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POSTER ABSTRACTS

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

Single-Cell Transcriptomics Points to a Role of Stemness and Bone Marrow Niche Interactions in the Poor Response to Induction Therapy in T-Cell Acute Lymphoblastic Leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) has a poor prognosis with a 15-20% relapse rate. The induction therapy response is the strongest predictor of relapse and shows great variation between patients. However, whether intra-patient leukemic cell heterogeneity plays a role for the induction therapy response has not yet been investigated.

We studied 13 patients with T-ALL (11 pediatric and 2 young adult, median follow-up 37 months). Of these, three were good responders (end of induction (EOI) minimal residual disease (MRD) undetectable/non-quantifiable by flowcytometry and PCR), while ten had a poor response (EOI MRD>0.01%, (range 0.05-97%). Of the ten poor responders, three experienced relapse and one died from resistant disease.

Bone marrow samples from diagnosis (n=13) and paired samples from relapse (n=3) or MRD (n=4, MRD >5%) were subjected to combined single-cell RNA sequencing, single-cell surface marker profiling by antibody-derived tag (ADT)-sequencing, and single-cell TCR sequencing, using the 10X Genomics platform. A published healthy bone marrow dataset was used for cell annotation and clear distinction was observed between healthy cell subsets and leukemic blasts. TCR sequencing data revealed a hyperexpanded clone in six patients, with identical clonal sequences at diagnosis and relapse(n=2)/MRD(n=1). Surface marker profiling using a panel of 134 surface antigens showed good correspondence between leukemia-associated immunophenotypes found by ADT-sequencing and by flow cytometry for 16 common clinically used markers.

To examine factors associated with a poor therapy response, we compared average gene expression of leukemic cells in poor and good responders at diagnosis using differential gene expression (DGE) and gene set enrichment analysis (GSEA). At diagnosis, poor responders showed upregulation of genes involved in extracellular matrix (ECM) degradation/organization, including the adhesion molecules *ITGA5* and *PECAM1*, and *MMP11*, while showing downregulation of cell cycle genes. Accordingly, the fraction of cells predicted to be in G0/G1 phase at diagnosis of poor responders positively correlated with EOI MRD (r=0.52, p=0.07, Spearman's rank correlation). To investigate if this high fraction of quiescent cells in poor responders was associated with a more stem-like cell state, we examined the expression of a published hematopoietic stem cell (HSC) signature (Forsberg et al, 2010) that we previously selected for investigation in BCP-ALL (Modvig et al, Molecular Oncology, 2022). Indeed, patients with a poor response had a higher percentage of leukemic blasts with an HSC signature module score above zero (p=0.028, Kolmogorov-Smirnov, distribution in Figure 1), suggestive of stemness as a contributing factor to therapy resistance.

To define molecular changes in leukemic blasts during therapy, we compared the leukemic blast gene expression profiles in matched diagnostic and MRD samples using DGE and GSEA. This revealed that the leukemic blasts within the first month of therapy downregulated cell cycle genes, while upregulating chemokine receptors, such as *CXCR4*. To identify specific genes associated with high MRD, we combined genes upregulated in poor responders (n=314, p<0.01, DGE) with genes

associated with EOI MRD levels (n=98, r>0.8 and p<0.001, rank regression). This resulted in a 30-gene signature, comprising several genes previously associated with cancer stem cells, such as *MICAL3, ELF4, and NLRP3*, and genes encoding proteins involved in cancer drug resistance. These included sialyl transferases, acid ceramidase (*ASAH1*) and zyxin (*ZYX*). *ZYX* silencing was previously shown to downregulate the anti-apoptotic protein BCL-2 in leukemia. Accordingly, the percentage of cells with detectable *BCL2* expression at diagnosis correlated with EOI MRD (r=0.58, p=0.04), supporting a role of BCL-2 inhibitor therapy in T-ALL.

