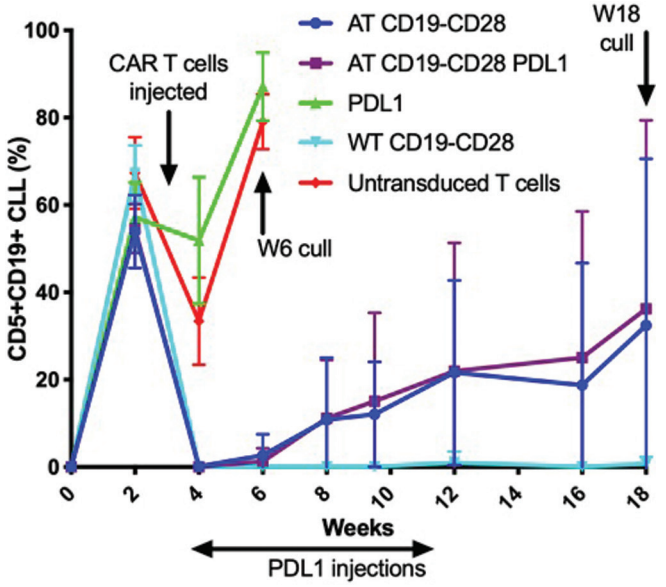
cyclophosphamide on D-1. On D0 the CAR groups received 1.5–2x10^6 CAR T cells with aPDL1 continuing until W12. Mice were bled every two weeks starting D+7 from CAR T cells to assess CLL load, CAR/T cell subsets and groups were culled together when they got sick or peripheral blood (PB) CLL>70%.

Results: Compared to WT CAR T cells, AT CAR T cells proliferate less in culture, skew towards CD8 cells, exhibit significantly lower transduction efficiencies in CD8 cells and have higher expression of PD1$^+$ in both CD4 and CD8 cells. All mice treated with CAR T cells cleared their CLL and normal B cells at D+7, and those mice treated with WT CAR T cells all remained in remission until W18, whereas 50–60% of mice treated with AT CAR T cells slowly relapsed from D+21. Spleen weights in WT CAR treated mice were equivalent to age matched controls whilst AT CAR treated mice had variable size by W18. The addition of aPDL1 to AT CAR T cells did not alter their performance from AT CAR T cells alone in any parameter examined. CAR T cell expansion in all groups was greatest at D+7 and the majority of CAR$^+$ cells were CD8$^+$ and PD1$^+$. PD1$^+$ expression was significantly higher in AT compared to WT CAR T cells at D+7.



Summary/Conclusion: CD19+ relapse post CAR T cells is determined by T cell fitness as WT CAR T cells can prevent relapse and can normalise PD1+ expression and spleen size. CAR T cells derived from CLL T cells are less able to prevent CD19+ relapse and this is not improved using concurrent aPDL1, which also seems to have limited activity in treatment alone of AT TCL1 CLL. Ongoing studies using CAR plus immunotherapy and BTK inhibitor combinations are addressing which steps are necessary to optimize CAR T cell fitness.

Hemolytic Anemias: From Diagnostics to Treatment

S899 INHIBITION OF C3 WITH APL-2 CONTROLS HAEMOLYSIS AND INCREASES HAEMOGLOBIN LEVELS IN SUBJECTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA (AIHA)

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Background: Autoimmune haemolytic anaemia (AIHA) is characterized by haemolysis mediated by autoantibodies directed against red blood cells (RBCs). Warm antibody (wAIHA; 60%>70%) and cold agglutinin disease (CAD; 20%>25%) subclasses are defined by the immunoglobulin (Ig) isotype involved and its thermal optimum for binding RBCs. wAIHA is mediated by IgG while 90% of CAD is mediated by IgM isotypes. CAD and wAIHA outcomes can be life-threatening, but no therapies are approved. Complement C3b deposition on RBCs plays a central role in AIHA, accelerating phagocytosis in the liver and/or spleen (extravascular haemolysis) and forming membrane attack complexes (intravascular haemolysis). By blocking formation of C3b, APL-2, a cyclic peptide, has potential as a novel therapeutic for AIHA.

Aims: To assess whether inhibiting complement C3 with daily subcutaneous (SC) doses of APL-2 (270 mg/day or 360 mg/day) prevents intra- and extravascular haemolysis in AIHA subjects, reducing anaemia associated signs and symptoms.

Methods: Subjects with primary AIHA were eligible for this Ph 2 open-label study to assess the safety, tolerability, efficacy, and pharmacokinetics of APL-2. Subjects had haemoglobin (Hb) levels < 11 g/dL, signs of haemolysis, and a positive direct agglutinin test (DAT) for IgG and/or complement C3. Twenty-four subjects (13 CAD, 11 wAIHA) were recruited and will be treated with APL-2 for 48 weeks. Efficacy was assessed by change from baseline in Hb, transfusion requirements, absolute reticulocyte counts (ARC), lactate dehydrogenase (LDH), haptoglobin, indirect bilirubin (INDBIL), and Functional Assessment of Illness Therapy (FACIT) fatigue score. Data are reported after 16 weeks (Day 112).

