PF153 MLL-AF4 CAUSES ABERRANT UPREGULATION OF PROM1 (CD133) IN ACUTE LYMPHOBlastic LEUKEMIA BY CONTROLLING ENHANCER-PROMOTER INTERACTIONS

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Background: MLL rearrangements (MLLr) are a common cause of aggressive, incurable acute lymphoblastic leukemias (ALL) in infants and children, most of which originate in utero. The most common MLLr produces an MLL-AF4 fusion protein. MLL-AF4 is thought to promote leukemogenesis by activating key target genes, mainly by altering the epigenetic profile of the cell through recruitment of DOT1L and increasing histone H3 lysine 79 methylation (H3K79me2/3). One of these key genes is PROM1 which encodes the cell surface protein CD133 (Prominin-1; PROM1) gene. Inhibition of MLL-AF4 complex activity upon treatment with DOT1L inhibitors causing loss of gene. Inhibition of MLL-AF4 complex activity was elucidated enhancer-promoter structure at the PROM1 locus.

Methods: We characterised normal fetal haematopoietic stem and progenitor cells (HSPC), blasts from patient leukaemia samples, and SEM cells (a MLL-AF4+ cell line) by immunophenotyping and transcriptome analysis. ChIP-Seq and Capture C (a high resolution chromosome conformation capture technique) was used to elucidate enhancer-promoter structure at the PROM1 locus.

Results: We find that the MLL-AF4 complex aberrantly upregulates PROM1 transcription by controlling enhancer promoter-interactions. Immunophenotyping and transcriptome analysis: Immunophenotypic analysis of normal human fetal bone marrow HSPC confirmed that CD133 expression is confined to the stem cell compartment and is of potential therapeutic interest. However, the mechanism by which MLL-AF4 dysregulates PROM1 expression and the impact of PROM1 expression on leukemia cell survival remains unclear.

Aims: To determine the mechanism by which MLL-AF4 causes aberrant expression of PROM1 in leukaemia.

Methods: We characterised normal fetal haematopoietic stem and progenitor cells (HSPC), blasts from patient leukaemia samples, and SEM cells (a MLL-AF4+ cell line) by immunophenotyping and transcriptome analysis. ChIP-Seq and Capture C (a high resolution chromosome conformation capture technique) was used to elucidate enhancer-promoter structure at the PROM1 locus.

Results: We find that the MLL-AF4 complex aberrantly upregulates PROM1 transcription by controlling enhancer promoter-interactions. Immunophenotyping and transcriptome analysis: Immunophenotypic analysis of normal human fetal bone marrow HSPC confirmed that CD133 expression is confined to the stem cell compartment and is downregulated through B cell commitment. In contrast, MLL patient blasts and SEM cells showed high levels of surface CD133 expression. Analysis of RNA-Sequencing data showed that PROM1 gene expression in normal fetal B progenitors, patient blasts and SEM cells reflected the pattern of surface CD133 expression.

Functional assays: Upon inhibition of MLL-AF4 binding by a DOT1L inhibitor, CD133 expression is downregulated in SEM cells and nascent RNA-Seq shows down-regulation of PROM1 at the transcriptional level. Direct knock down of PROM1 in SEM cells confers slower growth rates, less colony formation and increased cell death.

ChIP-Seq and Capture C: ChIP-sequencing identified MLL-AF4 and associated complex components binding to the PROM1 promoter and within the gene body at putative enhancer elements. Capture C provided intragene and intronic enhancer elements within the PROM1 gene body and within the nearby TAP71 gene. Inhibition of MLL-AF4 complex activity upon treatment with DOT1L inhibitors causing loss of H3K4K79me2/3 disrupted gene expression as well as enhancer-promoter interactions.

Summary/Conclusion: Together these results indicate that MLL-AF4 directly up-regulates PROM1 expression in ALL cells and promotes their proliferation. Therefore, CD133 expression should not only be a useful marker for diagnosis and residual disease monitoring but might also be exploited as a therapeutic target in MLL re-arranged ALL.

