected by acute leukaemia are scant and specific guidelines on prophylaxis of VTE in patients affected by leukaemia and lymphoma are not available, while single consensus documents have been developed for patients affected by multiple myeloma and chronic myeloproliferative neoplasm (MPN) with several limits; guidelines on the prevention of VTE in patients with cancer have been recently updated by several scientific societies, and one National Society (AIOM 2020). These recommendations, however, have not been specifically conceived for patients with haematological malignancies, thus the Società Italiana di Ematologia (SIE) has recently developed the first national evidence-based clinical practice guidelines on the administration of pharmacological primary prophylaxis of VTE in patients affected by haematological malignancies. The SIE guidelines have used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology and adopted as reference international guideline, the last updated guidelines from ITAC. The primary aim of these guidelines was to define the primary prophylaxis of VTE schedule in patients affected by haematological malignancies, managed in national structures, including available options, approved for this indication in Italy and published evidences. The target population of these guidelines includes patients affected by acute leukemia, lymphoma and multiple myeloma, not under chronic anticoagulant treatments. The SIE guideline working group (WG) involved Italian haematologists expert in this field (AF, VdS ,MM, AV), three methodology experts (MM,MP, PB) one coordinator (MN), volunteer representatives from scientific societies (MdN, AT,PT,GL) and patients (AP). Guidelines were peer reviewed by 3 external academic clinicians (SS, FR, RS). During the first WG teleconference encounter, recommendations from ITAC guidelines were selected for their further analysis. The following benchmarks were selected: Patients were deemed at high risk of VTE for an estimated VTE risk of more than 5% at 6 months; A pharmacological prophylaxis of VTE was judged acceptable for a ratio of the number needed to treat (NNT) for symptomatic VTE prevention and the number needed to harm (NNH) for major bleeding complications of more than 3; The availability of oral pharmacological prophylaxis of VTE was deemed relevant for patients' quality of life.PICO questions and recommendations are summarised in Table 1 and 2. Further studies in this field will allow to improve the intricate management of VTE in haematological malignancies.

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Table 1. PICO questions

In patients affected by haematological malignancies, is it recommended to apply a clinical risk score of VTE, specifically validated in this population for the prevention of symptomatic VTE?
In patients with haematological malignancies, a specific clinical risk score should be adopted to define an adequate prophylaxis of VTE ***(+C, with low evidence)***
In patients affected by haematological malignancies, is it recommended to apply a clinical risk score of bleeding, specifically validated in this population, with the aim to prevent major/clinically relevant bleeding complications?
It is recommended to not adopt bleeding risk scores validated in the general population or in patients affected by solid tumours under anticoagulant treatment to estimate the bleeding risk of patients with haematological malignancies ***(-S, with very low evidence)***
Should patients affected by haematological malignancies be periodically re-evaluated for their risk of VTE, instead of only once at diagnosis, with the aim to prevent symptomatic VTE and major/clinically relevant bleeding complications?
In patients affected by haematological malignancies, the risk of VTE should be evaluated at diagnosis, six months after treatment and at any disease relapse or progression, after the administration of a new treatment with potential thrombotic risk ***(+C, with low evidence)***
In patients affected by haematological malignancies at high risk of VTE, is it recommended a pharmacological prophylaxis of VTE, instead of no prophylaxis to prevent symptomatic VTE?
In patients affected by haematological malignancies at high risk of VTE in absence of high bleeding risk, a pharmacological prophylaxis of VTE is recommended ***(+S, with low evidence)***
In patients affected by haematological malignancies at high risk of VTE, eligible for pharmacological prophylaxis of VTE, is it recommended the administration of Low Molecular Weight Heparin (LMWH) instead of other available therapies with the aim to prevent symptomatic VTE and major/clinically relevant bleeding complications?
In patients affected by haematological malignancies, deemed eligible for prophylaxis of VTE, the administration of LMWH is suggested ***(+C, with very low evidence)***
In hospitalised patients affected by haematological malignancies VTE prophylaxis is recommended instead of no prophylaxis in order to prevent symptomatic VTE and major/clinically relevant bleeding complications?
In hospitalised patients affected by haematological malignancies, VTE prophylaxis with Unfractionated Heparin (UFH), LMWH or fondaparinux, is recommended in absence of contraindications ***(+C, with low evidence)***
In patients affected by haematological malignancies with central venous catheter (CVC), is pharmacological prophylaxis of VTE recommended instead of no prophylaxis for symptomatic VTE and major/clinically relevant bleeding prevention?
In patients affected by haematological malignancies pharmacological prophylaxis of CVC related VTE is not recommended ***(-C, with low evidence)***

Legend VTE= Venous thromboembolism, + =Positive ;-=Negative; C=Conditional;S=Strong ;N= Neutral.

