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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

In Vivo Modeling of T-Cell Acute Lymphoblastic Leukemia Reveals Synergistic Oncogenic Pathways and *Bcl11b* Haploinsufficiency As a Potential Therapeutic Vulnerability

Maximilian Schinke, PhD[1], *Marc-Jens Kleppa, PhD*[1], *Dirk Heckl, PhD*[2,3], *Michael Heuser, MD*[4], *Axel Schambach, MDPhD*[5], *Adrian Schwarzer, MDPhD*[6,7,4]

[1] Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany
[2] Department of Pediatric Hematology and Oncology, Martin-Luther-University Halle-Wittenberg, Halle, Germany
[3] Institute for Experimental Pediatric Hematology and Oncology, Goethe University Frankfurt, Frankfurt, Germany
[4] Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
[5] Institute of Experimental Hematology, Hannover Medical School, Hannover, DEU
[6] Department of Internal Medicine C, University Hospital Greifswald, Greifswald, Germany
[7] Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany

For decades, T-ALL models were based on retroviral overexpression of *NOTCH1* in murine hematopoietic stem cells (HSCs). This induces aggressive T-ALL but does not reflect the genetic and clinical heterogeneity of the disease. Large-scale genomic analyses have mapped the detailed landscape of T-ALL driver events over recent years, but how these diverse oncogenic events interact in T-ALL pathogenesis and which vulnerabilities this creates remains incompletely explored. This is in part due to the lack of appropriate mouse models that recapitulate the complex disease biology and are suitable to identify and study potential therapeutic targets and interventions in a more realistic context.

We modeled the genetic heterogeneity of T-ALL through *in vivo* multiplexing of gain-of-function and loss-of-function oncogenic events known to drive the disease. We developed a novel mouse strain that expresses Cre-recombinase and an inducible Cas9 only in the thymus (*LSL.Cas9 x Lck-cre*) along with a lentiviral vector system into which T-ALL specific oncogenes are cloned in antisense flanked by LOX66/71 sites. Gain-of-function events, such as the overexpression of transcriptional regulators (*Tlx1, Tal1, or Lmo2)* and signal transducers (*NRAS* ^{G12D}, *PIK3CD* ^{E1021K}) were induced in developing thymocytes by Cre-mediated inversion together CRISPR-Cas9 editing of recurrent T-ALL loss-of-function drivers. This combinatorial approach allowed to probe more than 2000 possible oncogenic mutational combinations *in vivo*.

Transplantation of HSCs from *LSL.Cas9 x Lck-cre* mice transduced with our vector system into sublethally irradiated mice led to T-ALL development with different morphology and disease phenotypes. Developing leukemia harbored reproducible phenotypes, covering very immature CD4-CD8-CD25+CD44+ T-ALL in the Tlx1_NRAS subgroup, in contrast to the classical cortical CD4+CD8+CD3- phenotype (Tlx1_PIK3CD subgroup), as well as mature single-positive CD4+CD3+ or CD8+CD3+ blasts (Tal1_Lmo2 subgroup). We identified *Tlx1*, *Tal1*, *Lmo2*, *NRAS* ^{G12D}and *PIK3CD* ^{E1021K}as the main driver of disease phenotype and gene expression profile, whereas the loss-of-function mutations contributed to and accelerated the onset of the disease. Tumor-derived cell lines could be easily established and kept their phenotypic characteristics and mutation patterns. The resulting T-ALL tumors contained up to eight different disease-relevant genetic alterations, thus recapitulating the genetic complexity in humans and overcoming the long latency for spontaneous mutations in less complex mouse models. Among these, co-evolution of insertions and deletions in *Notch1*, *Cdkn2a*, *Bcl11b*, and *Pten* were among the most frequently observed mutations in definitive T-ALL, whereas others, e.g., in *Dnm2*, *Phf6*, *Etv6*, and *Lef1*, occurred more randomly.

While gene editing by CRISPR-Cas9 mostly resulted in frame-shift mutations in both alleles, *Bcl11b* exhibited a strong haploinsufficient phenotype with exactly one knock-out and one intact allele in each case examined. Following this observation, further suppression of Bcl11b by shRNAs in *Bcl11b* $^{+/-}$ cell lines derived from our tumor models demonstrated rapid induction of cell death, which was more intense in T-ALL cells compared to non-malignant thymocytes. In addition, re-analysis of CRISPR-based loss-of-function screens of human hematopoietic cell lines retrieved from the Cancer Dependency Map confirmed BCLL11B as a strong selective dependency in human T-ALL.

In summary, we model subgroup-specific T-ALL transformation through conditional and multiplexed *in vivo* gene editing and oncogene overexpression, thus creating a resource of novel mouse models that recapitulate the genetic and biological

heterogeneity of the disease. Using our resource, we identified *Bcl11b* as a strong and selective context-specific dependency in T-ALL. Although Bcl11b is a transcription factor, our results could serve as an starting point for novel therapies that interfere with Bcl11b function, such as molecular glue degraders or protein interaction inhibitors.

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