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Title: THE ROLE OF THE BONE MARROW IMMUNE NICHE IN PREVENTING RELAPSE IN B-ALL FOLLOWING REDUCED INTENSITY CONDITIONING ALLO-HSCT

Abstract Type: Poster Presentation

Topic: Acute lymphoblastic leukemia - Biology & translational research

Background:

The UKALL14 trial suggested that reduced intensity-conditioned allogeneic haematopoietic stem cell transplantation (alloHSCT) can lead to durable event-free survival in patients with B-cell acute lymphoblastic leukaemia (B-ALL) aged >40 years (Marks et al., Lancet Haematol. 2022). However, persistence or reappearance of minimal residual disease (MRD) is a strong predictor of relapse after alloHSCT in ALL (Della Starza et al., Front. Oncol. 2019) and suggests ineffective graft-versus-leukaemia immunity.

Aims:

This study aimed to characterize differences in the bone marrow (BM) immune cell population architecture in adult patients with B-ALL who either did or did not relapse following alloHSCT.

Methods:

We used samples collected after alloHSCT from patients participating in the UKALL14 study to assess the bone marrow (BM) immune cell composition. 21 BM samples from 12 patients aged 41-60 were analyzed by bulk RNA-sequencing (RNA-seq) in 2 groups: MRD- ALL which remained in remission, and MRD+ ALL which subsequently relapsed. Longitudinal sampling was employed to assess how the BM composition changed temporally following alloHSCT. For higher level resolution of changes, single cell RNA-sequencing (scRNA-seq) was performed on BM samples from 10 patients aged 36-59 derived from the same groups. Bulk RNA-seq was performed on matched diagnostic samples from 7 patients for which post-transplant analysis had also been carried out.

Results:

Compared to patients whose ALL remained MRD- and in continuous remission, we found that patients with MRD+ ALL prior to relapse demonstrated increased expression of several neutrophil-related genes (e.g., *CTSG*, *LCN2*; L2FC>2, adjusted $p<0.05$) and this was mirrored by enrichment for several gene pathways for neutrophil degranulation. scRNA-seq also showed differences in immune cell architecture between patients in continuous remission versus those who subsequently relapsed. Cytotoxic CD8+ T cells and NK cells were increased in patients who remained in remission compared to those who later relapsed, whereas CD4+ naïve and central memory T cell populations were more prominent in the pre-relapse samples. Furthermore, a myeloid cell cluster with a gene expression signature comparable to that seen in the bulk RNA-seq was also detected and its presence positively correlated with an increase in CD4+ central memory T cell cluster frequency (R=0.6760, $p<0.02$). Interactome analysis revealed stronger putative interactions in pre-relapse specimens, most notably involving the CD4+ T cell and myeloid populations. Bulk RNAseq of matched BM samples at the time of diagnosis and before treatment also identified a myeloid gene signature that was associated with subsequent relapse after alloHSCT; this signature overlapped with the myeloid signature found in post-transplant MRD+ samples pre-relapse (hypergeometric test $p=8.5x10-36$).

Summary/Conclusion:

We have identified BM immune signatures associated with continuous remission versus future relapse after alloHSCT. Our preliminary data suggest that a neutrophil signature is enriched in both the diagnostic and post-transplant samples of patients destined to relapse. To determine whether this myeloid population predicts

eventual treatment failure after alloHSCT, we will interrogate diagnostic samples from >400 UKALL14 trial patients. Further work is being performed to evaluate the profile of the neutrophil-like populations in the bone marrow samples of relapsing patients and to determine how they might regulate anti-tumour immune surveillance.

Keywords: B cell acute lymphoblastic leukemia, Allogeneic hematopoietic stem cell transplant, Bone marrow microenvironment