Table 2. VTE prophylaxis in multiple myeloma, lymphoma and acute leukaemia

VTE prophylaxis in patients affected by multiple myeloma

In patients with multiple myeloma (MM)at high risk of VTE, eligible for primary pharmacological prophylaxis of VTE, is it recommended the administration of apixaban and rivaroxaban instead of LMWH with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

Available evidences on primary prophylaxis of VTE with apixaban and rivaroxaban in MM are currently too limited, thus the panel suggests to adopt LMWH over apixaban or rivaroxaban§ for the primary prophylaxis of VTE in patients with MM at high risk of VTE ***(-C, with low evidence)***

In patients with MM at low risk of VTE is it recommended the administration of primary prophylaxis with low dose acetyl salicylic acid instead of no prophylaxis with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

In patients with MM at low risk of VTE at diagnosis o during the follow-up it is suggested to evaluate pharmacological prophylaxis of VTE with low dose acetyl salicylic acid ***(+ C, with low evidence)***

VTE prophylaxis in patients affected by lymphoma

In outpatients with lymphoma requiring long-term primary prophylaxis of VTE, is it recommended the administration of UFH or LMWH instead of apixaban or rivaroxaban with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

In outpatients with lymphoma requiring long-term prophylaxis of VTE, it is suggested to preferably adopt LMWH or UFH instead of apixaban or rivaroxaban § ***(-C, with low evidence)***

In patients affected by lymphoma treated with CAR-T, is it recommended to administer any specific primary prophylaxis of VTE instead of common measures of prophylaxis with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

It is currently not possible to elaborate specific recommendations for the prophylaxis of VTE in patients with lymphoma treated with CAR-T. The panel suggests to adopt general recommendations, available for hospitalised patients with lymphoma (N, studies not available)

VTE prophylaxis in patients affected by acute leukaemia

In patients affected by non-APL acute leukaemia, is it recommended routine primary prophylaxis instead of personalised prophylaxis of VTE with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

Primary prophylaxis of VTE is not routinely recommended in patients with acute leukaemia due to the high bleeding risk mainly related to thrombocytopenia ***(-S, with moderate evidence)***

In patients affected by acute lymphoblastic leukaemia (ALL) under treatment with asparaginase, is it recommended primary prophylaxis of VTE with LMWH plus antithrombin infusion instead of no prophylaxis, with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

In patients affected by ALL under treatment with asparaginase, prophylaxis of VTE with LMWH is recommended, it is also suggested to administer antithrombin to reach therapeutic target levels of 80-120% ***(+C, with low evidence)***

In patients affected by acute lymphoblastic leukaemia is it recommended to regularly evaluate the risk of VTE and bleeding with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

It is recommended to regularly evaluate the risk of thrombosis by monitoring clinical and laboratory parameters, including fibrinogen and antithrombin levels, in each patient affected by ALL under treatment with asparaginase ***(+C, with very low evidence)***

In patients with acute leukemia under prophylaxis with LMWH, is it recommended to assay anti-Xa levels instead of no assay of anti-Xa with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

In patients under prophylaxis with LMWH, the panel suggests to evaluate, when available, anti-Xa levels ***(+C, with very low evidence)***

In patients affected by APL, is it recommended a personalised prophylaxis of VTE instead of routine prophylaxis of VTE with the aim to minimize symptomatic VTE and major/clinically relevant bleeding?

The prophylaxis of VTE in not recommended on a routine basis in patients affected by APL due to the high bleeding risk: a risk/benefit evaluation of VTE prophylaxis should be evaluated on an individual basis ***(-S,with very low evidence).***

Legend

+ =Positive;-:Negative; C=Conditional; S=Strong ;N= Neutral; APL= acute promyelocytic leukemia

§ apixaban and rivaroxaban are currently approved only for secondary prophylaxis of VTE

CLINICAL PRACTICE FOR MULTIPLE MYELOMA PATIENTS

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Introduction: Multiple myeloma (MM) is a malignant disease characterized by proliferation of plasma cells in the bone marrow that results in bone, renal, and hematologic complications.[1,2] Similar annual incidences of MM are seen across Europe and the United States, in Italy the incidence was estimated at 11.1/ 100,000 persons (5.759 new cases per year) [AIRTUM 2020]. The treatment of MM has drastically changed in the past decade with the incorporation of novel agents into therapeutic strategies resulting in substantial improvements in overall survival.[3,4] The median number of treatment lines for MM patients is two[5] and the health care cost in Italy is more than €15,000/year[6-8] with a higher social cost.[9] Considering the availability of several different classes of approved agents, which can be combined in doublet, triplet, or even quadruplet, the choice of the optimal strategy at diagnosis and at relapse represents a challenge for physicians.

