



Memorial Sloan Kettering
Cancer Center

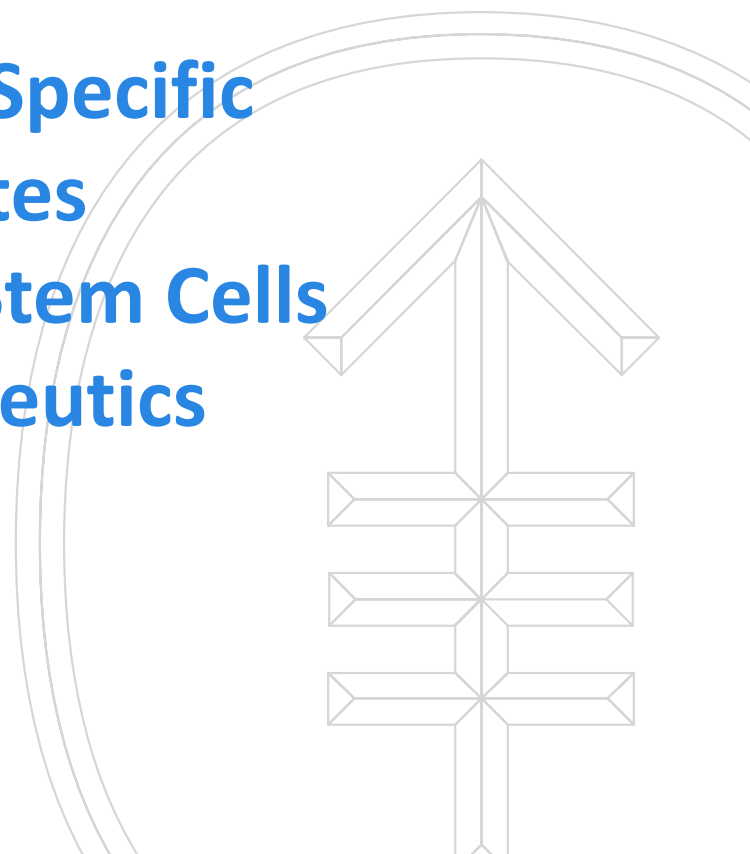
Fcete
THERAPEUTICS

Generation of Clonal Antigen Specific $CD8\alpha\beta^+$ Cytotoxic T lymphocytes from Renewable Pluripotent Stem Cells for Off-the-Shelf T Cell Therapeutics

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Michel Sadelain Lab

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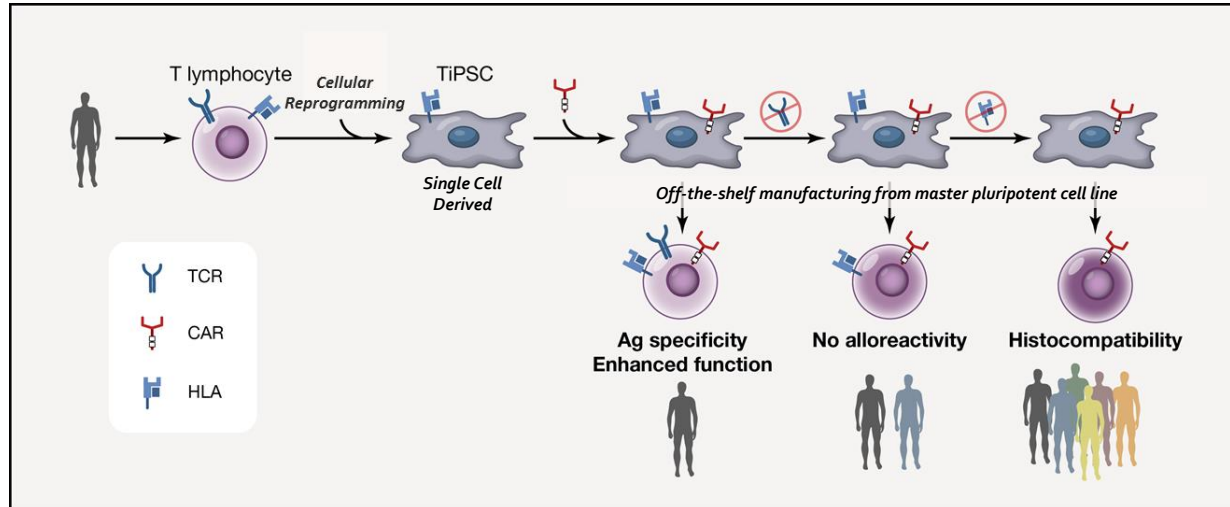


Goal: Generate Potent, Off-the-Shelf CAR T cells from a Clonal Population of Pluripotent Stem Cells

“Genetically engineered T cells are powerful new medicines, offering hope for curative responses in patients with cancer.”

