**S112 MLL FUSIONS AFFECT SPLICING TO INDUCE EXPLOITABLE METABOLIC DEPENDENCIES**

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**Background:** MLL fusions are aberrant transcription factors that induce highly aggressive and hard-to-treat leukemia with dismal prognosis. A better understanding of leukemia specific properties may reveal potential therapeutic intervention points.

**Aims:** Known direct downstream targets of MLL-ENL were scored for their importance for leukemia cell survival and genes with a positive readout were further investigated for their targeting potential.

**Methods:** A shRNA screen interrogating known MLL-ENL target genes identified the splice-modulator PTBP1 (polypyrimidin tract binding protein 1) as rate limiting for proliferation of MLL-ENL transformed murine cells and also in a human patient cell line.

**Results:** Reduction of PTBP1 slowed down cell cycle without inducing apoptosis and it reduced the competitive fitness of knockdown cells compared to controls. This was accompanied by a conspicuous phenotype characterized by reduced medium acidification during culture. Examination of the splicing pattern of PKM transcripts (pyruvate kinase muscle-type), a known PTBP1 splicing-target, revealed a shift towards the PKM1 isoform upon PTBP1 ablation. PKM1/PKM2 differ in the utilization of an alternate exon and the ratio of the isoforms is one of the major control points switching from anaerobic (PKM2 high) to aerobic (PKM1 high) metabolism. PKM2 restricts the outflow of glycolytic products into oxidative phosphorylation enabling the provision of proliferating cells with necessary anabolic intermediates. PKM2 fosters glycolysis at the expense of oxidative phosphorylation thus reducing energy yields obtained from glucose (Warburg effect). As a consequence cells increase glucose uptake and recycle reduction equivalents through production of lactate. PTBP1 knock-down reverted this phenotype, reduced glucose consumption and lowered lactate production compared to controls. Because MLL fusions obviously hardwire cells into an anabolic-proliferative state that requires large amounts of building blocks we tested the effect of starvation mimicking drugs (SMD) affecting glucose utilization, amino acid synthesis and energy production on MLL transformed and on primary hematopoietic precursors (Kit+ fraction of bone marrow). Whereas the immediate impact of SMDs on highly proliferative normal and MLL-ENL transformed cells was comparable, the long term effects after an intermittent treatment were dramatically different. In contrast to their normal counterpart MLL-ENL transformed cells lost a significant portion of their colony forming potential upon treatment indicating substantial damage suffered during episodes of starvation. On a molecular level this was accompanied by drastically higher ROS (reactive oxygen species) and an altered p53 response in MLL transformed cells.

**Summary/Conclusion:** The feasibility to exploit a differential sensitivity towards selective starvation for therapeutic purposes is currently explored and options for a possible translation into a clinically applicable strategy will be discussed.

**S113 GENETICS AND MODELING OF HUMAN ACUTE ERYTHROID LEUKEMIA**

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**Background:** Acute erythroid leukemia (AEL) is a subtype of acute myeloid leukemia (AML) characterized by an accumulation of variable proportions of erythroid progenitor cells and myeloblasts. Two subgroups of AEL have been proposed: 1-pure erythroid leukemia (PEL, AML-M0b) characterized by an accumulation of > 80% of erythroid progenitors and 2-AML-M6a characterized by an accumulation of both myeloid and erythroid progenitors, a group that has now been integrated into myelodysplastic syndrome (MDS) by the 2016 World Health Organization (WHO) classification. Earlier studies indicated that mutations of TP53 and epigenetic modifiers genes (e.g. TET2, DNMT3A) are prevalent alterations in AEL. However, the underlying molecular mechanisms driving the erythroid phenotype remain poorly understood.

**Aims:** The aims of this study were to better characterize the mutational and transcriptional landscape of AEL and to model the functional consequence of these alterations.

**Methods:** We collected AEL patient samples and performed transcriptomic (RNAseq) and genetic (exomes) analyses, on 31 and 11 samples respectively. Candidate alterations were then expressed in mouse erythroid progenitors and hematopoietic stem and progenitor cells (HSPC), in vitro and in vivo. Erythroblasts from a TET2-/- GATA1 s double mutant transgenic mouse model showed high long-term proliferation capacity in vitro and, when engrafted thereafter in mice, to leukemia presenting characteristics of AEL. Similarly, purified erythroblasts from a TET2-/- GATA1 s double transgenic mouse model showed high long-term proliferation capacity in vitro and subsequent murine AEL. In contrast, transplantation of hematopoietic stem and progenitor cells transduced with SKI retrovirus or purified from TET2-/- GATA1 s double transgens led to the development of a more myeloid disease, respectively mimicking MDS with erythroid component or
more homogeneous myeloid leukemia. Therefore, altered activity of these factors in erythroid progenitors led to pure erythroid phenotypes and to mixed erythroid and myeloid phenotypes upon expression in multipotent progenitors.