PF155 COMBINATION OF FLOTETUZUMAB, A CD123 X CD3 BISPECIFIC DART® MOLECULE, AND BLINATUMOMAB, A CD19 X CD52 BITE MOLECULE, TO PREVENT ANTIGEN ESCAPE IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Targeting CD19 antigen by BiTE molecule blinatumomab or CAR-T cells has shown great efficacy and survival benefits in patients with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (B-CP-ALL). However, a significant number of patients will relapse and up to 60% of relapses are CD19 negative. CD123 is highly expressed in B-CP-ALL and as shown by our group (EHA 2018) can be targeted with flotetuzumab, a bispecific DART molecule that is currently being clinically evaluated for treatment of acute myeloid leukemia. For both blinatumomab and flotetuzumab, cytokine release syndrome has been observed, which is dose dependent for both bispecific constructs.

Aims: 1) To verify if blinatumomab and flotetuzumab act synergistically in primary B-CP-ALL, and
2) to decipher if the release of cytokines can be reduced by using a suboptimal dose of both constructs while maintaining optimal activity.

Methods: Bone marrow cells were isolated from B-CP-ALL patients and incubated either with flotetuzumab alone (0.01 to 1 ng/ml) or blinatumomab alone (0.1 to 10 ng/ml), or with their combination. T-cell activation and cytokine production were measured after 24 h. The number of T cells and blasts was determined on days 4 and 6. Statistical significance was determined by unpaired Student’s t-test.

Results: Flotetuzumab, blinatumomab and their combination induced dose-dependent T-cell activation, proliferation, cytokine production and cytotoxicity. After 6 days, maximal killing of primary B-CP-ALL blasts was induced either with 0.1 ng/ml flotetuzumab or 1 ng/ml blinatumomab (median 97.01% (95.88% - 98.75%) and 98.39% (98.26% - 99.38%) killed blasts respectively, vs 45.44% (39.58% - 60.43%) in untreated control group, n = 3, Figure 1A). Although both 0.01 ng/ml flotetuzumab and 0.1 ng/ml blinatumomab resulted in suboptimal cytotoxicity, their combination induced the significant killing of B-CP-ALL blasts (median 85.78% (67.33% - 90.68%) killed blasts, vs 45.44% in untreated control group, p = 0.025, n = 3, Figure 1A). In addition, we compared the cytokine release by T cells in primary B-CP-ALL samples treated with optimal concentrations of flotetuzumab or blinatumomab + flotetuzumab combinations that resulted in significant blast killing (Figure 1B). Compared to the samples treated with 0.1 ng/ml flotetuzumab, secretion of cytokines was significantly lower in samples treated with the combination of 0.01 ng/ml flotetuzumab and 0.1 ng/ml blinatumomab (median 388.65 pg/ml vs 4.33 pg/ml IL-2, p = 0.042; median 40.75 pg/ml vs 7.73 pg/ml IL10, p = 0.043; and median 769.5 pg/ml vs 24.37 pg/ml TNFα, p = 0.0074; n = 3). Furthermore, compared to optimal concentration of 0.1 ng/ml flotetuzumab TNFα production (median 769.5 pg/ml) was significantly lower in samples treated with combination of higher flotetuzumab and blinatumomab doses (0.025 ng/ml flotetuzumab + 0.1 ng/ml blinatumomab: median 40.31 pg/ml, p = 0.0088, n = 3; and 0.5 ng/ml flotetuzumab + 0.1 ng/ml blinatumomab: median 96.98 pg/ml p = 0.014; n = 3). Finally, TNFα concentrations was significantly low in the samples treated with 0.01 ng/ml flotetuzumab + 0.1 ng/ml blinatumomab combination than in those treated with 1 ng/ml blinatumomab (median 24.37 pg/ml vs 214.93 pg/ml, p = 0.039, n = 3).
PF156  SINGLE-CELL TRANSCRIPTIONAL HETEROGENEITY SUGGESTS NOVEL FINGERPRINT OF RELAPSE IN ACUTE MYELOID LEUKEMIA

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Background: Some rare subgroups of leukemia cells harboring relapse-inducing genes were selected after chemotherapy. Transcriptome analysis showed that these leukemia cells exhibited unique gene expression patterns. In particular, the expression of cytokine receptors and cytokines is often altered in leukemia relapse. These changes in cytokine signaling can lead to the development of chemotherapy-resistant leukemia cells. To better understand the molecular mechanisms underlying leukemia relapse, we performed single-cell RNA sequencing (scRNA-seq) on bone marrow samples from leukemia patients at different stages of progression.