Sadelain, Rivière & Riddell, *Nature*, 2017

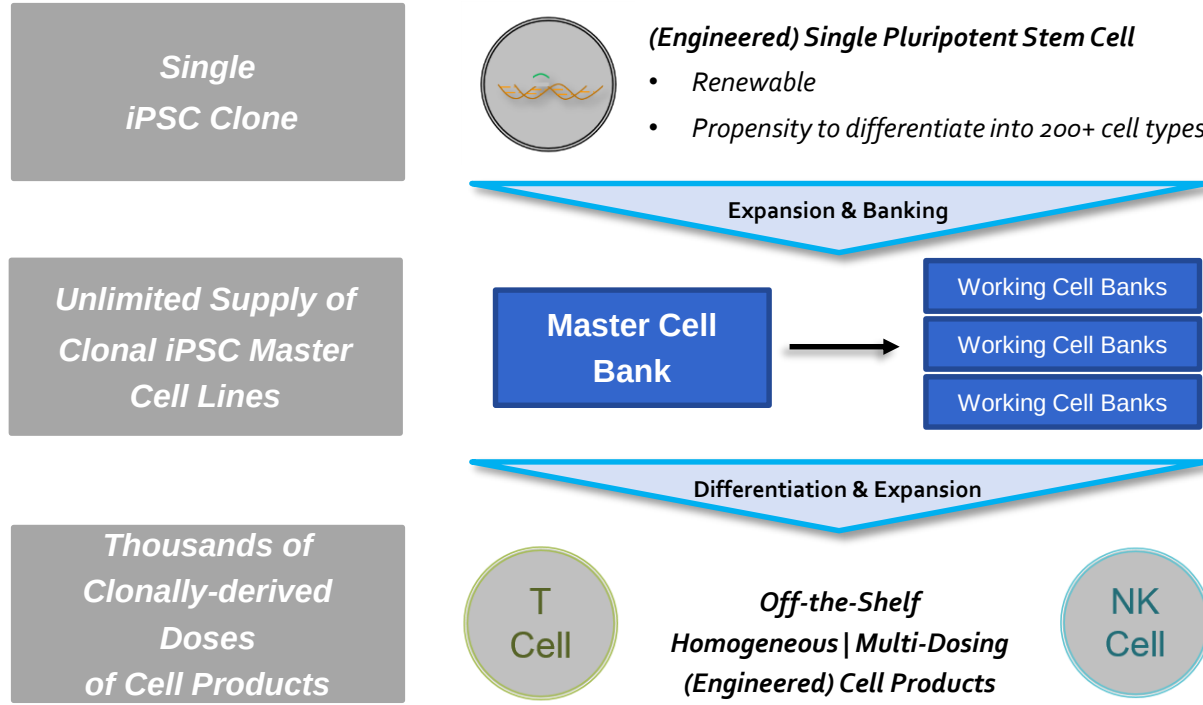
From Autologous to Off-the-Shelf
T Cell Therapy



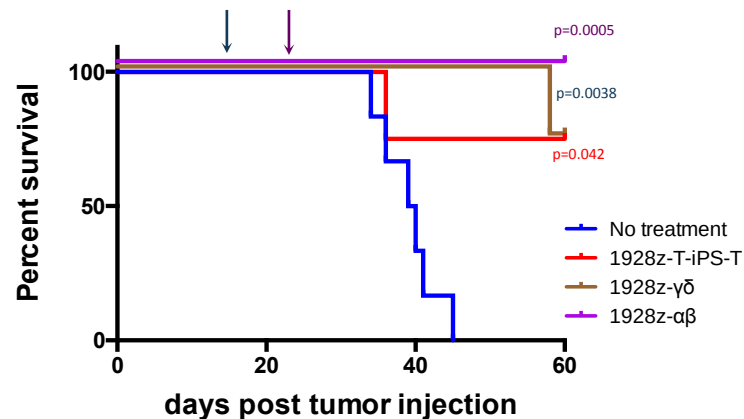
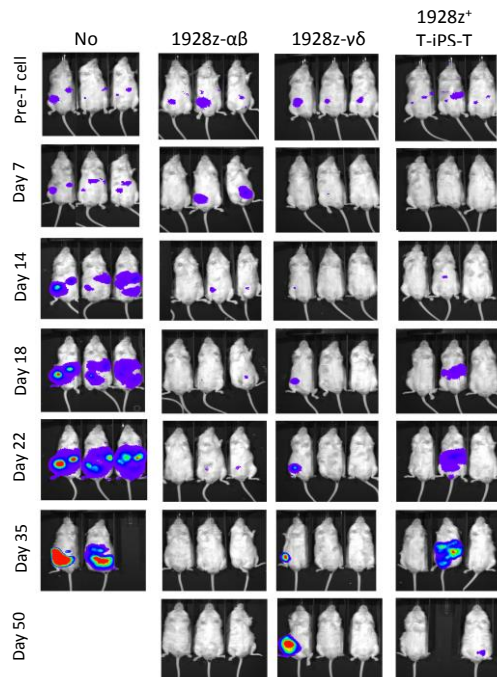
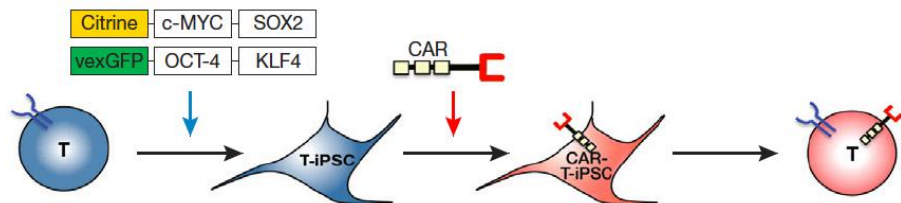
Adapted from: Themeli, Riviere & Sadelain, *Cell Stem Cells*, 2015

