In vivo modeling of T-Cell Acute Lymphoblastic Leukemia (T-ALL) reveals oncogenic cooperations and *Bcl11b* haploinsufficiency as a potential therapeutic vulnerability

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Abstract: *(max. 400 words)*

Background: Although large-scale genomic analyses mapped the landscape of T-ALL driver genes over recent years, how these diverse oncogenic events interact in T-ALL pathogenesis remains incompletely explored. This is primarily because there is a lack of mouse models that accurately replicate the complex disease biology and are suitable for studying therapeutic targets in a more realistic setting.

Methods: We modelled T-ALL by using *in vivo* multiplexing of known gain-of-function and loss-of-function driver mutations. We developed a mouse strain that expresses a Cre-recombinase and an inducible Cas9 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 and oncogenes were induced in developing thymocytes by Cre-mediated inversion together CRISPR-Cas9 knockout of tumor suppressors. This combinatorial approach allowed to probe more than 2000 possible oncogenic mutational combinations *in vivo*.

Results: Transplantation of HSCs from *LSL.Cas9 x Lck-cre* mice transduced with our vector system into mice led to T-ALL development, reaching from very immature T-ALL to the classical cortical phenotype, as well as mature T-ALL. The resulting T-ALL contained up to eight different disease-relevant genetic alterations, recapitulating the genetic complexity in humans. 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 intact allele in each case. Following this observation, further suppression of *Bcl11b* in cell lines derived from our tumor models and demonstrated the 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

Conclusion: We created a pipeline to realistically model subgroup-specific T-ALL leukemogenesis using conditional and multiplexed gene editing and overexpression *in vivo.* We identified *Bcl11b* as a context-specific dependency in T-ALL that may serve as a starting point for rational targeted therapies, such as molecular glue degraders and protein interaction inhibitors.