**Immune repertoire dysregulation and recovery in B cell acute lymphoblastic leukemia**

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**Background:** B cell acute lymphoblastic leukemia (B-ALL), the most common pediatric cancer, is treated with intensive combination chemotherapy and immunotherapy which compromise immune function and pose risk of infectious complications. High-throughput sequencing (HTS) of the clonally expanded immunoglobulin (Ig) gene rearrangement(s) unique to each B-ALL is routinely used to monitor minimal residual disease (MRD) to refine treatment intensity. In addition to tracking leukemia-associated Ig rearrangements across treatment timepoints, this assay also captures information about the diversity of the normal B cell receptor (BCR) repertoire at each time point that it is performed, providing valuable insight into the state of the adaptive immune system. In this study, we leverage existing clinical Ig HTS data to test the impact of BCR repertoire diversity and dynamics on adaptive immunity in patients with B-ALL.

**Methods:** In an IRB-reviewed and exempt protocol, we performed a retrospective review of Ig HTS clinical data available via the electronic health records from patients with B-ALL treated at a single academic medical center after September 2018 (when the clinical Ig HTS assay became FDA-cleared and readily clinically available). We compiled data pertaining to the number of unique Ig heavy (IgH) and light chain kappa and lambda (IgK and IgL) gene rearrangements in each bone marrow (BM) sample measured across key treatment timepoints, as markers of BCR diversity. We also collected data pertaining to functional immunoglobulin G (IgG) levels to test whether the repertoire of normal B cell Ig gene rearrangements corresponds to hypogammaglobulinemia in patients with B-ALL.

**Results:** We observed broad variability in the extent of BCR repertoire diversity at B-ALL diagnosis across 27 subjects, as well as in paired end of induction (EOI) samples from 19 (of 27). The mean numbers of unique BCR sequences at diagnosis were as follows: IgH 7876±12750, IgK 9020±11149, and IgL 2541±4096. At EOI – when most patients had achieved clinical remission – we observed higher numbers of unique IgH, IgK, and IgL sequences compared to diagnosis ($P<.0001$), indicating BCR recovery. In turn, the number of unique IgH sequences strongly corresponded to the number of IgK sequences at EOI ($R^2=0.9481$; $P<.0001$), consistent with functional B cell recovery. IgG levels (n=206 total) were highly variable across all timepoints (mean 447.5±228.8 mg/dL). Surprisingly, we found that BCR repertoire diversity at neither diagnosis nor EOI corresponded to IgG level

within the first 4 months from diagnosis, although IgG evaluation timepoints were inconsistent in this retrospective pilot analysis.

**Conclusions:** Our data suggest that BCR repertoire diversity is highly variable at B-ALL diagnosis, and that achievement of clinical remission is associated with recovery in BCR repertoire diversity and IgH/IgK concordance in patients undergoing treatment for B-ALL. While BCR repertoire diversity did not correspond to hypogammaglobulinemia in this pilot analysis, further evaluation in larger cohorts will be needed to test whether BCR diversity may serve as a biomarker of adaptive immune competence in patients with B-ALL. Future directions include expanded analyses in larger cohorts, testing the impact of IVIG repletion, and integrating clinical infectious toxicity data to test clinical relevance.