Induced Pluripotent Stem Cell Product Platform

Renewable Source for Off-the-Shelf Cell Products



Successful Derivation of Functional CAR T Cells from TiPSC



MSKCC : FATE Collaboration

To develop off-the-shelf engineered T cells

Objectives

Generation of Single Cell-Derived Genetically Edited Master Pluripotent Cell Line

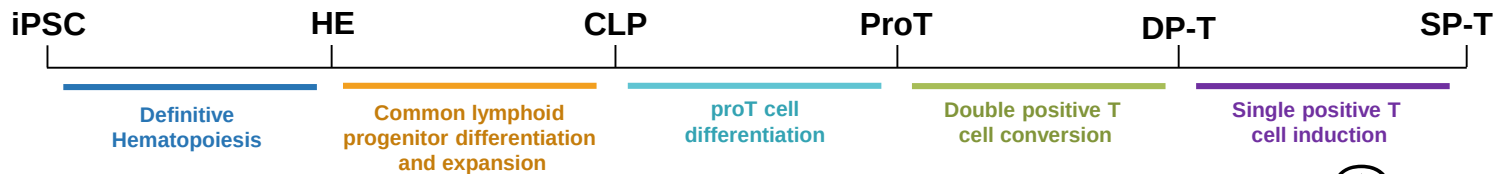
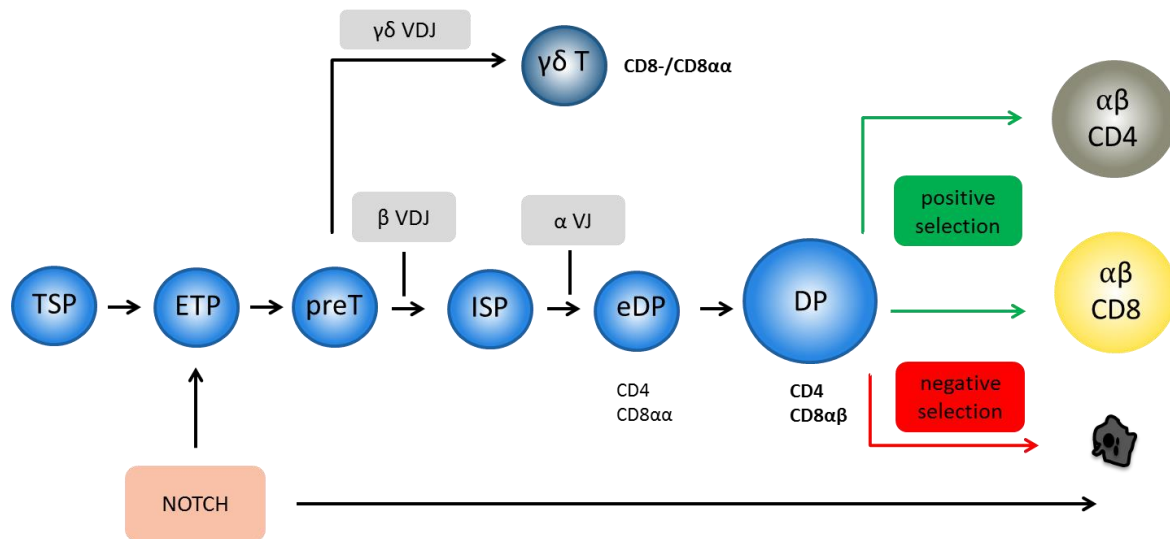
Derivation of Potent Engineered T cells ($\alpha\beta$ and $\gamma\delta$)

