Abstract

Autologous chimeric antigen receptor T (CAR) T-cell therapy has shown remarkable success in treating relapsed/refractory B-cell malignancies; however, even with high complete response rates, not all patients respond or have durable responses after CAR T-cell treatment. Furthermore, autologous CAR T-cell therapies have yielded the same promising outcomes in solid malignancies to date. A major limitation of autologous CAR T-cell therapy may be the dysfunctional state of a patient’s T-cell populations, used for manufacturing of a chimeric product. Allogeneic therapies can bypass this limitation by enabling the use of healthy donor starting material. Moreover, healthy donor material that enables specific CAR T-cell attributes can be engineered 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 difficult. We have performed functional and molecular characterization of CAR-T cell samples from multiple healthy and CLL donors using functional assays to uncover both gene expression and population differences associated with CAR-T cell performance. scRNA seq analysis revealed enriched heterogeneity among starting populations as well as CAR-T T cells 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 2: Healthy donor CAR-T cells show greater in vitro efficacy than CLL-derived CAR-T cells

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

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

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.