cell lines before and after treatment with a panel of anti-CD20 antibodies that included Type I and Type II anti-CD20 antibodies. This assay utilises the differential activity of four mitochondrial inhibitors to calculate basal respiration, ATP production, and maximal and spare respiratory capacity of the cell. Using the same method we then assessed whether chemically manipulating oxidative phosphorylation added to the effect on the bioenergetic profile observed following treatment with anti-CD20 antibodies. Finally, we performed clonogenic survival assays to assess whether cytotoxicity of anti-CD20 antibodies was enhanced by simultaneous treatment with Metformin, a well-established inhibitor of oxidative phosphorylation.
Results: We have observed that treatment with anti-CD20 antibodies has a significant effect on the bioenergetic profile of all DLBCL cells in our panel. Each of the antibodies in our panel had a differential ability to increase or decrease bioenergetic activity, in a cell-line specific manner. Further, we have shown that treatment with Metformin causes a significant reduction in the amount of energy produced by oxidative phosphorylation in both groups of cells in our panel. Finally, when analysing the clonogenic survival of cell lines we have found that the cytotoxicity of Type II anti-CD20 antibodies, was enhanced by simultaneously treating cell lines with an oxidative phosphorylation inhibitors.
Summary/Conclusions: With regard to clonogenicity of DLBCL cells, our data suggests that compounds that inhibit oxidative phosphorylation enhance the cytotoxicity of Type II CD20 antibodies. We believe that understanding the mechanism of loss of clonogenic survival will allow us to establish effective treatment combinations to significantly improve the efficacy of anti-CD20 antibody therapy in DLBCL.

E1390

CORRELATION OF PRE-TREATMENT SERUM CYTOKINE ABNORMALITIES AND BLOOD MARKERS OF IMMUNOSUPPRESSION IN PATIENTS WITH LYMPHOMA

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Background: Multiple studies have demonstrated that higher ratios of pre-treatment absolute lymphocyte counts (ALC) and absolute monocyte counts (AMC) are associated with improved outcomes in non-Hodgkin (NHL) and Hodgkin lymphoma (HL). Conversely, elevated serum cytokines at diagnosis are associated with inferior outcomes. Lymphocytes and monocytes have been implicated in immune surveillance, suppression of host anti-tumor immunity, and alterations of the tumor microenvironment supporting growth and survival of lymphoma cells. The relationship between pre-treatment serum cytokines and ALC and AMC remains unknown. We hypothesized that patients with elevated serum cytokines would be more likely to have suppressed ALC/AMC ratios.
Aims: To evaluate the relationship between pre-treatment serum cytokines and ALC/AMC ratios in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), T-cell lymphoma (TCL), and HL.
Methods: We studied pre-treatment samples from 725 patients with lymphoma (DLBCL=202, FL=216, MCL=88, TCL=69, HL=150) who enrolled in the University of Iowa / Mayo Clinic Lymphoma SPORE between 2002 and 2011 and were part of previous studies on cytokine secretion in lymphoma. Three-hundred seventy-six of these patients also had ALC/AMC ratios available, obtained from pre-treatment complete blood counts. Serum cytokine concentrations in patients and controls were measured using a standard ELISA (Invitrogen, Camarillo, CA) and analyzed using the Luminex-200 system. Data were acquired using STarStation software (Applied Cytometry, Dinnington, UK). Twelve cytokines passed quality control and were determined to have adequate measurements within the dynamic ranges of the assays: IL-1Ra, IL-2R, IL-8, IL-12p40p70 (IL-12), EGF, HGF, FGF-β, EOTAXIN, MIP-1β, MCP-1, IP-10, and MIG. The cytokines used for analysis were median-normalized to correct for plate effects. To assess the relationship between pre-treatment serum cytokines and ALC/AMC ratios, Spearman's rank correlation coefficients were calculated. P-values below 0.001 were considered statistically significant.