Generation of Off-The-Shelf TCR^{-/-} CAR T cells

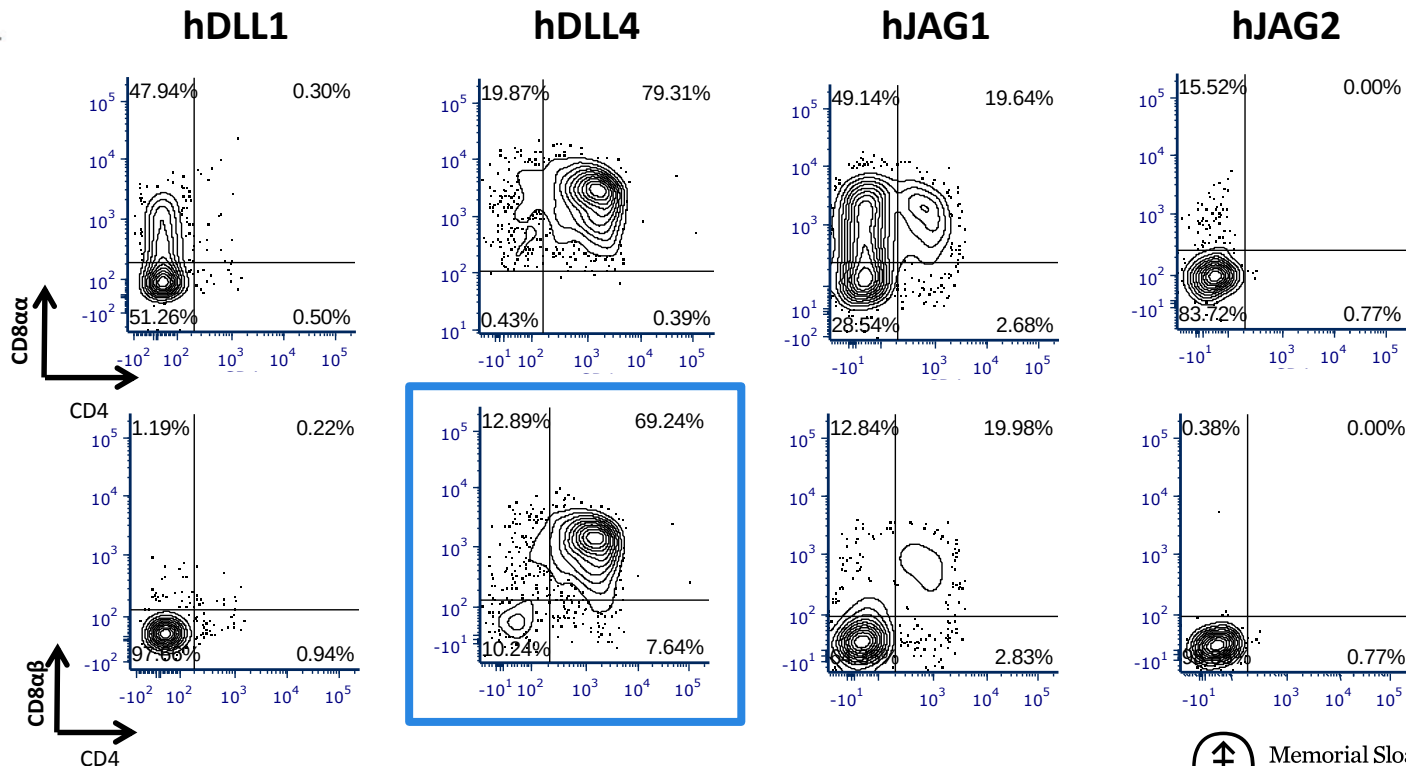
Clonal Cell Bank for Manufacturer of Drug Product

