ELSEVIER

Blood 142 (2023) 600–601

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

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

Single-Cell Transcriptomic Profiling of T Cells from Blinatumomab-Treated Patients with B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) Reveals Circulating CD8 T Cell Subsets Associated with Treatment Response

Francesco Corrado, MD[1,2], *Jan Wulf*[2], *Tobias Straub, PhD*[3], *Daniel Nixdorf, MSc*[2,4], *Anetta Marcinek, M.Sc.*[2,4], *Nora Philipp*[2,4], *Chang-Feng Chu*[5], *Christina Zielinski*[5], *Michael von Bergwelt-Baildon*[6,4], *Viktoria Blumenberg, MD*[4,6,2], *Maryam Kazerani, PhD*[4,2], *Marion Subklewe, MD*[6,2,7], *Veit L. Buecklein, MD*[2,4]

[1] Department of Biomedical Sciences, Humanitas University, Pieve Emanuele,Milan, Italy
[2] Laboratory for Translational Cancer Immunology, Gene Center, LMU Munich, Munich, Germany
[3] Core Facility Bioinformatics, LMU Biomedical Center, LMU Munich, Munich, Germany
[4] Department of Medicine III, LMU University Hospital, LMU Munich, Munich, Germany
[5] Department of Infection Immunology, Leibniz-Institute for Natural Product Research and Infection Biology, Jena, Germany
[6] German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany
[7] Department of Medicine III, LMU University Hospital, LMU Munich, Muenchen, Germany

Background Blinatumomab (Blina), a bispecific CD3xCD19 T-cell engager (BiTE®), is approved for patients with relapsed/refractory (R/R) and measurable residual disease (MRD) positive B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL). However, a significant percentage of patients do not respond to Blina, and the determinants of resistance are currently unknown. Here, we used Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) to identify pre-treatment T cell features associated with response.

Methods PBMCs of 13 patients with BCP-ALL who received Blina at the LMU Munich University Hospital from 2016 to 2021 were collected before treatment start. CITE-seq(BD Rhapsody Platform) was performed on a total of 34.907 cells isolated via magnetic-activated cell sorting based on the expression of CD3. An AbSeq panel containing 7 surface markers (CD4, CD8, CCR7, CD45RA, CX3CR1, CD127 and PD1) and a customized T-cell targeted RNA sequencing panel (477 genes) were employed. A Monte-Carlo permutation test was used to compare the proportions of cells in different clusters between Responders (R) and Non-Responders (NR). Significant differences were assumed with a fold change threshold of at least 1.5 (log2FC (LFC) $\geq$ 0.58) and a false discovery rate (FDR) cutoff of $<$ 0.05.

Results We used CITE-seq to characterize T cells in peripheral blood (PB) samples obtained from R/R (n=3) and MRD+ (n=10) BCP-ALL patients. Ten patients (9 MRD+, 1 R/R) responded to the Blina treatment. An integrated strategy based on Reciprocal Principal Component Analysis(RPCA) was implemented and bioinformatic analysis of gene expression mapped in two dimensions via uniform manifold approximation and projection (UMAP) identified 12 CD3^{pos} distinct clusters. Within CD8^{pos} T cells of R, we found an increased proportion of CCR7^{low} Granzyme K(GZMK)^{high} Effector Memory-like cells (GZMK^{high} TEM-like, LFC 0.78, 95CI: 0.66 to 0.92; FDR=0.0012) and a reduced percentage of Naïve cells(CCR7^{high} LEF1^{high} CD62L^{high}), when compared to NR (LFC -0.59, 95CI: -0.54 to -0.64; FDR =0.0012, Figure1A). Atrend towards an increased proportion of CCR7^{low}CX3CR1^{high}GZMB^{high} terminal effector-like cells (GZMB^{high} TE-like, LFC 0.57, 95CI: 0.45-0.70; FDR=0.0013) was also noted in CD8^{pos} T cells of R vs NR. Peripheral CD4^{pos} T cells of R exhibited a tendency towards an increased proportion of GZMK^{high} cytotoxic T cells (LFC 0.43, 95CI: 0.26 to 0.58; FDR=0.0019) and a reduced percentage of FOXP3^{high} T regulatory cells (LFC -0.19, 95CI: -0.03 to -0.34; FDR=0.025), compared to NR. Furthermore, we investigated gene expression differences between R and NR cells in each cell cluster. Naïve T cells (CD8 and CD4) of NR showed a significantly increased (LFC>1, FDR<0.05, Figure1B) PRDM1 (Blimp-1) expression, a key promoter of T-cell terminal differentiation that is known to be involved in CD19 CAR-T dysfunction. CD8GZMB^{high} TE-likecells of NR showed upregulated expression of genes involved in chemokine production and cytokine signaling (CCL3, IFNGR1) and downregulated LAIR2, a decoy receptor protecting CD8 T cells from collagen-induced exhaustion. Additionally, TOX, a regulator of T cell exhaustion, was moderately upregulated in CD8GZMB^{high} TE-like cells of NR (LFC 0.41, FDR=0.007).