Table 1. Correlations between blood cytokines and ALC/AMC ratios by disease.

	All	DLBCL	FL	MCL	TCL	HL
	(n=376)	(n = 128)	(n = 101)	(n = 37)	(n = 37)	(n = 73)
IL-2R	**-0.37 ***	**-0.43 ***	**-0.33 ***	-0.24	-0.24	**-0.38 ***
IP-10	**-0.21 ***	**-0.43 ***	-0.17	0.10	0.01	-0.18
MIG	**-0.30 ***	**-0.38 ***	-0.22	0.03	-0.41	-0.38
IL-12	-0.16	-0.17	-0.23	0.06	**-0.53 ***	-0.03

Values denote Spearman's rank correlation coefficients (for p<0.001)*

Results: The median age at enrollment was 60 years (18 - 93), 407 patients (56%) were male. Ann Arbor stages were I-II (35%), III-IV (64%), or unknown (1%). Nineteen percent were experiencing B symptoms and 30% had bone marrow involvement. International prognostic index scores were 0-1 (49%), 2 (28%), 3 (16%), or 4-5 (6%). Table 1 shows the statistically significant correlations by lymphoma type.
Summary/Conclusions: Patients with NHL or HL with high pre-treatment serum cytokines tended to have lower ALC and higher AMC. Similar results were observed in all subsets except MCL. These data support the notion that high levels of serum cytokines are immunosuppressive and add to our understanding why the ALC/AMC ratio is of prognostic significance in lymphoma. It also provides further rationale to target immunosuppressive monocytes and the tumor microenvironment for therapeutic benefit.

E1391

KPT-8602, A SECOND GENERATION CLINICAL STAGE SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) COMPOUND SHOWS ENHANCED ANTI-TUMOR ACTIVITY WHEN COMBINED WITH VENETOCLAX OR BENDAMUSTINE IN DLBCL

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Background: Selinexor, the first-in-class oral selective inhibitor of nuclear export (SINE) compound, is currently in Phase 1/2 clinical trials for the treatment of solid and hematological malignancies, including relapsed/refractory diffuse large B-cell lymphoma (DLBCL; NCT02227251). XPO1, the target of selinexor, has been shown to export >200 cargo proteins from the nucleus including major tumor suppressors (TSPs). SINE compounds prevent the nuclear export of many of TSPs facilitating suppressor reactivation. KPT-8602 is a second generation clinical stage oral SINE compound currently undergoing a Phase 1/2 open label study in patients with relapsed/refractory multiple myeloma (NCT02649790).
Aims: In preclinical studies KPT-8602 demonstrated improved tolerability over selinexor, possibly due to its reduced brain penetration. The goal of this study was to test whether single agent or combination of KPT-8602, with either venetoclax (selective BCL2 inhibitor) or bendamustine (DNA damaging agent) can further enhance the anti-tumor effect of KPT-8602 in DLBCL.
Methods: The effects of KPT-8602, bendamustine and venetoclax as single agents and KPT-8602 in combination with either bendamustine or venetoclax on cell viability were tested on a panel of DLBCL cell lines (i.e. RL, DB, SUDHL4, SUDHL10, Pfeiffer, U937, and Farage including the double hit lymphomas Toledo, DoHH2, and SUDHL6) using MTT assays. Total RNA and whole protein cell lysates from DLBCL cells were extracted and analyzed by qPCR and immunoblots. DoHH2 sub-cutaneous xenograft in mice were treated with KPT-8602, venetoclax or bendamustine alone or in combinations of KPT-8602-bendamustine or -venetoclax. Percent tumor growth inhibition (%TGI) and overall survival were determined for each treatment condition. Tumors were collected and analyzed using standard immunohistochemistry (IHC) methods.
Results: Combinations of KPT-8602 with bendamustine or venetoclax were highly effective both *in vitro* and *in vivo*. Using an MTT assay, we showed KPT-8602 was potent against a panel of DLBCL cells (median IC_{50}: ~100 nM) and was synergistic/additive when combined with bendamustine or venetoclax. In the KPT-8602-bendamustine combination DoHH2 xenograft, treatment with each drug showed a%TGI of 52% (KPT-8602) and 76% (bendamustine) while the combination%TGI was 107%. Western and IHC analyses showed that KPT-8602 reduced the expression of key DNA Damage Response (DDR) proteins preventing treated cells from repairing the damage induced by bendamustine. In the KPT-8602-venetoclax *in vivo* study, the individual drugs had similar%TGI (52%; KPT-8602 and 56%; venetoclax). However, when the two drugs were combined, the treatment showed an additive effect (98%). Although, the anti-BCL2 proteins, Bax and Bim, were upregulated in KPT-8602 treated xenograft tumors, these pro-apoptotic pathway proteins were elevated to a greater extent in the combination-treated tumors suggesting the two drugs induced non-redundant mechanism of apoptosis activation.
Summary/Conclusions: KPT-8602 shows single agent activity as well as enhanced antitumor activity in combination with bendamustine or venetoclax through modulation of the DDR and BCL2 pathways in models of DLBCL (including double hits). These data provide rational support for the study of single agent KPT-8602 and in combinations with bendamustine or venetoclax in future DLBCL clinical trials.