Aim: The aim of this Clinical Practice Guideline is to define the minimal level of therapeutic assistance and routine practice of MM patients followed in the Italian centres according to the currently available international data and the drug accessibility in Europe. No recommendations for the treatment of plasma cell leukaemia, POEMS and amyloidosis as well as diagnostic process and supportive care are provided here.

Method: An interdisciplinary panel of clinical experts on MM, members of scientific societies (SIES, GITMO, AIOM) and a patient representatives developed these recommendations that, before publication, were sent for comments to external reviewers. According to the methodology manual of SIE (Italian Society of Haematology) guidelines, the ASCO and CCO (American Society of Clinical Oncology / Cancer Care Ontario) Joint Clinical Practice Guideline published in 2019 were used as backbone, and articles, available since 2018, in EMBASE, Cochrane, MEDLINE/PubMed were selected to provide the additional systematic review. The Expert Panel met via teleconference, webinars, and corresponded through e-mail. During the first meeting the panel decided to review the clinical questions concerning the high quality of new evidence published in the last 18 months and the new treatments approved by EMA and decided which are the recommendations that can be "adopted" from the ASCO/CCO guidelines and which that are to develop "de novo". Since January 2019, EMA approved different drug combination:

1. Daratumumab-Bortezomib-Talidomide-Desametasone for NDMM (Newly diagnosed) transplant eligible (TE) patients
2. Daratumumab-Bortezomib-Melfalan-Prednisone for NDMM no transplant eligible (NTE) patients
3. Daratumumab-Lenalidomide-Desametasone for NDMM-NTE
4. Bortezomib-Lenalidomide- Desametasone for NDMM-NTE
5. Isatuximab-Pomalidomide-Desametasone for refractory/relapse (RRMM) > 3^ line
6. Elotuzumab-Pomalidomide-Desametasone for refractory/relapse (RRMM) > 3^ line
7. Bortezomib-Pomalidomide-Desametasone for refractory/relapse (RRMM) > 2^ line (lenalidomide in one of the previous line of therapy) and the subcutaneous administration of daratumumab as well as the generic drug

1. Bortezomib Fresenius Kabi (Committee for Medicinal Products for Human Use - CHMP 20-9-2019)
2. Lenalidomide Accord (CHMP 7-01-2020)

Based on these criteria 7 clinical questions are identify:

1. NDMM-TE: Dara-VTD vs SoC (standard of care) (VTD)
2. NDMM-TE: VRD vs SoC (VTD)
3. NDMM-NTE: Dara-VMP vs SoC (VMP, Rd)
4. NDMM-NTE: VRD vs SoC (VMP, Rd)
5. NDMM-NTE: DaraRd vs SoC (VMP, Rd)
6. MM refractory/relapse (RRMM) – 1^ or 2^ relapse: triplet's vs doublets
7. MM RRMM – 3^ relapse or more: Belantamab Mafodotin, Selinexor

Questions adopted from ASCO/CCO guidelines are:

1. Which are the response goals for the transplant-eligible patient? How to assess it?
2. What are the options for initial therapy before transplant?
3. How many stem-cell collections we need to collect for NDMM-TE?
4. Which are the patient candidate to transplant?
5. How select NDMM-TE patients?
6. Which conditioning regimen for ASCT?
7. When tandem ASCT must be recommended?
8. Which patients must receive consolidation therapy?
9. Which patients must receive maintenance therapy?
10. Which strategy for high risk cytogenetic patient?
11. When the response assessment is recommended?
12. It's possible to make modifications to maintenance therapy based on depth of response?
13. Which factors must be considered to decide the initial therapy for transplant ineligible patients?
14. Which are the response goals for the NDMM-NTE patient?
15. Which therapy should be preferred for the NDMM-NTE patient: continuous or fixed?
16. How personalize the treatment for the NDMM-NTE patient?
17. When treatment at relapse need to be start?
18. Which therapy should be recommended for the first and second relapse?
19. How long relapse treatment need to be continued?
20. Can response to second line treatment be consolidated with ASCT?
21. When allogeneic transplant should be recommended?

QUESTIONS/RECOMMENDATIONS

TRANSPLANT-ELIGIBLE POPULATION

Which are the response goals for the transplant-eligible patient? How to assess it

The goal of initial therapy for transplant-eligible patients should be the achievement of the best depth of remission. The quality and depth of response should be assessed by IMWG criteria (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

What are the options for initial therapy before transplant?

At least four (maximum 6) cycles of induction therapy including an immunomodulatory drug, proteasome inhibitor, and steroids for NDMM-TE patients. (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

For NDMM-TE patients DaraVTD or VTD?

Daratumumab should be add to VTD (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (de novo recommendation)

How many stem-cell collections we need to collect for NDMM-TE?

Ample stem-cell collection (sufficient for more than one SCT) should be considered up front, due to concern for limited ability for future stem-cell collection after prolonged treatment exposure (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

Which are the patient candidate to transplant?

