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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Genomic Determinants of Therapy Response in *ETV6::RUNX1* Leukemia

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ETV6::RUNX1 leukemia is the second most common childhood B-cell acute lymphoblastic leukemia subtype. Although it has a low overall relapse risk, a significant proportion of relapses occur within this subtype due to its relatively high incidence. No consistent genomic biomarkers have been identified that predict therapy response beyond early therapy response as measured by minimal residual disease at the end of induction.

In this study, our aim was to use multiomic data from 175 *ETV6::RUNX1* leukemias treated according to the NOPHO ALL2000 and ALL2008 protocols to identify genomic features that predict therapy response at presentation. Cases were classified into three responder groups based on MRD at end-of-induction (EOI) therapy: fast responders with negative MRD, intermediate responders with positive but <0.1% MRD, and slow responders with >0.1% MRD. Response data was also collected at mid-induction (day 15) and end-of-consolidation (EOC), when available. Diagnostic and remission DNA were analyzed using array-based copy number data (n=142) or whole genome sequencing (WGS) (n=33), and diagnostic bulk RNA sequencing (n=51) was complemented with single cell multiomics (RNA, surface protein and VDJ sequencing) data from both healthy (n=8) and leukemic bone marrow cells upon chemotherapy (n=8).

Overall, the frequency of somatic genetic changes was not significantly associated with treatment response suggesting that genetic diversification through elevated random mutagenesis events may not underlie the drug resistant phenotype. However, analysis of mutational signatures revealed that fast-responding leukemic blasts frequently exhibited the APOBEC mutational signature and had higher cell cycle activity (Figure 1A). Accordingly, we show that expression of *A3B* is highly specific to cycling cells also in healthy bone marrow hematopoietic progenitor cells, pro- and pre-B cells. We also examined RAG-mediated genetic instability that manifests in focal structural variations and has highest activity in *ETV6::RUNX1*leukemia (Figure 1B). We confirmed several recurrent RAG off-target sites, including significant enrichment of the known risk locus *TBX1LR1* in slow responding cases. Strikingly, slow-responding leukemias had also frequent rearrangements in the immunoglobulin kappa genes and the kappa-deleting elements, despite their pro-B-like transcriptome state upon diagnosis. Based on the single cell multiomics profiles, we found that upon treatment the blast phenotype shifts towards an immature-B-like transcriptome, with concomitant expression of Ig light chains with productive sequence, thereby permitting activation of BCR signaling that provides a survival advantage.

At CNV level, we found that the chromosomal alterations related to the translocation event associated with treatment response. Specifically, slow responders had more often the amplification of der(21)t(12;21) chromosome, impacting expression

of *USP5*, *TIGAR* and *SLC2A3*, associated in earlier studies to chemoresistance and tumorigenesis. In comparison, fast responders were often missing a region of chromosome 12p with lower expression of several genes including oncogenic *KRAS*.
In summary, combining multiomic and response data, we characterized genetic alterations and mutational processes that affect the treatment response in *ETV6::RUNX1*leukemia. Our findings highlight the genetic and transcriptomic heterogeneity of this common pediatric leukemia subtype and its implications for treatment sensitivity, paving the way for better classification of the disease at presentation.

Disclosures No relevant conflicts of interest to declare.

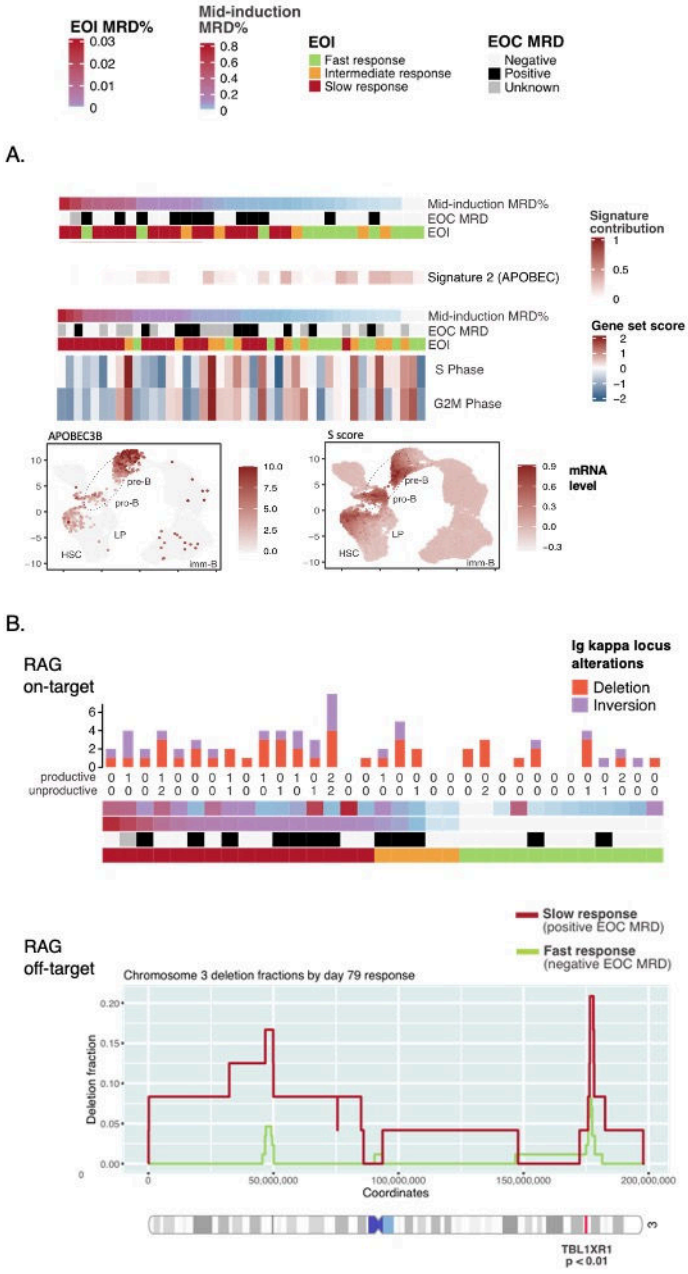

Figure 1. Mutational processes distinguishing fast (A) and slow (B) treatment response in ETV6::RUNX1+ acute lymphoblastic leukemia.

Figure 1

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