E1392

ANALYSIS OF DIFFERENT BIOLOGICAL FACTORS IN PATIENTS AFFECTED BY PERIPHERAL T-CELL LYMPHOMAS NOT OTHERWISE SPECIFIED: GATA-3 EXPRESSION IS ASSOCIATED TO REFRACTORY DISEASE AND POOR OUTCOME

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Background: Peripheral T-cell lymphomas (PTCL) have an aggressive clinical course with a poor 5-year overall survival with conventional therapy. Autologous stem cell transplantation (autoSCT) and allogeneic stem cell transplantation (alloSCT) can improve long-term disease control in first and second remission, respectively. Prognostic factors are not able to discriminate patients with chemorefractory disease. Preliminary evidence from gene expression profiling (GEP) and immunoistochemical studies have suggested that the majority of PTCL-not otherwise specified (PTCL-NOS) can be subdivided in two different groups based on GATA3 and Tbet (transcription regulators of T-helper 2 and T-helper 1 lymphocytes) expression with a poorer prognosis in the former group.
Aims: Because GEP studies are not feasible in routine clinical practice, we investigated whether expression changes of those proteins could be evaluated by immunohistochemistry (ICH) and used to predict prognosis
Methods: We collected paraffin tissues from 47 consecutive patients (pts) with a diagnosis of PTCL-NOS treated at different Italian Institutions, and 38 were available for analysis. Histology was centrally reviewed. Sections were analyzed for Ki-67, GATA3, T-bet expression by IHC. Results were expressed as mean percentages of positive tumor cells. Cases were regarded as immunoreactive for GATA-3 and T-bet if at least 27% and 25% of neoplastic cells exhibited positive staining, respectively. In case of GATA-3, we performed a quantitative analysis [from score 0 (<1%) to score 4 (>80%)] combined with staining intensity [score 1 (weak) to score 3 (strong)] and we defined high score for a summary value of 6-7. Median age was 57 years (range,18-79 years); 13 (38%) pts were characterized by IPI>2; 29 pts (76%) were candidated to transplantation whereas 9 (24%) were not due to age >65 years (n=8) or limited stage/IPI (n=1). All the pts received an anthracycline-based induction chemotherapy followed by autoSCT in 9 patients (7 in first remission); 16 pts (42%) underwent alloSCT in first remission (n=3) or at relapse (n=13). The median follow-up of alive pts was 33 months.
Results: The mean value of Ki67 expression was 60% (range, 10%>95%). Only 5 of 38 (13%) pts were immuno-reactive for T-bet whereas 17 of 38 (45%) were positive for GATA-3. Only two (5%) pts were characterized by double expression of GATA-3 and T-bet. 5 pts (31%) had a high GATA3 score that was associated with a median PFS of only 6 months. We did not observe differences baseline clinical characteristics between pts with positive (n=17) and negative (n=21) immunostaining for GATA-3. Pts immunoreactive for GATA3 were characterized by poor response to anthracycline therapy: 10 of 17 PD (58%) as compared to 4 of 21 (19%) in the negative cohort (p=0.01). Cases with positive GATA3 expression were significantly associated with a reduced 5-year PFS as compared to those with no expression [PFS: 6% (95%CI:0%>22%) *versus* 39% (95%CI:18%>59%), (p=0,03); OS: 38% (95%CI:13%>63%) *versus* 58% (95%CI:30%>78%),(p=ns), respectively]. By multivariable analysis, GATA-3 and Ki67 retained prognostic value on PFS whereas both GATA-3 and IPI influenced significantly the OS.
Summary/Conclusions: Our analysis identifies GATA3 ICH expression as a strong prognostic and predictive biomarker among PTCL-NOS. Pts with positive GATA3 expression values were characterized by chemorefractory disease and poorer outcome even with transplantation strategies. Validation of this biomarker in a larger series of patients is ongoing.