Up-front transplant should be offered to all transplant-eligible patients. (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

How select NDMM-TE patients?

To determine eligibility for SCT, patients must be evaluated for risks diseases, depth of response to treatment, type of induction treatment (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

Which conditioning regimen for ASCT?

High-dose melphalan is the recommended conditioning regimen for ASCT (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

When tandem ASCT must be recommended?

Tandem ASCT should not be routinely recommended. It's important to evaluate biological risk factors, response to first ASCT and patient's clinical conditions. (Type: evidence based; Evidence quality: intermediate, benefit equals harm; Strength of recommendation: strong). (adopted recommendation)

Which patients must receive consolidation therapy?

Consolidation therapy is not routinely recommended. For patients ineligible or unwilling to consider maintenance therapy, consolidation therapy for at least two cycles may be considered (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

Which patients must receive maintenance therapy?

Lenalidomide maintenance therapy should be routinely offered to all patients starting at approximately day 90 to 110 at 10 to 15 mg daily until progression. (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

Which strategy for high risk cytogenetic patient?

Induction therapy must be consolidated with tandem ASCT and maintenance therapy. When patients cannot receive lenalidomide as maintenance therapy two cycles of consolidation treatment must be considerate. (Type: evidence based; Evidence quality: low, benefit outweighs harm; Strength of recommendation: weak). (adapted recommendation according to Italian contest)

When the response assessment is recommended?

It is recommended that depth of response be assessed with each cycle. Frequency of assessment once best response is attained or on maintenance therapy may be assessed less frequently but at minimum every 3 months (Type: evidence based; Evidence quality: low, benefit outweighs harm; Strength of recommendation: weak). (adopted recommendation)

It's possible to make modifications to maintenance therapy based on depth of response?

There is insufficient evidence to make modifications to maintenance therapy based on depth of response, including minimal residual disease (MRD) status (Type: informal consensus/evidence based; Evidence quality: low/intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

TRANSPLANT-INELIGIBLE POPULATION

Which factors must be considered to decide the initial therapy for transplant ineligible patients?

Initial treatment recommendations for patients with multiple myeloma who are transplant ineligible should be individualized based on shared decision making between physicians and patients. Multiple factors should be considered: disease-specific factors such as stage and cytogenetic abnormalities, and patient-specific factors including age, comorbidities, functional status, frailty status, and patient preferences should also be considered (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

Which are the response goals for the transplant-ineligible patient?

The goal of initial therapy for transplant-ineligible patients should be achievement of the best quality and depth of remission (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

Which therapy should be preferred: continuous or fixed?

Continuous therapy should be preferred over fixed-duration (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation)

Which therapy should be preferred: DaraVMP or standard of care (VMP or Rd)?

DaraVMP therapy should be preferred over the standard of care (VMP and Rd) (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (de novo recommendation)

Which therapy should be preferred: VRd or standard of care (VMP or Rd)?

VRd therapy should be preferred over the standard of care (VMP and Rd). Patients need to be selected with attention due to toxicity profile of VRd. (Type: evidence based; Evidence quality: low, benefit outweighs harm; Strength of recommendation: weak). (de novo recommendation).

Which therapy should be preferred: DaraRd or standard of care (VMP or Rd)?

DaraRd therapy should be preferred over the standard of care (VMP and Rd) (Type: evidence based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (de novo recommendation).

How personalize the treatment?

It is recommended that patients be monitored closely with consideration of dose modifications based on levels of toxicity, neutropenia, fever/infection, tolerability of adverse effects, performance status, liver and kidney function, and in keeping with the goals of treatment. (Type: informal consensus; Evidence quality: low, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation)

RELAPSED POPULATION

When treatment at relapse need to be start?

All clinically relapsed patients with symptoms due to myeloma should be treated immediately (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adopted recommendation).

Which therapy should be recommended for the first and second relapse?

Triplet therapy should be administered on first relapse, though the patient's tolerance for increased toxicity should be considered. A triplet is defined as a regimen with two novel agents (PIs, immunomodulatory drugs, or monoclonal antibodies) (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: strong). (adapted recommendation: to second relapse too)

How long relapse treatment need to be continued?

Treatment of relapsed multiple myeloma may be continued until disease progression. There are not enough data to recommend risk-based versus response-based duration of treatment (such as MRD) (Type: evidence-based; Evidence quality: intermediate, benefit outweighs harm; Strength of recommendation: moderate). (adopted recommendation).

Can response to second line treatment be consolidated with ASCT?

ASCT, if not received after primary induction therapy, should be offered to transplant eligible patients with relapsed multiple myeloma. Repeat SCT may be considered in relapsed multiple myeloma if progression-free survival after first transplant is > 24-36 months or greater (Type: evidence-based; Evidence quality: low, benefit outweighs harm; Strength of recommendation: weak). (adopted recommendation).