Results: Two of 13 CAD subjects withdrew; 7 of 11 remaining subjects reached Day 112. Mean Hb (standard error [SE]) increased from 8.9 (0.4) [n = 13] to 11.6 (0.5) [n = 7] g/dL, and mean LDH, ARC, and INDBIL returned within normal range. Mean FACIT scores (SE) increased 9.9 (3.4) points from baseline. Two subjects had pre-study and 1 had on-study transfusions (normalized to 112 days). All 13 CAD experienced ≥1 treatment-emergent adverse event (TEAE), mainly Grade 1–2 and unrelated. Four subjects reported 10 unrelated serious adverse events (SAEs) (6 SAEs in 1 subject). Three subjects experienced 3 unrelated Grade 3 TEAEs (pneumonia, dyspnoea, hypertension); 1 subject reported 2 unrelated Grade 4 TEAEs (high calcium, high creatinine) and withdrew.

Eight of 11 wAIHA subjects were DATmonoC3+ and 3 were DATmonoC3-. Two of 3 DATmonoC3- withdrew for lack of response and efficacy data are reported for 8 DATmonoC3+ subjects. Six of these reached Day 112. Mean Hb (SE) increased from 9.2 (0.3) [n = 8] to 10.8 (0.5) [n = 6] g/dL, and mean ARC and INDBIL returned within normal. Mean FACIT scores (SE) increased 2.7 (2.8) points from baseline. One subject had pre-study and 2 had on-study transfusions (normalized to 112 days). Ten of 11 wAIHA (C3+ and C3-) experienced ≥1 TEAE, mainly Grade 1–2 and unrelated. Five subjects reported 8 unrelated SAEs. Five subjects experienced 13 unrelated Grade 3 TEAEs (oral squamous cell carcinoma, haemolytic flare, purpura, acute kidney injury, dyspnoea, chest pain); 1 subject reported 1 unrelated Grade 4 TEAE (haemolytic flare) and withdrew. Six-month data for 10 CAD and 6 wAIHA C3+ is planned for June 2019.

Summary/Conclusion: APL-2 increases Hb values in CAD and wAIHA C3+ within the first weeks of treatment with sustained benefit with longer exposure. APL-2 reduces intra- and extravascular haemolysis shown by reductions in LDH, INDBIL, and ARC. APL-2 appears to be safe and well-tolerated.

S900 EVIDENCE BASED USE OF ERYTRHOPOIETIN IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: A MULTICENTER INTERNATIONAL STUDY

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Background: Autoimmune hemolytic anemia (AIHA) is clinically heterogeneous, from chronic compensated to abrupt hemolysis. Together with

the rate of antibody mediated erythrocyte destruction, bone marrow reticulocyte compensation is a recently recognized determinant of outcome. Erythropoietin (EPO) has been anecdotally used in AIHA to ameliorate bone marrow response, but only one systematic series has been published and predictors of response are not known

Table 1

Age years, median(range)	68 (35-92)
M/F	16/13
CAD, N(%)	12 (41.4)
WAIHA IgG, N(%)	5 (17.2)
WAIHA IgG+C, N(%)	9 (31)
MIXED, N(%)	2 (7)
DAT neg, N(%)	1 (3.4)
Haematologic parameters at diagnosis	
Hb g/L, median(range)	73 (41-118)
LDH U/L, median (range)	468 (193-6000)
Ret x109/L, median(range)	122 (57-310)
BMRI, median(range)	69 (35-193)
EPO, median(range) N=15	35 (8-670)
Previous therapy lines	
steroids, N(%)	22 (76)
rituximab, N(%)	19 (66)
splenectomy, N(%)	2 (7)
immunosuppressor, N(%)	13 (45)
time from diagnosis to EPO days, median (range)	1740 (209-1760)
time on EPO days, median (range)	209 (21-2464)
Haematologic parameters at EPO initiation	
Hb g/L, median(range)	87 (62-109)
LDH U/L, median (range)	338 (193-1030)
Ret x109/L, median(range)	117 (34-310)
BMRI, median(range)	85 (30-222)
EPO, median (range) N=17	27 (9.3-620)
Concomitant therapy, N(%)	20 (69)
Response rates, ORR (%), CR/PR	
day+15, N=29	18(64), 3/15
day+30, N=23	16(69), 4/12
month+3, N=24	17(71), 9/8
month+6, N=15	9(60), 9/0

Aims: To evaluate EPO efficacy and its predictors in a cohort of AIHA patients

Methods: Data on primary and secondary AIHA cases who had received EPO either alone or concomitantly to other therapies were retrospectively collected using a preformed survey. Efficacy was evaluated at 15 and 30 days, and then at 3,6 and 12 months; Hb response was considered partial (PR, ³2 g/dL Hb increase or >10 g/dL) or complete (CR, >12 g/dL) and hemolytic markers (LDH, reticulocytes) were registered