Conclusions Taken together, we were able to identify CD8 T cell subsets that are associated with a response to Blina treatment, as well as gene sets involved in BiTE-mediated T-cell function. Interestingly, the PRDM1 overexpression we found in both CD4

and CD8 naive T cells of Non-Responders has already been linked to T-cell dysfunction in the context of CD19 CAR-T cell therapy. We plan to confirm the results of our analysis in an independent patient cohort. Concomitantly, we will enrich the dataset with results from multi-parameter flow cytometry and in vitro T-cell functional assays conducted using longitudinally collected PB samples from Blina-treated patients.

Figure1 A) Log Fold Change(log FC) of the proportion of Responders vs Non Responders CD8 ^{pos} cells in each cluster. The dots represent the observed logFC, the horizontal line the bootstrapped 95%Confidence Intervals; PTGDR2= prostaglandin D2 receptor 2; ns= not significant **B)** Differentially expressed genes in Non Responders vs Responders CD8 ^{pos} naïve T cells. FDR=False Discovery Rate; CCL5= Chemokine (C-C motif) ligand 5 ; PRDM1= PR/SET Domain 1;SGK1= serum/glucocorticoid regulated kinase 1.

Disclosures von Bergwelt-Baildon: *Roche:* Research Funding, Speakers Bureau; *Astellas:* Research Funding, Speakers Bureau; *Mologen:* Research Funding, Speakers Bureau; *Gilead:* Research Funding, Speakers Bureau; *BMS:* Research Funding, Speakers Bureau; *Novartis:* Research Funding, Speakers Bureau; *MSD Sharpe & Dohme:* Research Funding, Speakers Bureau; *Miltenyi Biotec:* Research Funding, Speakers Bureau. **Blumenberg:** *Gilead:* Consultancy, Research Funding; *Novartis:* Honoraria, Research Funding; *BMS:* Research Funding; *Janssen:* Honoraria, Research Funding; *Roche:* Consultancy, Research Funding. **Subklewe:** *Molecular Partners:* Consultancy, Honoraria, Research Funding; *Pfizer:* Consultancy, Honoraria, Other: Travel Support, Speakers Bureau; *Gilead/Kite:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *BMS/Celgene:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Ichnos Sciences:* Consultancy, Honoraria; *AstraZeneca:* Speakers Bureau; *Janssen:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Takeda:* Consultancy, Honoraria, Research Funding; *AvenCell:* Consultancy, Honoraria; *Roche:* Consultancy, Honoraria, Other: Travel Support, Research Funding, Speakers Bureau; *Incyte Biosciences:* Consultancy, Honoraria; *Amgen:* Consultancy, Honoraria, Research Funding; *Seagen:* Research Funding; *Miltenyi Biotec:* Consultancy, Honoraria, Research Funding; *Novartis:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *GSK:* Speakers Bureau; *LAWG:* Speakers Bureau; *Springer Healthcare:* Speakers Bureau; *AbbVie:* Consultancy, Honoraria; *Autolus:* Consultancy, Honoraria; *advesya (CanCell Therapeutics):* Consultancy, Honoraria; *Genmab US:* Consultancy, Honoraria; *Interius BioTherapeutics:* Consultancy, Honoraria; *Nektar Therapeutics:* Consultancy, Honoraria; *Orbital Therapeutics:* Consultancy, Honoraria; *Sanofi:* Consultancy, Honoraria; *Scare:* Consultancy, Honoraria. **Buecklein:** *Priothera:* Consultancy; *Pierre Fabre:* Other: Travel Funding; *Miltenyi Biotech:* Research Funding; *Pfizer:* Consultancy, Honoraria, Speakers Bureau; *Amgen:* Consultancy; *Gilead/Kite:* Other: Travel Funding, Research Funding; *BMS:* Research Funding; *Roche:* Honoraria, Research Funding.