When allogeneic transplant should be recommended?

Allogeneic transplant for multiple myeloma is not routinely recommended but may be considered in select high-risk patients or in the context of a clinical trial (Type: evidence based; Evidence quality: intermediate, harm outweighs benefit; Strength of recommendation: strong). (adopted recommendation).

CAR-T and other immunotherapy strategies can be recommended at the moment?

Immunotherapy strategies targeting BCMA or other antigens on the surface of myeloma cells, including chimeric antigen receptor T (CAR-T) and cells bispecific T-cell engagers (BiTEs), are under clinical investigation in RRMM patients. Results of phase III studies are awaited so, at the moment, it's not possible to give recommendations based on scientific evidence.

More information is available at: https://siematologia.it/raccomandazioni-linee-guida.html

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IMMUNE THROMBOCYTOPENIA IN THE ADULT: ITALIAN GUIDELINES

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The Italian Guidelines (GL) for the management of the adult patient with immune thrombocytopenia (ITP) represent an adaptation according to the AGREE II tool of the 2019 ASH GL, in accordance with the GRADE-ADOLOPMENT approach. The terminology proposed by the Consensus of the "International Working Group (IWG) on ITP", published in 2009, was used (Table 1).

Table 1. Terminology and definitions based on published literature.

TERMINOLOGY	DEFINITION
Corticosteroid dependent	Continued need for Prednisone >5 mg/day (or equivalent cortisone) to maintain platelet count ≥30,000/mm^3 and/or to prevent bleeding
Sustained response	Platelet count ≥30,000/mm^3 and at least a two-fold increase from baseline at 6 months
Early response	Platelet count ≥30,000/mm^3 and at least a twofold increase from baseline at 1 week
Response	Platelet count ≥30,000/mm^3 and at least a two-fold increase from baseline at 1 month
Major bleeding	Grade 3-4 bleedings according to the WHO classification
Minor bleeding	All bleedings not classifiable as major
Newly diagnosed ITP	Duration of ITP within 3 months
Persistent ITP	ITP duration 3-12 months
Chronic ITP	ITP duration > 12 months

Table 2. Clinical questions selected by the expert panel and agreement rate.

	Clinical questions	% of agreement
1	In adult patients with newly diagnosed thrombocytopenia and platelet count <30,000/mm^3 who are asymptomatic or have minor muco-cutaneous bleeding, is corticosteroid treatment or observation preferable?	100
2	In adult patients with newly diagnosed ITP, asymptomatic or with minor muco-cutaneous bleeding, and platelet count >30,000/mm^3, is corticosteroid treatment or observation indicated?	100
3	In adult patients with newly diagnosed ITP, asymptomatic or with minor muco-cutaneous bleeding, and platelet count <20,000/mm^3, is inpatient or outpatient management indicated?	100
4	In adult patients with newly diagnosed ITP, asymptomatic or with minor muco-cutaneous bleeding, and with platelet count >20,000/mm^3, is inpatient or outpatient management indicated?	100
5	Should adult patients with newly diagnosed ITP be initially treated with a prolonged course (>8 weeks including discontinuation) or short (<8 weeks) course of corticosteroid therapy?	80
6	In adult patients with newly diagnosed ITP, should the initial corticosteroid treatment be prednisone (0.5-2 mg/Kg/day) or dexamethasone (40 mg/day for 4 days)?	100
7	In adult patients with ITP of >6 months duration, and who are dependent or unresponsive to corticosteroids and candidates for TPO mimetics, is treatment with eltrombopag or romiplostim preferable?	100
8	In adult patients with ITP of >6 months duration who are dependent on or unresponsive to corticosteroids, is treatment with TPO mimetics or splenectomy preferable?	100
9	In adult patients with ITP of > 6 months duration who are dependent or unresponsive to corticosteroids, is treatment with splenectomy or rituximab preferable?	100
10	In adult patients with ITP of >6 months duration who are dependent on or unresponsive to corticosteroids, is treatment with TPO mimetics or Rituximab preferable?	100
11	In adult patients with ITP lasting >2 months but <6 months, dependent on or unresponsive to corticosteroids, is treatment with splenectomy or Rituximab preferable?	100