E1393

IN MANTLE CELL LYMPHOMA, BCR SIGNALING AND WNT PATHWAY INDUCE B-CATENIN STABILIZATION AND CELLULAR RE-LOCALIZATION BY DIFFERENT MECHANISMS

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Background: Mantle cell lymphoma (MCL) cells survival relies on the B-cell receptor (BCR) signaling pathway that also facilitates the interactions with the microenvironment. In more than 50% of MCL cases, the Wnt/β-catenin pathway is activated and contributes to cyclin D1 and c-myc expression. As both pathways are important for cell survival as well as tumor cell adhesion, we hypothesized that a cross talk between BCR signaling and β-catenin could affect the cell homeostasis and could be targeted by specific inhibitors.
Aims: The aim of the project is to identify the role of β-catenin in MCL and how it is activated. In parallel, the inhibition of a cross talk between the BCR signaling and the Wnt pathway by ibrutinib is analysed.
Methods: Peripheral blood B cells from MCL patients (n=8) were pretreated with Wnt/ β-catenin inhibitors: XAV-939 (25 μM), promoting degradation of β-catenin through the Axin dependent destruction complex or PKF118-310 (500 nM) blocking the interaction of β-catenin with the transcription factor TCF4. Cells were pre-treated with quercetine (20μM) a PI3K inhibitor, or Ibrutinib (5μM) a BTK inhibitor. BCR signaling pathway was then stimulated with soluble anti-IgM (10 mg/ml). As a control, Wnt/β-catenin pathway was activated by the conditioned media from human bone marrow stromal cells secreting large amount of Wnts. Apoptosis, β-catenin -dependent genes expression and β-catenin subcellular localization were analyzed by flow cytometry, RT-qPCR and cell fractionation respectively.
Results: β-catenin expression is detected in all leukemic MCL samples in variable amount. The inhibition of β-catenin/TCF transcriptional complex by PKF118-330 induces tumor cells apoptosis, suggesting an important contribution of β-catenin to MCL cell survival. In parallel, β-catenin level increases rapidly in response to BCR stimulation and this stabilization is inhibited by a pretreatment with Ibrutinib showing the existence of a cross talk between these two survival pathways. Wnt stimulation stabilizes β-catenin and its translocation into the nucleus leads to an increase of the target genes *i.e.* axin2, cyclin D1 and LEF1. After BCR stimulation, even though β-catenin rapidly translocates into the nucleus it does not induce the same transcriptional response, suggesting a different role of β-catenin when activated by BCR or Wnt. Moreover, stabilization of β-catenin degradation complex by a pretreatment with XAV939 does not induce its degradation after BCR stimulation suggesting that BCR signaling interferes with the β-catenin degradation process. Thus, the impact on β-catenin by BCR signaling is likely independent from Wnt.
Summary/Conclusions: The BCR signaling pathway leads to β-catenin stabilization and nuclear translocation. However, this nuclear translocation translates into a different transcriptional response than the one induced by Wnt. Most likely, β-catenin associates with different nuclear partners driving the expression or repression of BCR specific genes. Since β-catenin can stabilize cell adhesion structures, β-catenin likely represents another player through which the BCR signaling impacts on the interaction of MCL cells with the microenvironment. Importantly, this cross talk can be efficiently interrupted by Ibrutinib, currently used in mantle cell lymphoma treatment.