Results: 29 AIHA cases followed from June 2007 to February 2019 at 7 centers in Italy, France, Norway, Austria, and UK were included in the study. Main AIHA types (warm, cold, mixed, and DAT negative) were present, and 3 cases were secondary to a lymphoproliferative disorder (not active and without specific treatment at the moment of the study). Patients' characteristics are shown in table 1: at diagnosis 74% of cases presented with severe anemia and 95% displayed inadequate reticulocytosis (i.e. bone marrow responsiveness index<121). Bone marrow evaluation at diagnosis (N = 16) showed hypercellularity with dyserythropoiesis in 7 cases, and reticulin fibrosis in 3; a lymphoid infiltrate was found in 13 patients (T-cell in 4, B-cell in 7, mixed in 2), greater than 10% in the 3 secondary cases only. All patients had received at least one previous therapy, and the majority (69%) started EPO because of non-response to ongoing treatment (steroids 15, immunosuppressor 4, sutimlimab 1). Six patients had received rituximab during the 3 months before EPO start (median 1 month, range 0–5). At EPO initiation, 21% of cases displayed severe anemia, 73% had inadequate reticulocytosis, and 89% (of 18 tested) showed inappropriately low endogenous EPO levels. Patients were treated for a median of 7 months and responses were observed in about 70% of cases at month+1 and +3 (table1), with a median Hb and reticulocyte increase of 21.5 (2–48) g/L ($p < 0.001$) and 25(0–220)x10⁹/L at month+1; and 29 (0–66) g/L ($p < 0.001$) and 49 (0–195)x10⁹/L at month+3, respectively. Notably, 64% of patients responded as soon as at day+15; this finding supports an activity of EPO although recent or concomitant treatments may have contributed. At last follow up, 13 cases had discontinued EPO: 6 for long standing CR and 7 because of NR (3 with hemolytic flares). We observed an association of response to EPO and primary AIHA (73 vs 33% in secondary), inadequate reticulocytosis (76 vs 50% with adequate reticulocytosis), and not-warm (85 vs 50% in warm cases) not transfusion dependent cases (76 vs 50% transfusion dependent), although the small number did not allow statistical significance

Summary/Conclusion: Use of EPO is effective in about 70% of AIHA patients unresponsive to ongoing/previous treatments, particularly in cases with inadequate reticulocytosis. Although preliminary, these data advise EPO use to stimulate bone marrow compensatory response

S901 COMBINED GENE PANEL SEQUENCING AND EKTACYTOMETRY - THE NEXT GENERATION OF RED CELL DIAGNOSTICS

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Background: The Red Cell Gene Panel next generation sequencing (NGS) service has been offered at the Viapath Red Cell Centre at Kings College Hospital since 2016. To date we have reported 1500 diagnostic cases in the following categories: unexplained anaemia (membranopathy, enzymopathy, haemoglobinopathy and congenital dyserythropoietic anaemia), inherited bone marrow failure, congenital erythrocytosis and other (iron regulation and porphyria). The diagnostic yield of the unexplained anaemias was 46% in the first 1000 cases (patients in which a clear genetic diagnosis was found). However, in a further 34% of cases, a genetic variant of uncertain clinical significance (VUS) was detected and therefore the diagnosis was unclear.

Within haemolytic anaemia, blood film, EMA dye binding and enzyme assays have had a positive impact on the diagnostic yield. The membranopathy (spherocytosis, elliptocytosis and stomatocytosis) genes have yielded the largest amount of clear diagnoses. However, due to the large nature of the membrane genes (*PIEZO1*, ankyrin, alpha and beta spectrin, etc.) and their propensity for variation, they have also produced the largest amount of uncertain results. A further functional assay that could help to discriminate between the disease causing genetic variants and benign variation was sought to clarify cases with VUSs. Osmotic gradient ektacytometry presents us with such a methodology for looking at the functional properties of the red cell membrane.

Aims: Here we present data from the evaluation and validation study of the LoRRca Maxsis (RR Mechatronics) at aiding clinical interpretation of genomic variants. By using ektacytometry we aim to clarify the clinical significance of VUSs in cases where a diagnosis would previously have been uncertain.

Methods: Patients that were referred for EMA, membranopathy genetic analysis and those with a diagnosis of unexplained haemolytic anaemia were used in the validation study. All samples had genetic analysis and ektacytometry. These data were combined and the clinical utility of having genetic and ektacytometry results was assessed.

Results: A total of 96 patients to date have been analysed using genetic data and ektacytometry, 80 patients had the NGS panel performed and a further 16 were analysed solely for the familial variant detected in the proband. Whilst many cases that had a clear genetic diagnosis have shown concordant ektacytometry results, here we present 5 cases that we believe show a clear diagnostic benefit of this combined approach. The new phenotypic information has informed the classification of the genetic variants detected. This includes a suspected case of di-genic inheritance of variants in the