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; TAPT1 body and within the nearby TAP1T gene). Capture-C (a high resolution chromosome conformation capture technique) was used to elucidate enhancer-promoter structure at the PROM1 locus.

Methods: We characterised normal fetal haematopoietic stem and progenitor cells (HSPC), blasts from patient leukemia samples, and SEM cell line (a MLL-AF4+ cell line) by immunophenotyping and transcriptome analysis. ChIP-Seq and Capture C analysis identified intragenic enhancer elements within the PROM1 body and within the nearby TAPT1 gene. Inhibition of MLL-AF4 complex activity upon treatment with DOT1L inhibitors causing loss of H3K79me2/3 disrupted gene expression as well as enhancer-promoter interactions.

Results: Analysis of RNA-Sequencing data showed that PROM1 expression in normal fetal B progenitors, patient blasts and SEM cells showed high levels 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.

Summary/Conclusion: Together these results indicate that MLL-AF4 directly up-regulates PROM1 expression in ALL cells and promotes their proliferation and survival. 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 CD3 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 (BCP-ALL). However, a significant number of patients will relapse and up to 60% of relapses are CD19 negative. CD123 is highly expressed in BCP-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 BCP-ALL, and 2) to decipher if the release of cytokines can be reduced by using a sub-optimal dose of both constructs while maintaining optimal activity.

Methods: Bone marrow cells were isolated from BCP-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 BCP-ALL blasts was induced either with 0.1 ng/ml flotetuzumab or 1 ng/ml blinatumomab (median 97.01% (93.88% - 98.75%) and 98.39% (98.26% - 99.34%) 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 BCP-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 BCP-ALL samples treated with optimal concentrations of flotetuzumab or blinatumomab alone or in combination. 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 concentration was significantly lower 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).