What was recently published by the IWG in 2019, although obtained through the Consensus mode, was also considered as a reference. The Expert Panel (EP) was composed of 5 hematologists, 1 pediatric hematologist, 1 representative of ITP patients ITP, and 2 methodologic hematologists. The purpose of these GL was to produce clinical recommendations regarding the management of adult patients with ITP. With an estimated annual incidence of 1 to 6/100,000 people in the general population, ITP is listed in the Rare Diseases Registry in Italy. The absence of large, randomized trials characterizes its variability in clinical practice on the national territory. The main aim of these GL is to produce recommendations that take the indication to start treatment, the impact of individual treatments on the patient, the response in terms of platelet count, adverse events and outcomes into consideration, based on the systematic review and critical evaluation of the evidence available in literature, in order to optimize physician's care and improve patient outcomes. The targeted audience is represented by the health care providers involved, stakeholders, and patients. The questions of the ASH GL were presented by email to the panel members. The panel subsequently agreed on consensus by vote. Ten clinical questions were considered relevant, with the exception of the question regarding the use of Rituximab 1st line, because it conflicted with the current Italian legislation on the prescriptibility of the drug. Another aspect of deviation from the ASH 2019 GL regarded the non-prescribability on the national territory of Thrombopoietin (TPO) mimetic drugs before the 6th month from the diagnosis of ITP. This led to the need to introduce a recommendation in the form of an Indicator of Good Clinical Practice adapted to the national territory in the 2nd line treatment of patients before 6 months from diagnosis. The EP therefore formulated, in complete agreement, 11 clinical questions (Table 2), to be answered with strong (S) or weak-conditional (C) recommendation, according to the analysis of available evidence. "Notes" and "Indicators of Good Clinical Practice (IGCP)" were used as tools to express suggestions derived from the direct experience of the EP or slight deviations from the GL adopted as a model. Therapy of patients with ITP should be customized and should prevent major bleedings and optimize quality of life. In general, treatment should at least aim to achieve and

maintain a platelet count >20-30,000/mm^3, in the absence of additional bleeding risk factors.

In recommendation 1, the panel suggests treatment with corticosteroids rather than observation (C recommendation) in adult patients with newly diagnosed ITP, platelet count <30,000/mm^3 and asymptomatic, or with minor mucocutaneous bleedings.

In recommendation 2, the panel recommends observation rather than corticosteroid treatment (S recommendation, based on very low evidence certainty) in patients with platelet count >30,000/mm^3 who are asymptomatic or have minor mucocutaneous bleedings.

In recommendation 3, the panel recommends inpatient rather than outpatient management in newly diagnosed patients with platelet count <20,000/mm^3, asymptomatic or with minor mucocutaneous bleedings. The benefit of inpatient management is considered moderate, but elements such as confirmation of diagnosis, therapeutic choice, observation of platelet count trend, response to therapy, assessment of additional bleeding risk, and prompt therapy in case of major bleeding (C recommendation) are valued.

In patients with a previously confirmed diagnosis of ITP, asymptomatic or with minor mucocutaneous bleedings and with a platelet count <20,000/mm^3, the panel considered the benefit of inpatient management to be negligible.

In recommendation 4, the panel recommends outpatient management rather than hospitalization (C recommendation) in patients with a platelet count >20,000/mm^3, asymptomatic, or with minor mucocutaneous bleedings.

In recommendation 5, the EP considered a total duration of steroid therapy until definitive discontinuation of less than 6 weeks, as suggested by the U.S. panel, unfeasible. However, a total duration ≤8 weeks was considered more practicable. In fact, maintenance of the induction dose for 3-4 weeks is generally recommended. Moreover, in the last weeks of tapering, the steroid dose is low and therefore less undesired effects are observed. The panel considered that the evidence and the EtD framework related to the question of the duration of steroid therapy shorter or longer than 6 weeks did not differ significantly (except for the above-mentioned feasibility) from those of the same question with a threshold time of 8 weeks. Therefore, they adopted the evidence profile and the EtD tables, only modifying the text of the question and the corresponding recommendation. Therefore, a S recommendation was formulated, given the high-quality evidence regarding the potential side effects of prolonged cortisone therapy (>8 weeks) in other patient populations. Side effects considered included: hypertension, hyperglycemia, sleep and mood disorders, epigastralgia, glaucoma, myopathy, and osteoporosis.

In recommendation 6, with regard to the type of corticosteroid to be used, the panel suggests the use of prednisone (0.5-2 mg/Kg/day) or dexamethasone (40 mg/day for 4 days) (C recommendation), suggesting the preferential use of dexamethasone if a more rapid increase in platelet count is needed.

In recommendation 7, in patients with ITP of >6 months duration, dependent or unresponsive to corticosteroids, and candidates for TPO mimetics, the panel suggests treatment with eltrombopag or romiplostim, considering the choice of the drug based on the patient's preference in the method of administration (C recommendation) plausible.

In recommendation 8, in patients with ITP of >6 months duration who are corticosteroid-dependent or nonresponsive, the panel suggests treatment with TPO mimetics or splenectomy, the latter preferably performed after 12 months from diagnosis and after vaccination against capsulated bacteria (C recommendation).

In recommendation 9, in patients with ITP of >6 months duration, dependent or unresponsive to corticosteroids, the panel suggests treatment with Rituximab rather than splenectomy (C recommendation).