Open Clinical Trial

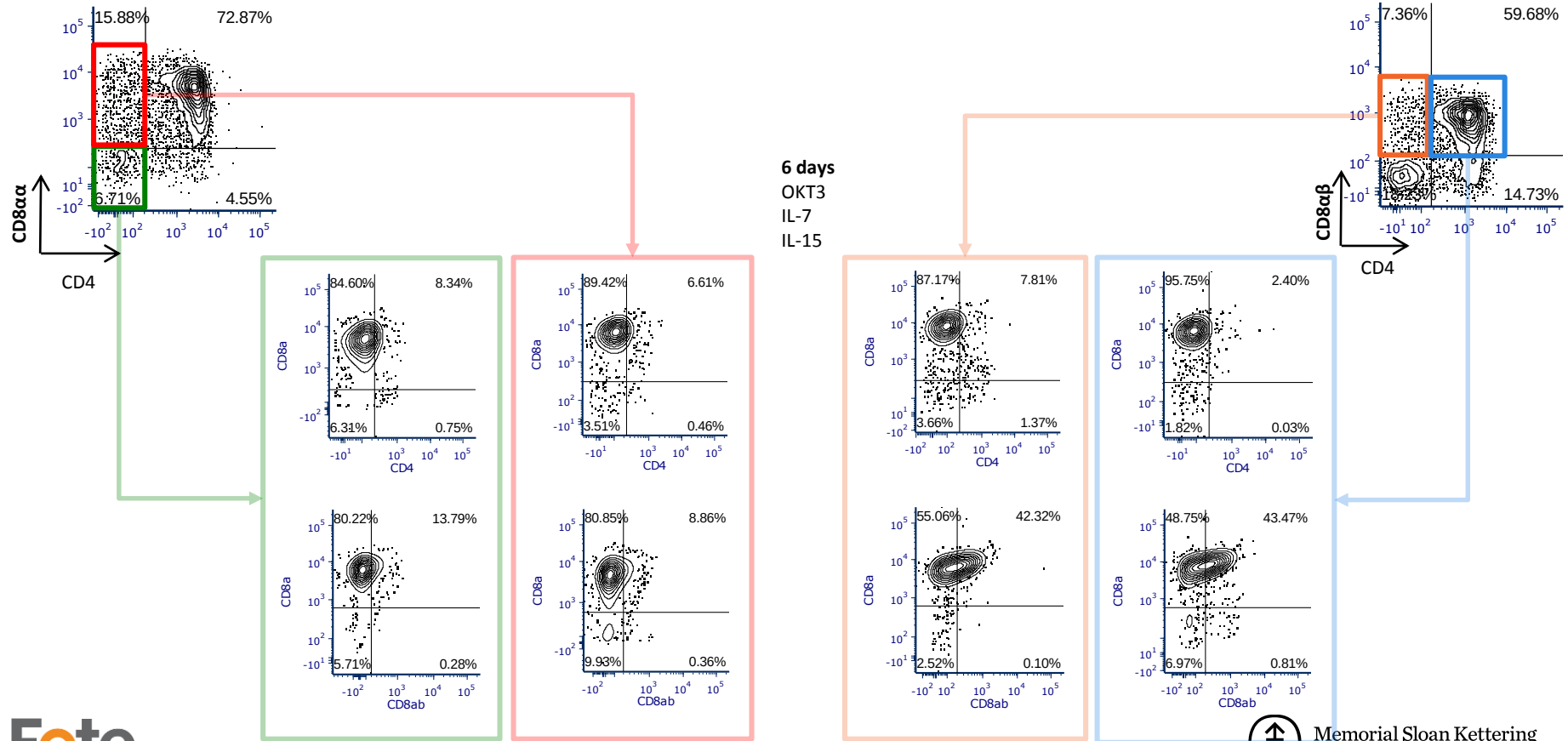
Human T cell development



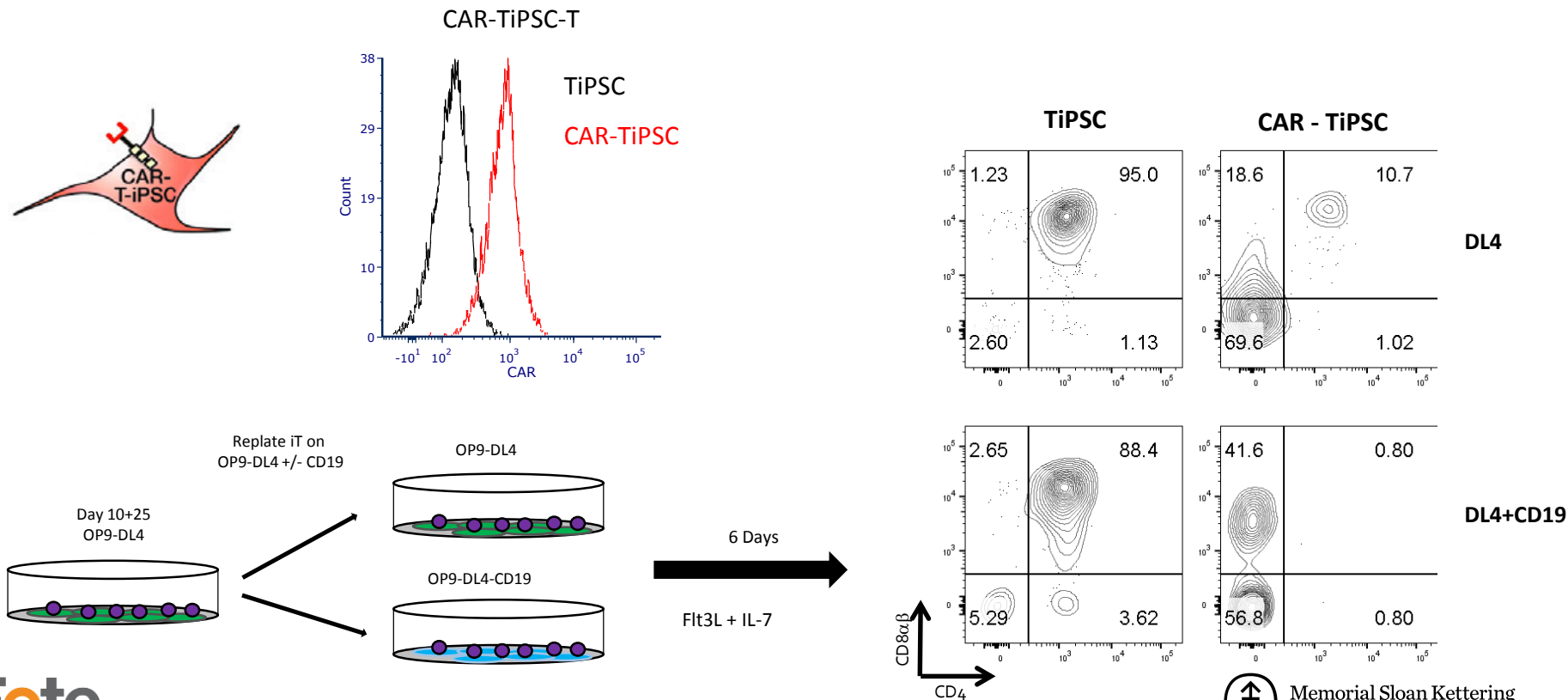
Notch Ligands Influence the Emergence of CD4⁺CD8 $\alpha\beta$ ⁺ DP T cells



