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Single-cell RNA sequencing and functional assessment of healthy donor- and cancer patient-derived T and CAR-T cells

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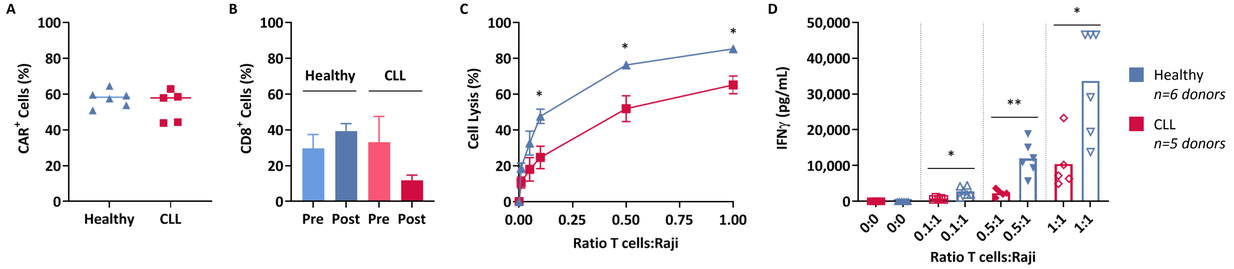
Abstract

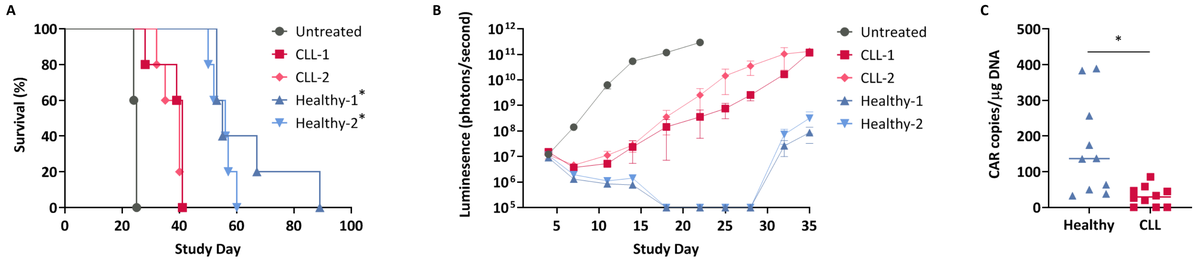
Autologous chimeric antigen receptor T (CAR-T) cell therapies have shown remarkable success in treating relapsed/refractory B-cell malignancies. However, even in indications with high complete response rates, not all patients respond or have durable responses after CAR-T treatment. Furthermore, autologous CAR-T treatments have not yielded the same impressive outcomes in solid malignancies to date. A major limitation of autologous CAR-T therapy may be the dysfunctional state of a patient's T cell populations used for manufacturing of a drug product. Allogeneic therapeutics can bypass this limitation by enabling the use of healthy donor starting material. Moreover, healthy donor material that exhibits specific T cell attributes can be selected for drug product manufacturing. To identify attributes that can be associated with improved performance of CAR-T cells we have characterized T cells from healthy donors as well as cancer patients, in particular from chronic lymphocytic leukemia (CLL) patients as these have been described previously to be dysfunctional. We show impaired function of cancer patient-derived CAR-T cells when compared to healthy donor-derived cells utilizing both *in vitro* and *in vivo* assays. We have performed single cell RNA sequencing (scRNA seq) on both starting material T cells and CAR-T cells from multiple healthy and CLL donors used in functional assays to uncover both gene expression and population differences associated with CAR-T cell performance. scRNA seq analysis revealed marked heterogeneity among starting populations as well as CAR-T lots from the cancer patient-derived T cells. Our analysis has allowed us to associate distinct cellular subpopulation and gene expression profiles with preclinical functional outputs.

Figure 1: Healthy donor CAR-T cells show greater *in vitro* efficacy than CLL-derived CAR-T cells