In conclusion, our data suggest that therapy-resistant cells are more stem-like and quiescent at diagnosis, display upregulation of genes involved in bone marrow niche ECM organization and adhesion, and genes related to drug resistance. Likewise, residual leukemic blast following therapy show less proliferation and *CXCR4* upregulation. Thus, single-cell transcriptomics provides important molecular insights into therapy-resistant leukemic cells and could comprise a valuable prognostic tool in T-ALL.

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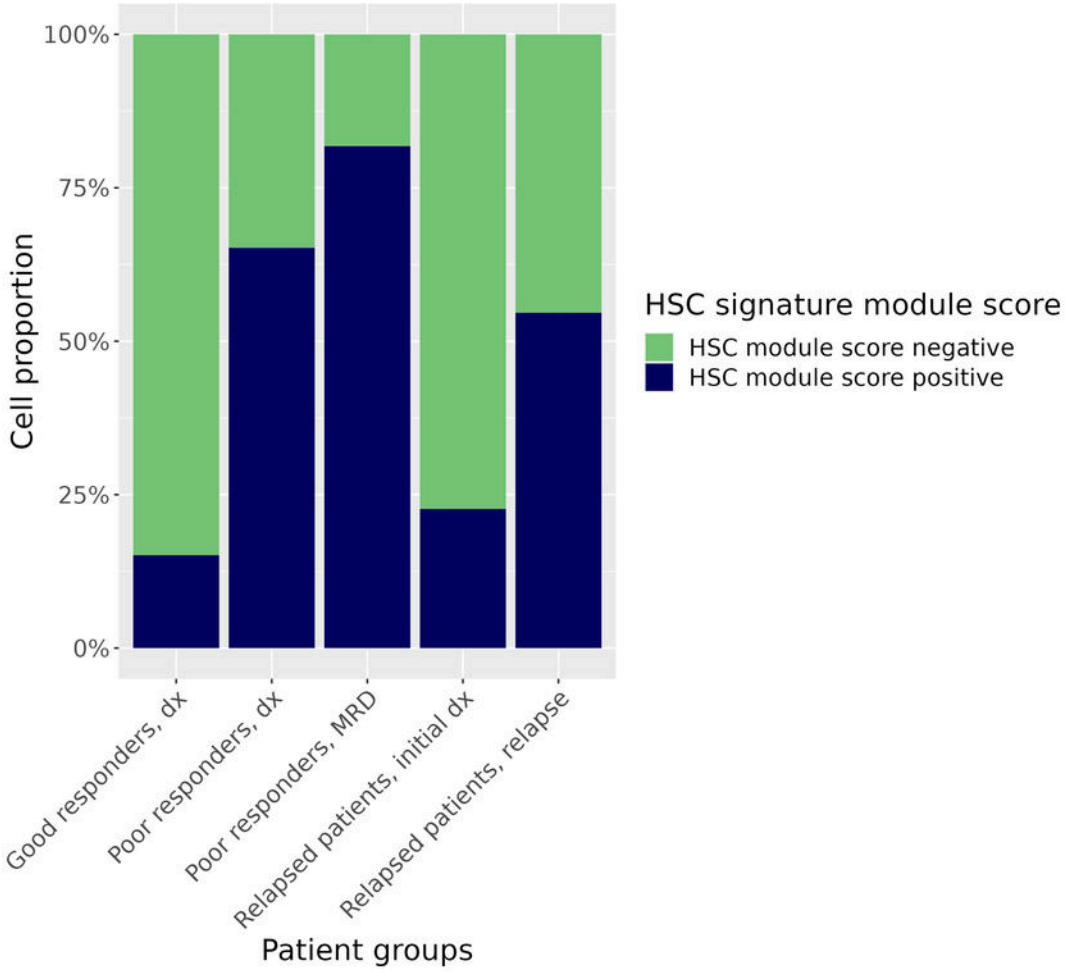

Figure 1 - Proportion of cells with an HSC signature module score above zero in patients grouped by clinical outcome

Dx: Time of diagnosis. Good responders: MRD undetectable/detectable below lower limit of quantitation at end of induction. Poor responders: MRD>0.01% at end of induction.

Figure 1

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