Establishing a model system for B cell acute lymphoblastic leukemia immunoglobulin variable gene diversity

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Introduction: B-cell acute lymphoblastic leukemia (B-ALL) is the most common pediatric cancer and is characterized by extensive diversity in clonal in immunoglobulin (Ig) variable (V), diversity (D), and joining (J) gene rearrangements at the Ig heavy chain (IgH) locus. Extensive VDJ rearrangement diversity in B-ALL has been associated with worse clinical outcomes and unique transcriptional features implicating metabolic activation and cellular proliferation, suggesting the potential for VDJ diversity to serve as a predictive biomarker for responsiveness to metabolic inhibition. As a critical first step to testing this, we applied IgH next generation sequencing (NGS) to patient-derived xenograft (PDX) B-ALL samples to test whether IgH clonal composition is preserved in an experimental model.

Methods: Using 2 human B-ALL PDX, we applied IgH NGS to define the composition of clonal IgH rearrangements in each human B-ALL sample. We then tested whether IgH composition and the extent of VDJ rearrangement diversity is preserved after engraftment in NOD-SCID mice. After genomic DNA purification from each primary human and post-PDX engraftment B-ALL sample, we performed multiplex PCR for amplification of IgH VDJ rearrangements for NGS (Illumina MiSeq; UR Genomics Research Center). We then applied established methods for IgH clonality determination to define the clonal composition of each sample and compare the IgH repertoire of human B-ALL samples with paired xenograft samples to test whether clonal diversity is preserved after engraftment.

Results: In one patient sample, a non-diverse IgH rearrangement profile was observed and maintained stable after engraftment, suggesting that clonal composition was preserved *in vivo*. However, in the second patient, we observed a significant shift in the IgH repertoire post engraftment: the human sample exhibited no diversity while the corresponding PDX sample displayed increased clonal diversity. Notably, the dominant rearrangement in the human leukemia sample was not the same one expanded in the xenograft sample, indicating that certain B-ALL subclones may possess greater fitness for engraftment and proliferation *in vivo*. These findings indicate that clonal dynamics can shift post-engraftment and that some leukemia cell populations may be better suited to survive and expand in the mouse model than others. Future studies will focus on uncovering the biological mechanisms that drive clonal shifts following PDX engraftment. We aim to

identify the properties that give certain B-ALL subclones a selective advantage i*n vivo*. Expanding the number of patient-PDX pairs and incorporating *in vitro* and *ex vivo* models may help determine how often these shifts occur and under what conditions. Ultimately, our goal is to better understand clonal dynamics in B-ALL and evaluate whether IgH diversity can serve as a biomarker for therapeutic response, particularly to metabolic inhibition.

Conclusions: Clonal dynamics can shift after PDX engraftment, suggesting that certain B-ALL subclones have greater *in vivo* fitness. More work is needed to understand these shifts and ultimately determine whether IgH diversity can serve as a biomarker to guide response to metabolic inhibition.