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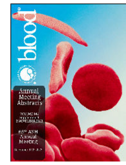

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Phenotypic, Transcriptomic and Proteomic Characteristics of CAR T-Cell Dysfunction Are Associated with Inferior CAR T-Cell Expansion and Treatment Failure in r/r B-NHL

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Introduction: Despite the curative potential of CAR T-cells, a significant number of patients fail to respond or relapse early. Recently, we and others showed that early expansion failure, and particularly CAR T-cell levels at day 7, are strong predictors for treatment resistance (Blumenberg et al, Blood Supplement 2022; Locke et al, Blood Adv 2020). Here we dissected early characteristics of CAR T-cell dysfunction based on immune checkpoint expression, transcriptomic signatures and cytokine profiles and investigated their link to CAR T-cell expansion failure and patient outcome of CD19-targeted CAR T-cell therapy.
Methods: Patients with r/r B-NHL who underwent treatment with axi-cel or tisa-cel between January 2019 and November 2021 in the third- or later-line setting at the LMU in Munich were included (n=55). EDTA-anticoagulated peripheral blood and serum was collected. CD19 CAR and immune checkpoint expression (IC; PD-1, TIM-3, LAG-3 and CD224) was assessed by multiparameter flow cytometry on day 14 after CAR T-cell infusion. A next-generation targeted single-cell proteogenomics approach (BD Rhapsody ™) to analyze the transcriptome of distinct CAR T-cell subpopulations on day 7 and day 28 after CAR T-cell infusion. A total of 92 human immuno-oncology related proteins were simultaneously measured using the Olink® Immuno-Oncology panel on day 14 after infusion. Signatures of CAR T-cell dysfunction have been compared between patients with low (loEx) or high (hiEx; $<$ vs $\geq$ 19 CAR T-cells/µl at day 7 post infusion)CAR T-cell expansion and were put into context of clinical outcome measures.
Results: CAR T-cell levels at day 7 were inversely correlated to co-expression of PD-1 ($r = -0.4923$, $p = 0.0011$, $n = 41$), TIM-3 ($r = -0.3634$, $p = 0.0195$, $n = 41$) and LAG-3 ($r = -0.5412$, $p = 0.0003$, $n = 41$) on CAR T-cells at day 14, suggestive of an association of early expansion failure and dysfunction of CAR T-cells. Indeed, loEx (n=26) showed higher frequencies of CAR T-cells coexpressing several IC, including LAG-3$^+$ (p=0.0036), LAG-3$^+$PD-1$^+$ ($p = 0.0048$), or LAG-3$^+$TIM-3$^+$ ($p = 0.0065$) compared to hiEx (n=15) on day 14 after transfusion. Conversely, we detected higher frequencies of non-dysfunctional CAR T-cell phenotypes negative for several IC, such as CD244$^+$PD-1$^-$TIM-3$^-$ ($p = 0.0006$), CD244$^+$LAG-3$^-$PD-1$^-$TIM-3$^-$ ($p = 0.001$) and CD244$^+$LAG-3$^+$PD-1$^-$TIM-3$^-$ ($p = 0.011$) in hiEx. Next, we applied uniform manifold approximation and projection (UMAP) data to examine the surface expression of IC on CAR T-cells at day 14 post-infusion according to treatment response. Unsupervised clustering revealed a higher abundance of CAR T-cell clusters positive for several IC (CD244$^+$LAG-3$^-$PD-1$^+$TIM-3$^+$) in NR (n = 16) compared to R (n = 23). By contrast, CAR T-cell clusters containing CAR T-cells expressing no IC were more abundant in R compared to NR. In addition, we found significantly increased levels of a highly dysfunctional CAR T-cell phenotype positive for all four IC in NR compared to R at day 14 post infusion ($p = 0.0178$, $n = 46$). When specifically comparing the transcriptome of CAR T-cells between NR (n = 6) and R (n = 6), immune inhibitory and exhaustion-related genes were

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upregulated in NR, such as GZMK, KLRC1 and NR4A2 (figure 1, cluster 1). Conversely, CAR T-cells in R overexpressed genes related to T-cell effector function and cell differentiation, such as TIMP-1, TNFDF10 and MYC (figure 1, cluster 0). Consistent with these findings, a multiplexed proteomics assay for immunomodulatory serum factors in day 14 samples revealed an upregulation of proteins linked to an immune inhibitory and inflamed milieu in patients with highly dysfunctional CAR T-cells (e.g., sPD-L1 and sLAG-3, $p = 0.0235$ and $p = 0.0264$, respectively, $n = 46$) as well as in non-responding patients (e.g., sPD-L1 and IL-6, $p = 0.0036$ and $p = 0.0053$, respectively, $n = 55$).

Conclusion: We were able to characterize a phenotypic, transcriptomic and proteomic signature of CAR T-cell dysfunction in post-infusion samples of both patients experiencing CAR T-cell expansion failure as well as lack of radiographic response. Our data show that treatment failure might be predicted not only by CAR T-cell kinetics but also by early assessment of the CAR T-cell phenotype, transcriptome and secretome, and thus enables identification of patients in need of salvage treatment.

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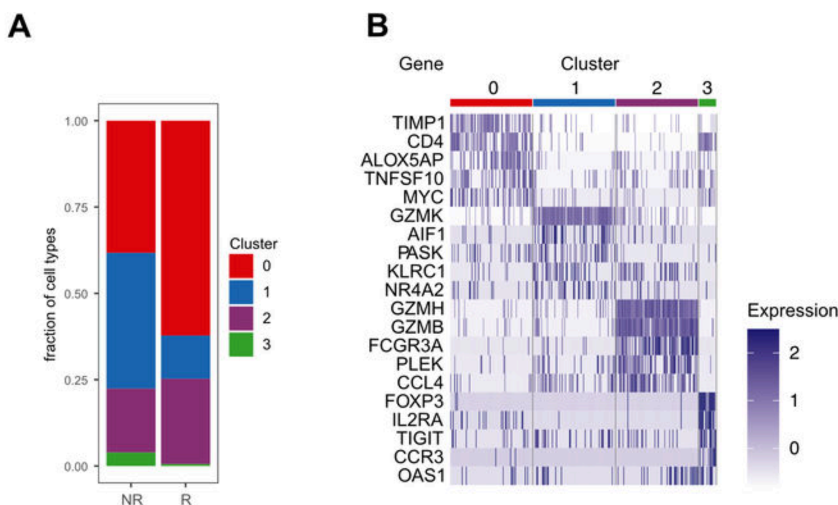

Figure 1. Transcriptomic correlates of CAR T-cell dysfunction. (A) Bar plot indicating fraction of cell types in non-responding (468 cells) and responding (368 cells) patients from each subgroup in each cluster. (B) Heat map indicating top 5 marker genes expression for each cell cluster.

Figure 1

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