Methods: We used the 10x Genomics Chromium platform to perform scRNA-seq on bone marrow samples from leukemia patients. We then used the Seurat package to analyze the data and identify distinct subpopulations within the leukemia cells. The expression of cytokine receptors and cytokine-inducing genes was used to identify subpopulations that were enriched in leukemia relapse.

Results: We identified several distinct subpopulations within the leukemia cells that were enriched in cytokine-inducing genes and cytokine receptors. The expression of these genes was associated with the development of chemotherapy-resistant leukemia cells. These findings suggest that targeting cytokine signaling pathways may be a promising strategy for preventing leukemia relapse.

PF157  THERAPEUTIC TARGETING OF CYTOKINE INDUCED PIM1 ACTIVATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA

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Background: PIM1 is an oncogenic kinase that recently emerged as an interesting novel therapeutic target for the treatment of T-cell acute lymphoblastic leukemia and lymphoma (T-ALL/T-LBL). Indeed, aberrant levels of PIM1 have been identified in primary T-ALL/T-LBL with T-cell receptor driven PIM1 translocations or activating mutations targeting IL7R/JAK/STAT signaling. However, based on these cell-intrinsic mechanisms, the absolute number of T-ALL/T-LBL patients that might benefit from PIM inhibition remains limited.

Aims: Besides intrinsic molecular genetic defects, non-cell autonomous mechanisms might also be able to drive therapeutically relevant aberrant signaling in T-ALL/T-LBL. Indeed, aberrant levels of PIM1 have been identified in primary T-ALL/T-LBL with T-cell receptor driven PIM1 translocations or activating mutations targeting IL7R/JAK/STAT signaling. Here, we show that specific hematopoietic cytokines, such as interleukin-7 (IL7), are able to induce PIM1 expression in specific primary T-ALL and T-LBL patient samples. As expected, this induction capacity is based on the pattern of cytokine receptor expression induced by IL7. Moreover, we show that IL7 mediated PIM1 induction is observed in a broad panel of T-ALL/T-LBL samples, whereas IL3, SCF, and FLT3LG driven effects on PIM1 induction were more restricted to immature T-ALL/T-LBL.

Results: Here, we show that specific hematopoietic cytokines, such as interleukin-7 (IL7), stem cell factor (SCF) and FLT3 ligand, are able to induce PIM1 expression in specific primary T-ALL and T-LBL patient samples. As expected, this induction capacity is based on the pattern of cytokine receptor expression induced by IL7. Moreover, we show that IL7 mediated PIM1 induction is observed in a broad panel of T-ALL/T-LBL samples, whereas IL3, SCF, and FLT3LG driven effects on PIM1 induction were more restricted to immature T-ALL/T-LBL.

Interestingly, we subsequently used patient derived xenografts to show that cytokine induced PIM1 activation renders T-ALL/T-LBL cells susceptible to in vivo treatment with PIM447, a PIM inhibitor that is currently in clinical trials for the treatment of acute myeloid leukemia. Of note, these in vivo anti-leukemic effects upon PIM inhibition were also observed for primary T-ALL/T-LBL patient samples that initially displayed low PIM1 levels at diagnosis. In line with this notion, paired analysis of diagnostic and xenografted material from these T-ALL/T-LBL samples revealed a profound increase in PIM1 expression upon xenotransplantation. Finally, we also confirmed that in cytokine induced T-ALL/T-LBL samples, PIM447 treatment displays a strong synergy with glucocorticoids.