In recommendation 10, in patients with ITP of > 6 months duration, dependent or non-responsive to corticosteroids, the panel suggests treatment with TPO-mimetics rather than Rituximab, given the longer duration of response achievable with the former (C recommendation).

In recommendation 11 (IGCP), in patients with ITP of ≤ 6 months duration, who are dependent or unresponsive to first-line corticosteroid therapy, treatment with repeated administrations of high-dose immunoglobulins or Rituximab is suggested, rather than splenectomy, after 12 months from diagnosis. Alternatively, in selected cases, maintenance therapy with low doses of cortisone or the use of other immunosuppressive drugs can be considered and are to be continued until the 6th month (see current Italian regulations on the prescriptibility of TPO-ra). A flowchart of the recommendations is illustrated in Figure 1.

The EP concluded that in clinical practice there is no single optimal second line for all ITP patients. Treatment should be tailored according to disease duration, frequency of bleedings requiring hospitalization or antihemorrhagic therapy, comorbidities, age, adherence to therapy, cost of medical and social support, patient values and preferences, and availability of the treatment options.

The EP is in agreement to postpone splenectomy to after the first year from ITP diagnosis, because of the possibility that some patients may achieve spontaneous remission within this timeframe. For patients with disease duration <12 months, the panel made a conditional recommendation, favoring TPO-mimetics over Rituximab, because of the longer duration of the response achievable with the former. Moreover, Rituximab might be preferred in patients who prefer to avoid long-term treatment. For patients with disease duration >12 months, the panel considered splenectomy, TPO mimetics, and Rituximab all to be possible choices.

Finally, the panel discussed the criteria for updating these GL, agreeing on a timeline consistent with the authorization for prescribing new drugs, or on any changes regarding the criteria for prescribing drugs already in use for the treatment of immune thrombocytopenia in the national territory.

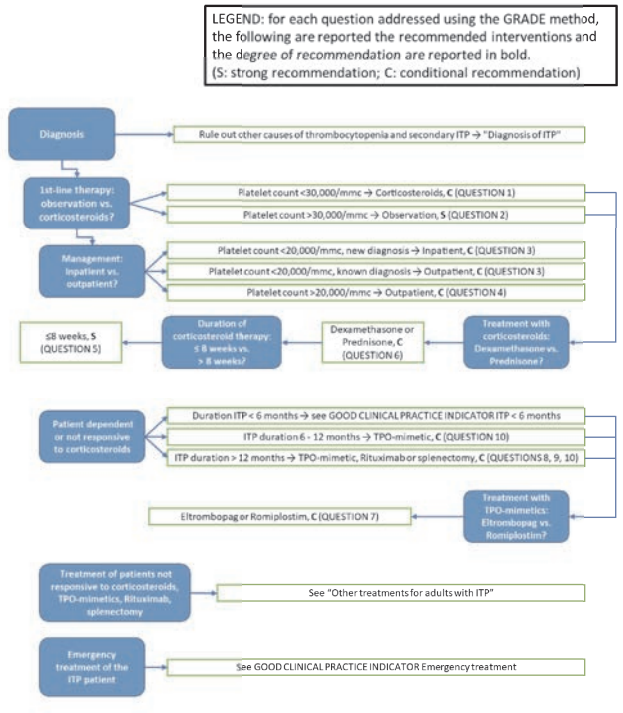

Figure 1. Flowchart of the questions and recommendations.

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MARGINAL ZONE LYMPHOMAS

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Marginal Zone Lymphoma (MZL) are a group of indolent lymphoproliferative disorders arising from post- germinal center marginal-zone B cell. MZLs accounts for 7% of all non-Hodgkin lymphomas in adults in the Western world.[1]

The current 2017 World Health Organization classification recognized three different subtypes of MZLs including the extranodal MZL (EMZL) of mucosa-associated lymphoid tissue (MALT) which can be subdivided into gastric and extragastric EMZL, the splenic MZL (SMZL) and the nodal MZL (NMZL) accounting for approximately 70%, 20%, and 10% of MZLs, respectively.[2,3,4] The median age at the diagnosis is 60 years for NZML and EZML and 65 years for SMZL and most patients experience long term survival. These three entities present a common immunophenotype pattern, expressing positivity for pan B-cell markers (CD19, CD20, CD22, CD79a) and negativity for CD5, CD10 and (usually) CD23; at the same time, they differ in diagnostic criteria, molecular cytogenetic characteristics, clinical courses and therapeutic approaches. For the diagnosis is mandatory an accurate tumor biopsy and a bone marrow aspirate and biopsy are required for NMZL and SMZL and highly recommended for EMZL.

The disease staging follows the Lugano modification of Ann Arbor staging system and requires specific blood tests, computed tomography (CT) scan of head, neck, chest, abdomen, pelvis and specific investingation for EMZL (upper gastrointestinal endoscopy, orbit MRI, etc).

