# Abstract Submission

*01. Acute lymphoblastic leukemia - Biology & Translational Research*

**EHA-1513**

**THE SHEDDASE DOMAIN OF ADAM10 AUGMENTS THE INTERACTION OF LEUKEMIA CELLS WITH THE BONE MARROW NICHE IN VIVO AS SHOWN BY RECONSTITUTING PDX LEUKEMIA CELLS WITH CRISPR-CAS9-INDUCED KNOCKOUT**

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**Background:** Tumor-microenvironment interactions are critically important determinants contributing to leukemia formation and maintenance. Interrupting the leukemia-bone marrow interaction represents an attractive therapeutic approach in acute leukemia (AL). Functional genomics significantly increases our understanding of the vulnerabilities and gene dependencies of individual tumors.
**Aims:** Here, we developed a CRISPR-Cas9 screening approach for functional analysis of surface molecules in patient-derived xenograft (PDX) AL models in vivo.
**Methods:** Size of CRISPR library was determined by genetic barcoding. Stable expression of fluorescently labelled Cas9 and sgRNA constructs in two PDX samples. Enrichment of double positive cells by MACS and injection into NSG mice. Gene depletion analysis using MAGeCK algorithm to screen and functional competitive *in vivo* assays to validate the candidates. Characterization of the ADAM10 KO or inhibitor (GI254023X) treated cells for engraftment capacity by homing assay, frequency of leukemic stem cells by competitive limiting dilution transplantation assay (LDTA), sensitivity towards routine chemotherapy by *in vivo* competitive chemotherapy trials in both lineages. Rescue assay by reconstitution of ADAM10 variants in functional competitive *in vivo* assays.
**Results:** When running a customized CRISPR-Cas9 screen targeting about 100 cell surface candidates in two AL PDX samples, several sample-specific, but also commonly depleted candidates were identified. CRISPR screen findings were confirmed on the level of single molecules, using a competitive molecular in vivo approach and testing the PDX cells with and without knockout in the same mouse. These experiments validated an essential function for the two well-known depleted candidates CXCR4 and ITGB1 in both PDX models in vivo. Of note, various members of the Solute Carrier Family (SLC) were among the list of drop-out candidates. ADAM10 was identified as a commonly depleted candidate in both PDX models. In vivo experiments confirmed the essential role of ADAM10 in PDX models from 6 additional patients with either acute lymphoblastic leukemia (ALL) or acute myeloblastic leukemia (AML), indicating a broad essential role of ADAM10 in both, ALL and AML, independent from their oncogenic-driver mutations and chromosomal abnormalities. Moreover, treating PDX cells with an ADAM10 chemical inhibitor resulted in significantly reduced engraftment capacity into the bone marrow (BM), indicating a role for ADAM10 in the early engraftment and homing process in the BM microenvironment. Knockout of ADAM10 reduced the frequency of leukemia stem cells, indicating that a relevant fraction of stem cells depended on ADAM10. ADAM10 KO ALL and AML PDX samples showed increased sensitivity towards routine chemotherapy treatments, indicating that inhibition of ADAM10 sensitizes AL towards conventional chemotherapy. When ADAM10 knockout cells were reconstituted with different recombinant ADAM10 variants, PDX in

vivo experiments revealed that wildtype ADAM10 rescued the phenotype, while an ADAM10 variant lacking the enzymatic domain did not, highlighting the importance of the sheddase activity for ADAM10 function in leukemia maintenance.

**Summary/Conclusion:** In summary, we established CRISPR-Cas9 drop-out screens in PDX models *in vivo* as technology to explore patient-specific tumor dependencies. Our data revealed a yet unknown function of ADAM10 to maintain patient leukemic cells in the bone marrow microenvironment niche. ADAM10 thus represents an attractive future therapeutic target for the treatment of acute leukemia.

**Keywords**: Acute lymphoblastic leukemia, Acute myeloid leukemia, Screening, Xenotransplantation