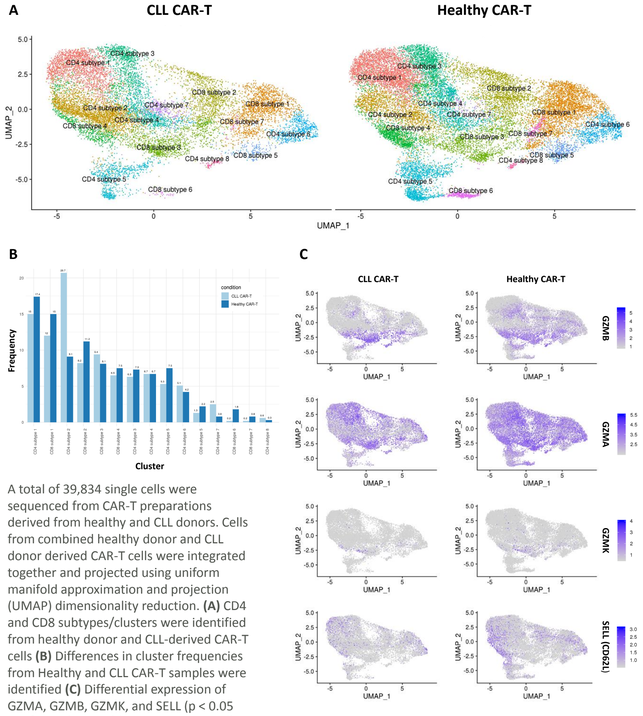
T cells from healthy donors or from donors with CLL were used to produce TCR-deficient CRISPR-edited CAR-T cells with the CAR construct knocked into the *TRAC* locus. **(A)** Equivalent levels of surface CAR expression were detected in healthy vs. CLL donor derived CAR-T cells. **(B)** CLL-derived CAR-T cells tended to contain a diminished level of CD8 cells. Healthy donor CAR-T cells had **(C)** increased cytolytic activity and **(D)** produced higher levels of INFγ compared to CLL-derived CAR-T cells. * p <0.05

Figure 2: Healthy donor CAR-T cells show greater *in vivo* efficacy than CLL-derived CAR-T cells



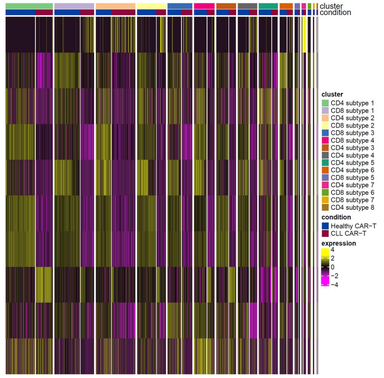
(A) CAR-T cells derived from healthy donors increased survival longer than CLL donor derived CAR-T cells in the CD19+ Nalm6-luciferase xenograft leukemia model in NOG mice (n=5 mice each). **(B)** Healthy donor CAR-T cells controlled leukemia burden longer than CLL-derived cells *in vivo*. **(C)** CAR-T cells from healthy donors were detected at higher levels than those from CLL donors at day 26 of the study. * p <0.05

Figure 3: scRNA seq analysis of healthy and CLL donor derived CAR-T cells reveals differences in the frequency of T cell subtypes and in the expression of genes related to T cell function



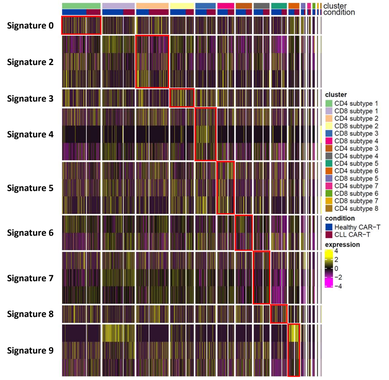
A total of 39,834 single cells were sequenced from CAR-T preparations derived from healthy and CLL donors. Cells from combined healthy donor and CLL donor derived CAR-T cells were integrated together and projected using uniform manifold approximation and projection (UMAP) dimensionality reduction. **(A)** CD4 and CD8 subtypes/clusters were identified from healthy donor and CLL-derived CAR-T cells **(B)** Differences in cluster frequencies from Healthy and CLL CAR-T samples were identified **(C)** Differential expression of GZMA, GZMB, GZMK, and SELL ($p < 0.05$ Bonferroni) in Healthy vs. CLL CAR-T cells was identified in specific T cell clusters.

Figure 4: Identification of differential gene expression between healthy and CLL donor CAR-T cells across T cell clusters



A gene expression signature was identified associated with healthy donors in multiple cell clusters. A heatmap is shown for the top 10 differentially expressed genes (p <0.05 Bonferroni) within any cluster and between healthy donor and CLL-derived CAR-T cells.

Figure 5: Identification of genes that are differentially expressed within T cell clusters between healthy and CLL donor CAR-T cells



scRNA seq analysis identifies cell cluster-specific gene expression changes between healthy and CLL donor derived CAR-T cells. A heatmap is shown for the top 3 differentially expressed genes (p <0.05 Bonferroni) within only one cluster and between healthy donor and CLL-derived CAR-T cells (red boxes). Cells are grouped by the assigned cluster (labeled at the top row) and further separated between healthy (blue) and CLL (red).

Conclusions from Preclinical Studies

- Healthy donor CAR-T cells can outperform cancer T cell-derived CAR-T cells in functional assays *in vitro* and *in vivo*
- scRNA seq can identify differences in CAR-T product cell composition and gene expression
- Gene expression changes across and within cell clusters can distinguish CAR-T products with superior functional performance
- Healthy donor CAR-T cells have increased expression of genes regulating memory and effector function in distinct T cell subtypes
- scRNA seq can thus serve as a basis for discovering novel features of CAR-T products