

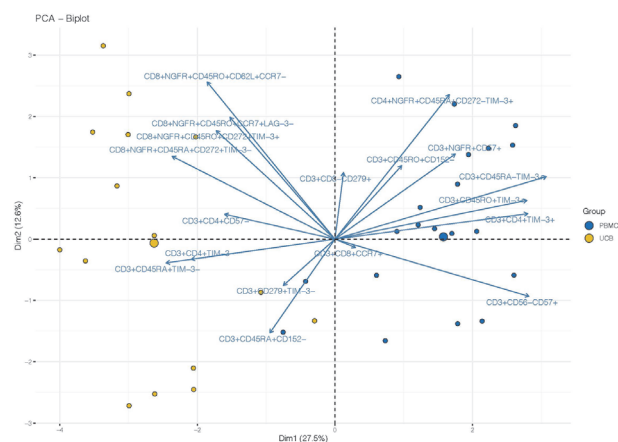
## THE DEEP PHENOTYPE CHARACTERIZATION OF 'OFF-THE-SHELF' CD19-CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS ALLOWS TO IDENTIFY THEIR SUBSET COMPLEXITY AND TO OPTIMIZE THEIR MANUFACTURING

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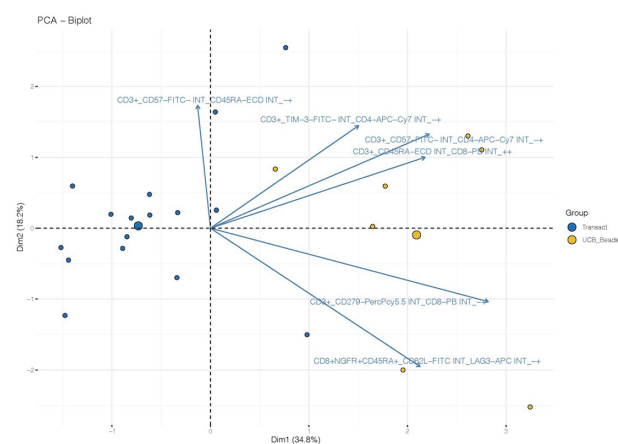
**Background** Umbilical cord blood (UCB) represents a promising source of T cells for the generation of 'off-the-shelf' T cells engineered to express a chimeric antigen receptor (CAR). This study is aimed at understanding the composition of T cell subsets within UCB-CAR-T cells.

**Methods** T cells, either from UCB or peripheral mononuclear cells (PBMCs) of healthy donors, were activated in vitro with CD3/CD28 mAbs either conjugated to magnetic beads (Dynabeads) or to a colloidal polymeric nanomatrix (TransAct; Miltenyi Biotec). T cells were then transduced with lentiviral vectors encoding for CD19-CD28z or CD19-4-1BBz CARs. The deep phenotype analyses of the CD19-CAR-T cells (N=32) was performed through a multidimensional flow cytometry to assess the expression/co-expression of T cell-associated markers (N=29). The NGFR was utilized as probe for the expression of CD19-CAR. To select the pertinent markers characterising the different groups, we applied a machine learning technique called L0-regularized logistic regression,<sup>1,2</sup> and implemented in the R package L0Learn. 5-fold cross-validation (CV) was used to select the optimal values of the tuning parameters. CD19-CAR-T cells have been also characterized for the transcriptomic profile by parallel quantitative PCR using the high throughput BioMark HD platform and for cytokines, perforin and granzyme B release upon the co-culture with CD19 expressing or not target cells.

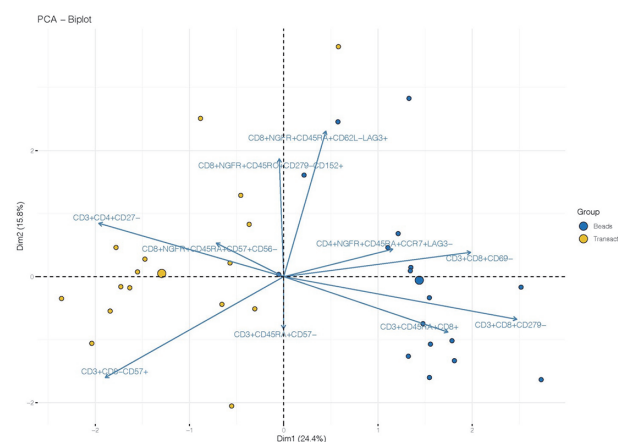
**Results** T lymphocytes UCB showed efficient expression of the CARs (40–70% of positive cells). Different T cell subsets could discriminate the composition of T cells activated with either Beads or TranAct. CD4+NGFR+CD45RA+ or CD8+NGFR+CD45RA+ T cells associated with different combinations of CCR7, CD62L, LAG3, CD57, CD56 could discriminate between cells activated with Beads vs. TranAct (figures 2–3). CD8+NGFR+CD45RO+CD279–CD152+ T cells were also differentially expressed in TranAct vs. Beads. The PCA analyses also highlighted differences in terms of CD19-CAR-T cell subsets (such as CD8+NGFR+CD45RO+CD62L+, CD8+NGFR+CD45RO+CCR7+, CD8+NGFR+CD45RO+CD272+TIM–3+, CD8+NGFR+CD45RO+CD272+TIM–3+, CD8+NGFR+CD45RA+CD272+TIM–3– and CD4+NGFR+CD45RA+CD272–TIM–3+) in PBMCs vs. UCBs (figure 1). In addition, bystander T cells with different phenotype not expressing the CARs were also detected within the populations of T cells with different origins. Similarly, different T subsets were found in relationship with the sources of T cells. These CD19-CAR-T cells were also characterized for the anti-tumor activity and transcriptomic profiling.



Abstract 102 Figure 1 PCA of CAR-T cells from UCB vs. PBMCs



Abstract 102 Figure 2 PCA of CAR-T cells from UCB to compare TranAct vs. beads



Abstract 102 Figure 3 PCA of CD19-CAR-T cells to compare TranAct vs. Beads irrespective of the source of the T cells

**Conclusions** The combination of deep phenotype characterization with novel statistical tools allowed to identify the complexity of subsets in the engineered T cells in relationship with the starting material and the methods for the activation of the lymphocytes. These findings have important implications for the optimization of the manufacturing of CD19-CAR-T cells.

#### REFERENCES

1. Antoine Dedieu, Hussein Hazimeh, and Rahul Mazumder. Learningsparse classifiers: Continuous and mixed integer optimization perspectives. *Journal of Machine Learning Research* 2021.
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