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Background

The goal of this study is to generate and optimize the manufacturing of "off-the-shelf" CD19-CAR-T cells utilizing umbilical cord blood (UCB) as starting material.

Methods

T cells were isolated from UCBs (N=15) through negative magnetic selection and upon activation in vitro using CD3 and CD28 mAbs. The T lymphocytes were then transduced with lentiviral vectors encoding for CD19-CD28z-or CD19-4-1BBz-CARs. CD19-CAR-T cells were also generated from the peripheral blood lymphocytes (PBL; N=5) as reference. The multiparametric phenotype analysis was performed assessing the expression of markers associated with T cell differentiation and activation by CAR-T cells. Functional assays, through either Elispot, FluoroSpot or Luminex platforms, were carried out to assess cytokine, chemokine and cytotoxic profiles of the T cells. A machine learning technique called L0-regularized logistic regression, implemented in the R package L0Learn, was used to select the optimal values of the tuning parameters. The metabolomic and transcriptomic profiles of CD19-CAR-T cells was determined upon the antigen-specific or not engagement of the CARs.

Results

The enrichment of both CD4+ and CD8+ CD19-CAR-T cells with stem memory-like or early stage of differentiation (CD45RA+) phenotype and co-expressing either ICOS or BTLA was observed in UCB- vs. PBL-derived CD19-CAR-T cells (p<0.0002-<0.05). Moreover, differential phenotype of CAR-T cells was associated with the variable costimulatory signals comprised in the structure of the CARs (CD28z or 4-1BBz). The differential antigen-specific anti-tumor activity of these CAR-T cells was identified, with diversities depending on the type and source of engineered T cells. Distinct metabolomic profiles, including pathways related to amino acid, tryptophan and nucleotide sugar metabolism and protein biosynthesis were detected in relation to the antigen-specific or unrelated stimulation and the manufacturing procedures. Integration of multi-omics results, including the transcriptomic profile allowed to identify the complexities of CD19-CAR-T cells phenotypes and functions.

Conclusions

The characterization of phenotype and functional properties of CAR-T cells through multi-omics platforms allowed to prove the suitability of UCB to generate "off-the-shelf" CAR-T cells and to identify sub-populations endowed with superior anti-tumor activity.

REFERENCES


Ethics Approval

The study obtained ethics approval from Sidra Medicine review board; approval #1500788. Participants to the study gave informed consent before taking part.