CD8 $\alpha\beta$ SPs arise from the DP population

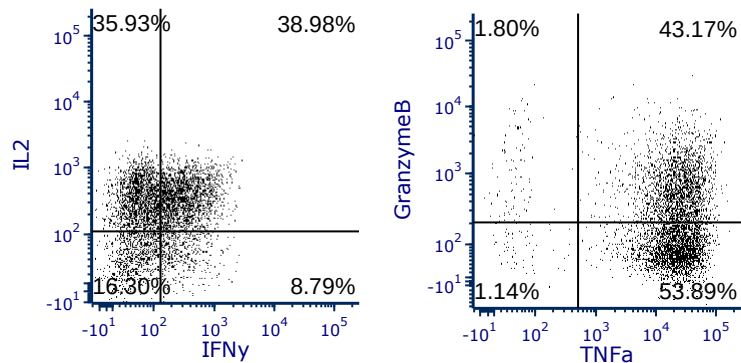


Antigen Stimulation Promotes Maturation to CD8 $\alpha\beta$ CAR T Cells

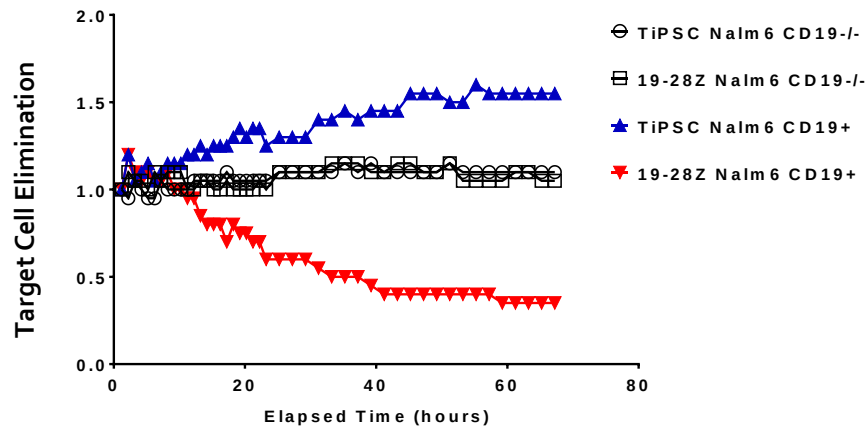


CD8 $\alpha\beta$ SP CAR T Cells secrete cytokines and have antigen specific cytotoxicity

