THE DEVELOPMENT OF ALLOGENEIC TIPS-DERIVED TCR$^-$ CAR$^+$ CD8αβ T CELLS

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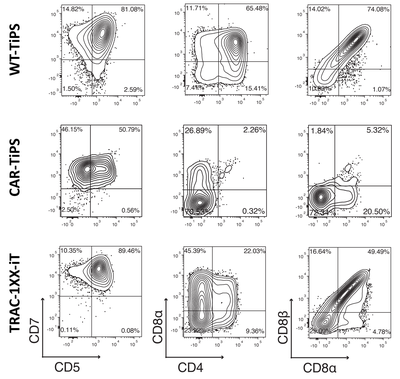
INTRODUCTION

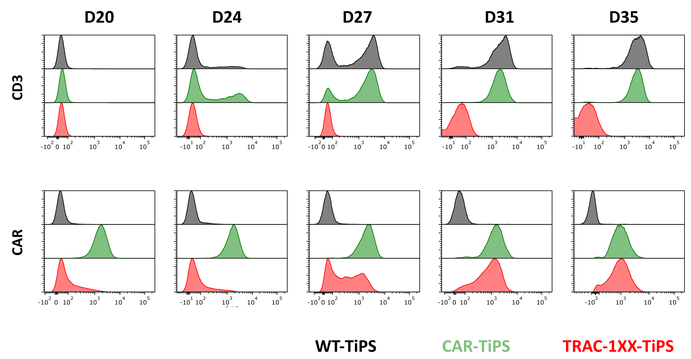
T cell-derived iPSCs (TiPS) can provide a self-renewing source for in vitro CAR T cell production, resulting in a homogenous CAR$^+$TCR$^+$ TiPS-derived T (iT) cell population. However, in vitro differentiation yields iT cells with suboptimal features, generating cells that divert towards a CD8αα innate-like phenotype. Premature expression of the T cell receptor (TCR) causes this diversion towards an innate-like phenotype. This can be partially averted by disabling the TCR but does not allow for maturation of the cells to a CD8αβ single-positive stage. In the absence of a TCR, careful control of CAR expression timing and calibrated signaling strength can uniquely drive adaptive iT cell differentiation to the CD4$^+$CD8αβ$^+$ DP stage. CAR engagement at the DP stage subsequently facilitates maturation to CD8αβ single positive T cells, substituting for the TCR. DP cells upregulate 4-1BB expression upon CAR engagement and providing 4-1BBL costimulation enhances iT cell proliferation, persistence and in vivo function.

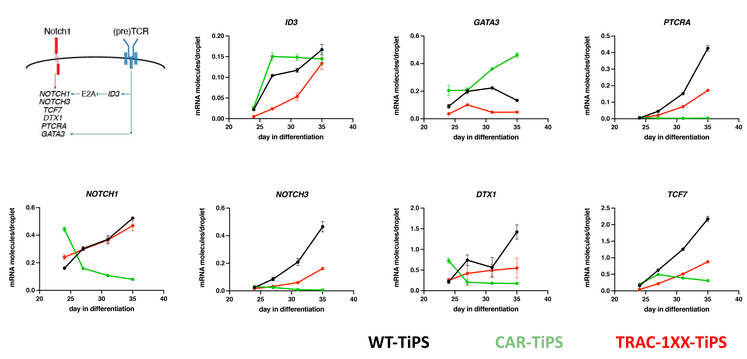
METHODS

T cell reprogramming and engineering
↓
TiPS clonal selection
↓
iCD34 differentiation
↓
DP iT differentiation
↓
iT maturation
↓
iT in vitro and in vivo functional assessment

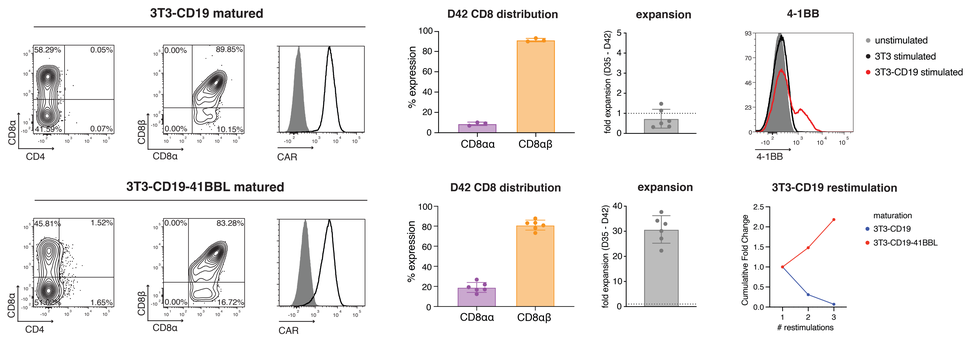
CONTROLLED CAR EXPRESSION CAN DRIVE CD4$^+$ CD8$\alpha\beta^+$ DP T CELL DEVELOPMENT

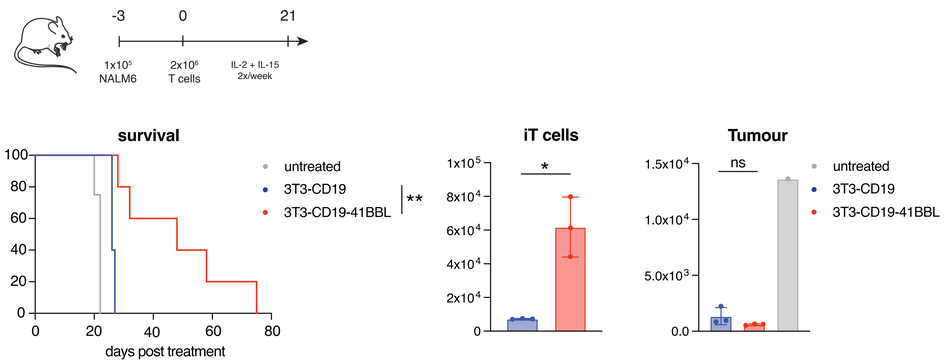






4-1BB COSTIMULATION IMPROVES IT CELL EXPANSION AND IN VIVO FUNCTION





CONCLUSION

Regulated CAR expression can substitute for the TCR to drive DP T cell differentiation and CD8 αβ SP T cell maturation. This remarkable finding suggests that the strength and timing of CAR signaling, together with the strength of Notch stimulation, are the essential drivers of αβ vs $\gamma\delta$ lineage commitment.

4-1BB costimulation provided during maturation to the CD8αβ SP stage increases iT cell in vitro proliferation, in vivo persistence and in vivo cytotoxic function.

Enhanced 4-1BB costimulation through expression of 4-1BBL in iT cells further increases iT cell proliferation upon antigen exposure and in vivo persistence, thereby increasing in vivo tumor control and survival.

Collectively, TCR$^-$CAR$^+$41BBL$^+$-iT cells have improved in vivo cytolytic function, without exposing to the risk of inducing graft-versus-host disease owing to the complete TCR$^-$ phenotype, and can be manufactured at scale and made available off-the-shelf for broad patient access

4-1BBL-iT CELLS HAVE IMPROVED IN VIVO PERSISTENCE AND CYTOLYTIC FUNCTION