**Summary/Conclusion:** We report that, in addition to previously described genetic alterations including TP53 and chromatin regulator mutations, human AEL is also characterized by aberrant expression of several genes interfering with GATA1 including ETS factors, ETO2 and SKI. Modeling their ectopic expression in different murine progenitors suggests that the prevalence of the erythroid phenotype is dependent on the targeted cell type. Together, alterations of the GATA1 transcriptional activity and targeting of different stages of the hematopoietic differentiation may explain the continuum of phenotype between MDS and pure erythroid in human AEL.

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**Alternative Donor Transplantation**

**S114 POST-TRANSPLANT CYCLOPHOSPHAMIDE VS ATG FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN T-REPLETE HAPLOIDENTICAL TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA: A REPORT OF THE ALWP/EBMT**

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**Background:** The two leading strategies for Graft-versus-Host Disease (GvHD) prophylaxis in the setting of non-F-cell-depleted (T-plete) haploidentical stem cell transplantation (HaploSCT) are post-transplant cyclophosphamide (PTCy) and anti-thymocyte globulin (ATG). We have previously compared these outcomes between two approaches in patients undergoing HaploSCT for acute myelogenous leukemia (AML) in the registry of the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT; Ruggeri A, Hematologia, 2017), however results may differ in patients treated for acute lymphoblastic leukemia (ALL).

**Aims:** To compare PTCy with ATG-based GvHD prophylaxis in patients undergoing HaploSCT for ALL.

**Methods:** We analyzed all adult patients (≥ 18 years) reported to the EBMT with ALL in first or second complete remission (CR) or advanced disease at time of transplantation who underwent HaploSCT between 2000–2015. Before first transplantation, Haplo was defined as related donor with ≥ 2 mismatched HLA alleles. Outcomes were compared using multivariable Cox regression analysis (MVA).

**Results:** A total of 434 ALL patients were included; 336 received PTCy-based regimen and 98 received ATG. Median follow-up was 24 months (interquartile range 12–40). Median age was 35.6 (range 18–76) years, and 63.5% of patients were male. B-ALL predominated (69%), with 32% of patients Philadelphia (Ph)-negative, 36% were Ph+ while 32% had T-ALL. The majority of patients (48%) was in first CR, with 20% of patients in advanced disease. Karnofsky performance status ≥ 90 was observed in 72% of patients. 53% of patients received peripheral blood (PB) grafts while 47% BM graft, and 75% of patients were treated with myeloblastic conditioning while 25% reduced intensity conditioning. Patients who received ATG were treated earlier than those who received PTCy, with median year of transplantation 2011 and 2015, respectively (p < 0.0001), and were more likely to have advanced disease (31% vs 16%, p = 0.01). Patients treated with PTCy were more likely to have received PB grafts (68% vs 48% ATG, p < 0.001) and TBI-based conditioning (45% vs 26%, p < 0.001).

Similar outcomes were seen for engraftment (92.7% ATG vs 93.5% PTCy), as for 100 day incidence of acute GVHD both > Gr II and severe (Gr. II+, 32.7% vs 30.5%; Gr. III+, 11.6% vs 14.1%), and chronic GVHD (27.7% vs 31.7%). In both groups, infection accounted for 32% and 30% of deaths. In MVA, relapse incidence was lower in PTCy vs ATG: 2 year RI: 33.8% vs 43%; hazard ratio [HR] 0.61 [95% CI: 0.59–0.94], p = 0.03, with a trend toward lower non-relapse mortality (NRM) as well (26.7% vs 32.9%; HR 0.68 [0.42–1.11], p = 0.12). Both 2 year leukemia-free (LFS) and overall survival (OS) were higher for PTCy when compared with ATG (40.3% vs 24.1%; HR 0.67 [0.46–0.96], p = 0.03, and 48.4% vs 27.4%; HR 0.60 [0.42–0.84], p = 0.003, respectively (Fig). Active disease and lower KPS were associated with lower LFS, OS and GRFS, while PB grafts were associated with higher incidence of both acute and chronic GVHD.

**Summary/Conclusion:** Among ALL patients receiving T-replete HaploSCT, GvHD prophylaxis with PTCy resulted in significantly lower RI and superior LFS and OS compared to ATG. These results are similar to previous observations in HaploSCT for AML.

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**S115 IMPACT OF MESURABLE RESIDUAL DISEASE POSITIVITY ON OUTCOMES FOLLOWING UNBILICAL CORD BLOOD TRANSPLANTATION: A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT AND EUROCORD**

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**Background:** Cord blood transplantation (CBT) has been associated with good transplantation outcomes in acute leukemia patients with...