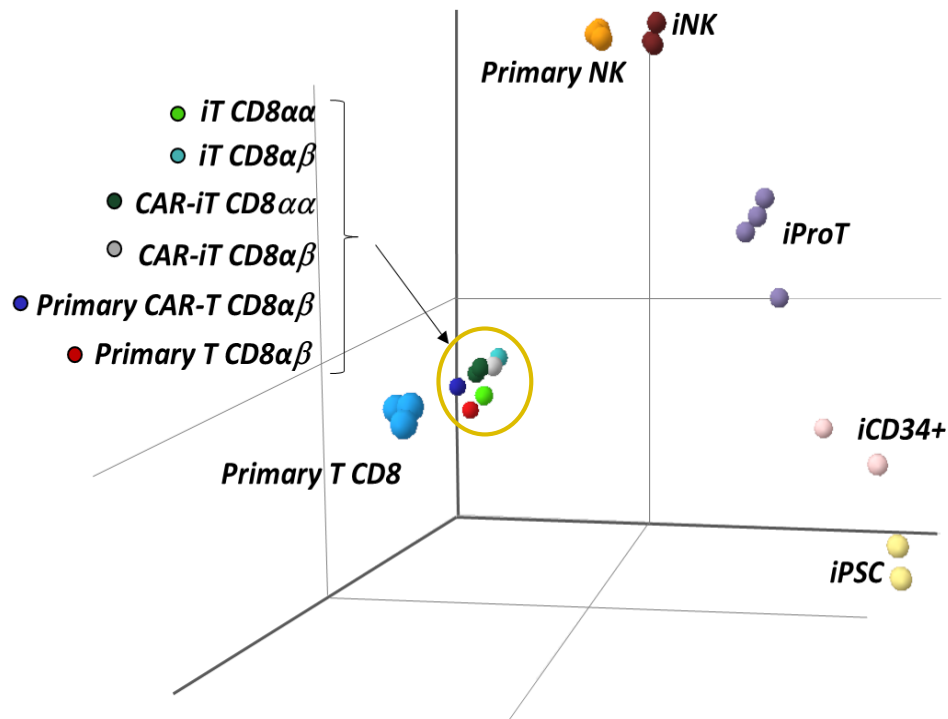
4hr PMA/Ionomycin



CD8 $\alpha\beta$ CAR T Cytotoxicity



Peripheral Blood- and iPSC-Derived CD8 $\alpha\beta$ T & CAR T cells Display Similar Global Gene Expression

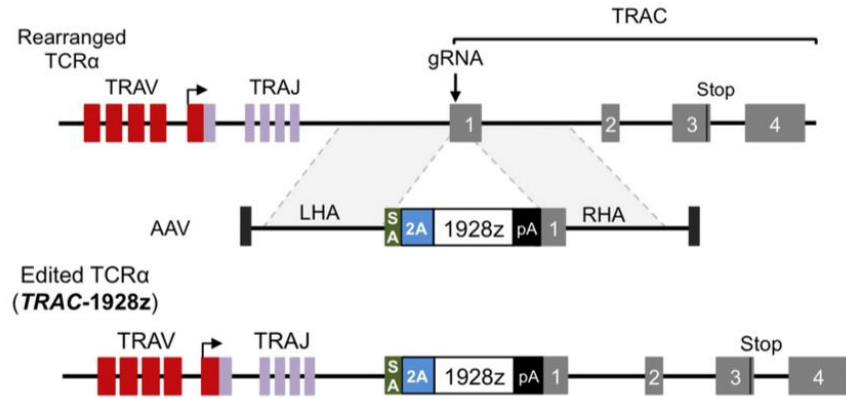


CAR⁺ TCR^{-/-} T cells to Facilitate Off-the-Shelf T cell Therapy

- TiPSC yield CD8 $\alpha\beta$ SP T cells through DLL4 induced differentiation and TCR stimulation
- CAR:antigen interaction can provide positive selection
- CAR stimulation can provide mature CD8 $\alpha\beta$ SP T cells in the absence of a TCR

TRAC-encoded CAR Expression

TCCTAACCTGATCCTCTTGTCACAGATATCCAGAACCCTGACCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAG...
AGGATTGGGACTAGGAGAACA**GGTGTCTATAGGCTTGGGAC**TGGGACGGCACATGGTCGACTCTCTGAGATTAGGTC...
TRAC exon 1



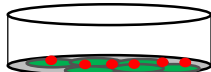
Eyquem et al. Nature, 2017

CAR and Notch support T cell development in absence of the TCR

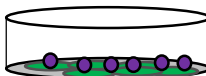
Engineered iPSC Clone
TRAC-encoded CAR



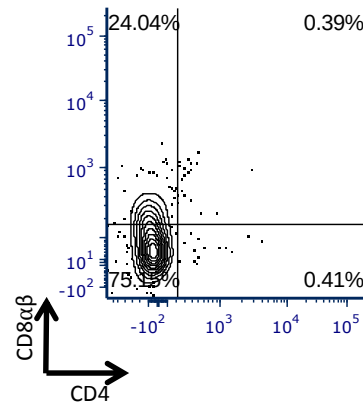
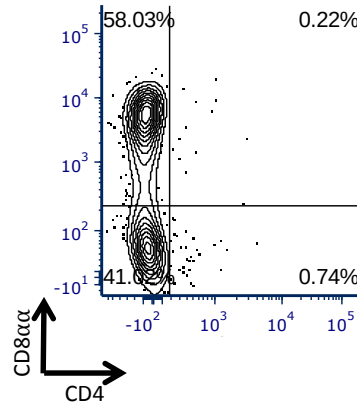
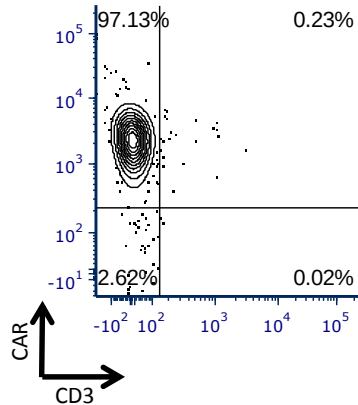
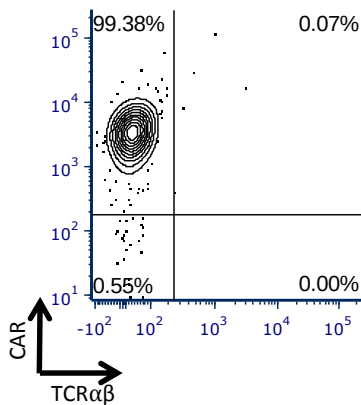
D10
iCD34