E1394

METHOTREXATE ELIMINATION AND TOXICITY: THE ROLE OF MTHFR 677C>T POLYMORPHISM IN PRIMARY CNS LYMPHOMA PATIENTS TREATED WITH HIGH DOSE METHOTREXATE MONOTHERAPY

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Background: Methylenetetrahydrofolate reductase (MTHFR) plays a key role in metabolism and homeostasis of intracellular folate, and substitution of C >T is reported to be associated with decreased MTHFR enzyme activity, contributing to low folate level. The genetic association of MTHFR 677C>T in methotrexate (MTX) toxicity was evaluated in a number of studies, however the results were conflicting. The substantial heterogeneity within the study population could compromise the effect of MTHFR 677C>T polymorphism on MTX toxicity, and conflicting published results may have attributed to this.
Aims: The aim of this study was to evaluate the role of MTHFR 677C>T polymorphism in MTX toxicity within a homogenous study population by limiting cancer type to primary central nervous system lymphoma (PCNSL) and chemotherapy protocol to the first four cycles of high dose methotrexate monotherapy and fixed leucovorin rescue regimen (HD-MTX & HD-ARA regimen).
Methods: Data of patients diagnosed with PCNSL treated with HD-MTX & HD-ARA regimen were retrieved. The effect of MTHFR 677C>T polymorphism on the incidence of MTX toxicity was evaluated using a generalized estimating equation analysis.
Results: A total of 111 patients (402 cycles) was included in the analysis. The hematologic toxicity and nephrotoxicity were most frequently presented in patient with heterozygous variant genotype, with an incidence of 57 (29.1%) and 7 (3.6%). The incidence rate of hepatotoxicity and oral mucositis requiring treatment was highest in patient with wild genotype (hepatotoxicity: 7.3%, oral mucositis: 4.1%). None of the patients with homozygous variant genotype experienced the oral mucositis. Twenty eight point six percent of nephrotoxicity occurred in cycles with delayed elimination, and delayed elimination was most frequently seen in patients with homozygous variant genotype (3.6%). The risk for developing clinically meaningful hematologic toxicity was higher in patients with heterozygous variant genotype than wild genotype (odds ratio; OR: 2.60, 95% confidence interval; CI: 1.32-5.09, *P*-value=0.0055). No valid difference was observed between patients with homozygous variant and wild genotype in terms of hematologic toxicity. Other explanatory variables shown to increase the risk of hematologic toxicity were the presence of delayed elimination (OR: 10.06, 95% CI:2.87-35.31, *P*-value=0.0003), high serum lactate dehydrogenase level exceeding the upper range of normal at the time of diagnosis (OR: 2.04, 95% CI:1.09-3.81, *P*-value=0.0257) and concomitant administration of penicillin antibiotics with MTX (OR: 2.88, 95% CI:1.17-7.07, *P*-value=0.0213). No correlation between age, sex, Eastern Cooperative Oncology Group performance status, concomitant administration of proton pump inhibitor and hematologic toxicity was demonstrated. For hepatotoxicity, MTHFR 677C>T polymorphism was the only explanatory variable included in the model. The MTHFR 677C>T polymorphism was not shown to be correlated with risk of