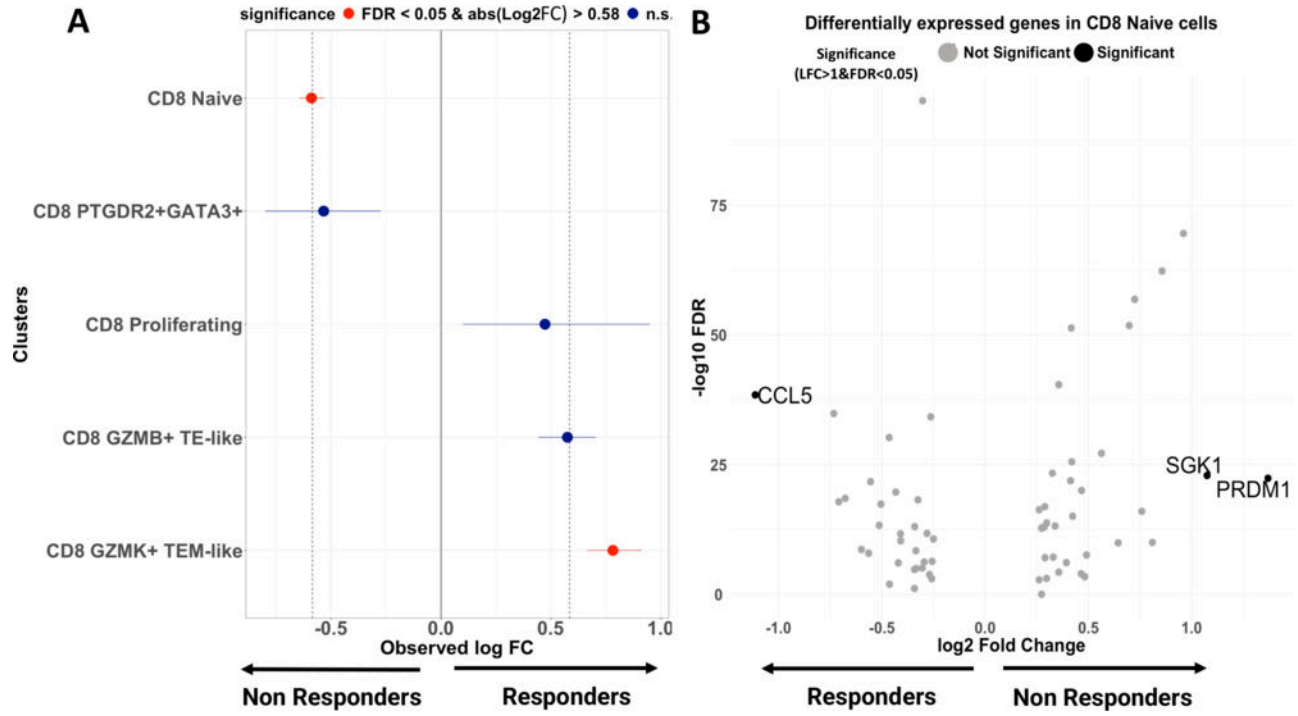

Figure 1

https://doi.org/10.1182/blood-2023-188970