Day 10+25
OP9-DL4

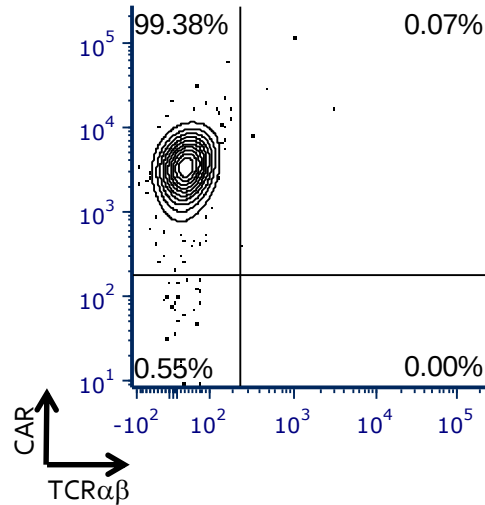


D10+25

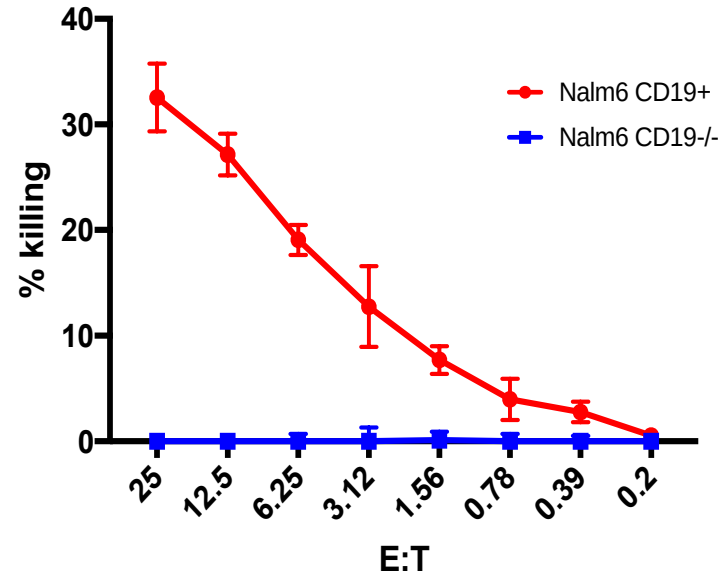


TRAC-encoded TCR^{-/-}CAR⁺ T cells display specific antigen toxicity

TRAC-encoded CAR Expression

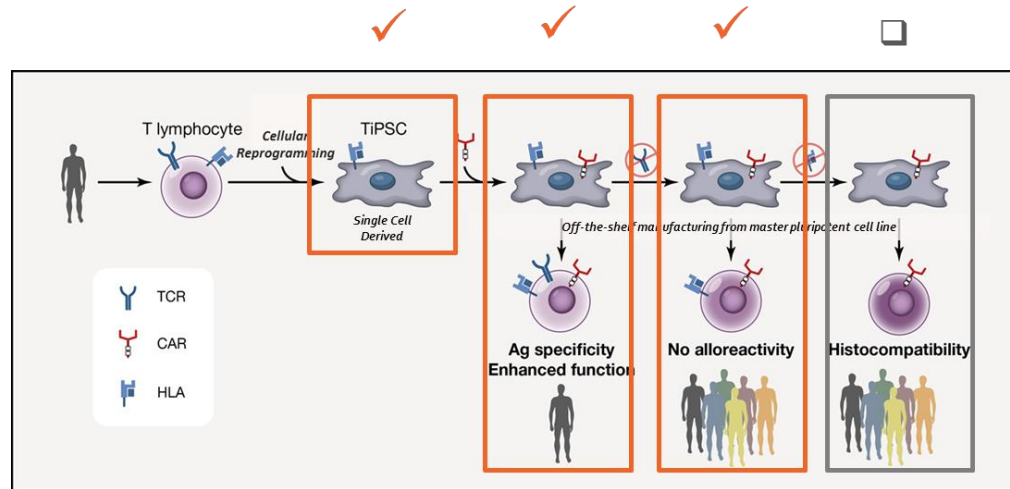


Specific Cytotoxicity



Conclusions

- TiPSC clones yield functional CD8 $\alpha\beta$ T cells
- Antigen specific CD8 $\alpha\beta$ CAR⁺ T cells can be generated from TiPSC
- TCR^{-/-} CD8 $\alpha\beta$ CAR T cells can be generated from TiPSC
- Planning first TiPSC-derived off-the-shelf CAR T cell therapy clinical trial



Adapted from: Themeli, Riviere & Sadelain, *Cell Stem Cells*, 2015

Acknowledgements

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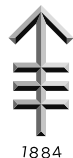
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