The use of positron emission tomography (PET) scan is still controversial and remains investigational. Frontline treatment is usually tailored on patients, depending on the MZL's subtype, lymphoma's stage and clinical features.

Asymptomatic patients (early or advanced stage) may benefit from a "watch and wait" approach with follow-up evaluation every 3- 6 months.

Treatment's criteria are rappresented by systemic B symptoms, cytopenias, bulky disease or rapid and symptomatic lymphoma enlargement. Involved site radiotherapy (ISRT)/involved field radiotherapy (IFRT) is indicated in localized NMZL or EMZL (stage I/II). Eradication therapy with antibiotics anti-Helicobacter Pylori is strongly recommended in all gastric MALT lymphomas.

Multiple terapeutic options are available for patients with SMZL ranging from 'watchful waiting' approach to hepatitis-C antiviral therapy in HCV-positive patients[5], splenectomy and immunotherapy. Chemoimmunotherapy or immunotherapy alone (with anti-CD20 monoclonal antibody, rituximab) are effective in all patients with MZL who require systemic treatment. In relapsed MZL a re-treatment with chemoimmunotherapy can be indicated after a long remission (> 24 months). Novel targeted therapies such as bruton tyrosine kinase (BTK) inhibitors or phosphoinositide 3-kinase (PI3K) inhibitors show promising results in chemo-immune-resistance MZL.[6]

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COVID-19 IN HEMATOLOGICAL MALIGNANCIES

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An outbreak caused by a novel human coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Wuhan (China) in December 2019.[1] Infection rapidly spread and on March 11, 2020, the World Health Organization declared 2019 Coronavirus Disease (Covid-19), caused by SARS-CoV-2, a global pandemic. The potential threat of Covid-19 to patients immuno-compromised because of cancer was thought to be significant from the beginning.[2] Hematological malignancies (HMs) can be cured or have a long survival in a sizeable fraction of cases, therefore infections can shorten life expectancy. Patients have usually long-lasting immunodeficiency because of malignancy itself, anticancer treatments, or hematopoietic cell transplantation. Thus, they are more susceptible to infections and less efficient in mounting an effective immune response.

In this scenario, the ITA-HEMA-COV project was developed to collect and analyze the data in adult HM patients requiring hospitalization for Covid-19. This study was registered in the ClinicalTrials.gov, NCT04352556 and now includes 2768 cases. The initial report analyzed 536 patients admitted during the first wave of Covid-19 in Italy.[3] As of June 22, death occurred in 198 subjects (37%). The ratio between observed death in the study cohort and expected death of the Italian popu-

lation was 2.0 in the whole population and 3.7 in individuals younger than 70 years. Older age (HR, 1.0), progressive disease status (HR, 2.1), diagnosis of acute myeloid leukemia (HR, 3.5), indolent (HR, 2.2) and aggressive non Hodgkin lymphomas (HR, 2.5), plasma cell neoplasms (HR, 2.5), and severe/critical Covid-19 (HR, 4.0) were associated with death. In addition, the analysis showed that withholding specific effective treatments during the pandemic is not justified. This information was subsequently confirmed by the American Society of Hematology Registry[4] and a meta-analysis including 3,210 persons with HM.[5]

Several studies have shown that 95-100% of immunocompetent patients with Covid□19 have seroconverted about 3 weeks after symptoms onset.[6] The ITA-HEMA-COV analyzed 237 HM patients (62 myeloid neoplasms, 121 lymphoid neoplasms, and 54 plasma cell neoplasms) to assess different patterns of immune response and found that Covid-19 elicits an impaired antibody response against SARS-CoV-2 in HM.(7) Overall, 69% of patients had detectable IgG SARS-CoV-2 serum antibodies. Serological negative patients (31%) were evenly distributed across patients with myeloid, lymphoid and plasma cell neoplasms. Overall, chemoimmunotherapy (OR: 3.4) was associated with a lower rate of seroconversion with an effect lasting more than 180 days from treatment withdrawal. Based on these data, a lower rate of seroconversion after Covid-19 vaccination is expected. At present, the anti-SARS-CoV-2 vaccination represents the most effective strategy for the prevention of Covid-19 in the general population. Limited information on the immunological response to anti-SARS-CoV-2 vaccines in patients with chronic lymphocytic leukemia,[8] multiple myeloma[9] and other HMs disclosed a low rate of seroconversion. Neutralizing monoclonal antibodies, that seem promising in immunocompetent patients to reduce viral load and hospitalization,[10] need to be studies in HM.

In conclusion, Covid-19 severely impacted outcome of HM patients. Vaccination of patients and caregivers should be favored, but SARS-CoV-2 swab monitoring of HM patients should be continued and early treatment with neutralizing monoclonal antibodies advised in the case of positive test.

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