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Screening iPSC lines for optimal characteristics of differentiation into immune effector cells for clinical programs

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Introduction

Engineered Induced Pluripotent Stem Cells (iPSCs) are the core foundational technology for Century Therapeutics. In our constant effort to identify iPSC lines with diverse functional activity, we have derived a large panel of clinical-grade peripheral blood mononuclear cell (PBMC)-derived and gamma-delta T cell-derived IPSC lines (PiPSCs and TiPSCs, respectively) from multiple donors. These lines have been screened by multiple criteria to select the top lines for clinical development in our iPSC-derived NK (iNK) and T cell (iT) programs.

Initial screening included in-depth genomic analysis of PiPSC and TiPSC lines in Century's genomic characterization pipeline to eliminate IPSC lines with any unwanted genetic abnormalities. Next, all lines were differentiated to iNK or iT effector cells and characterized throughout the differentiation process. In those studies, 86.5% of PiPSCs and 69.2% of TiPSCs lines were compatible with our protocols, were able to be successfully differentiated, and acquired NK cell and gamma delta T cell phenotypes respectively. Additionally, transcriptomics data was collected throughout the differentiation process, to build multi-omics datasets for correlating gene polymorphisms and transcript variation to function.

To evaluate the function of the differentiated immune effector cells, we measured *in vitro* cytotoxicity (innate and CAR-Mediated) for all clones that met our differentiation thresholds (>90% lineage commitment). Additionally, the post target-engagement persistence of the differentiated effector cells was evaluated.

For this presentation, we will be sharing the genomic characterization of both our PiPSC and TiPSC cell lines. For our process and screening methods to evaluate functional characteristics, we will focus on the evaluation of the TiPSC cell lines.

Donor Info

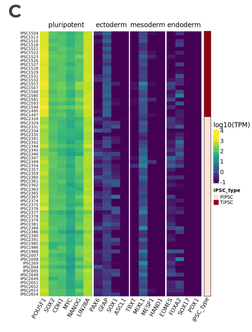
ID	Age	Sex	Source
DON087	27	F	AllCells
DON088	30	F	AllCells
DON089	25	M	AllCells
DON090	41	M	AllCells

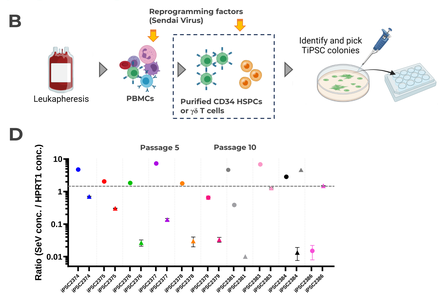
- Fresh Leukopaks Processed in controlled lab to PBMCs and cryopreserved
- All donors tested prior to and post-collection according to US/EU standards for infectious agents
- All donor material collected under informed consent for clinical use

Reprogramming Process

A

Source	Donors	# clones
Whole PBMCs	DON087, DON090	28
Purified CD34+	DON087, DON090	33
Purified γδ T cells	DON087, DON088, DON090	76

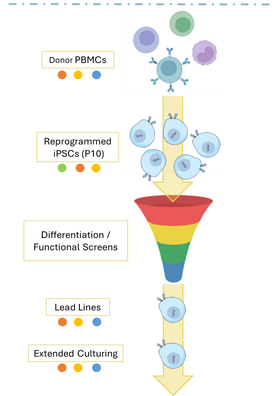




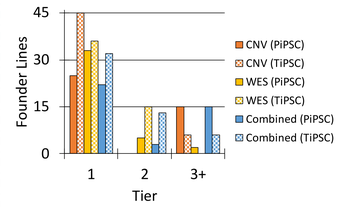
(**A**) iPSCs were generated from multiple cell type origins of clinical donors. (**B**) iPSC reprogramming by using CTS™ CytoTune™-iPS 2.1 Kit. Each single cell-derived colony was cloned and banked at P5 and P10. (**C**) Pluripotent and embryonic tri-lineage marker gene expression of iPSC lines. (**D**) Confirmation of the removal of the Sendai Virus (SeV) gRNA by ddPCR. Most lines had SeV gRNA under detection limit. All steps were processed by using cGMP materials and in the controlled environment.

Figure 3: Genomic Characterization

Donor derived small variants ●, CNVs ●, and structural variants ● are cataloged. iPSCs free of reprograming elements ●, are evaluated for reprograming derived events (● and ●). Functional testing identifies lead lines where structural variant analysis is used to identify karyotype abnormalities and smaller structural variants. Lead lines are cultured for an extended period and their genomic stability is evaluated (●, ●, and ●).



A tiering system is used based on impacts to Cancer Gene Census (CGC) genes.



CNV Tiers

Tier	Short description
1	No long CNV **and** no CNV intersecting CGC gene
2	Long CNV with **no known genes**
3	CNV impacting CGC **level 2** gene
4	CGC **level 1** gene **intron** impacted
5	CGC **level 1** gene **exon** impacted

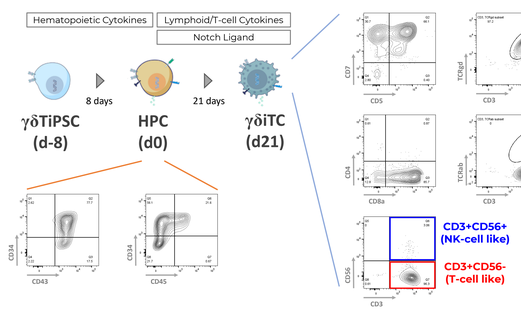
WES Tiers

Tier	Short description
1	No CGC gene impacted
2	1 CGC gene impacted
3	2+ CGC genes impacted

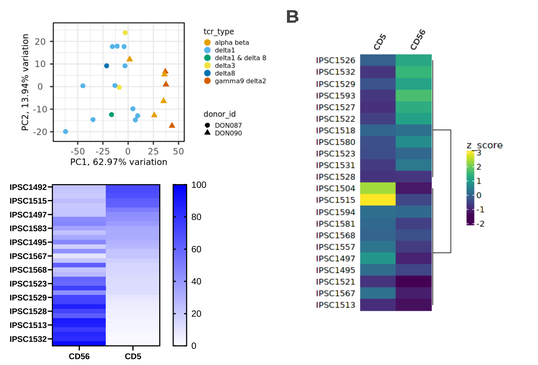
Combined (CNV & WES) Tier

Tier	Short description
1	CNV & WES tier 1
2	CNV tier ≤2 and WES tier ≤2
3	CNV tier ≥3 or WES tier 3

Figure 4: Differentiation Phenotype (& Transcriptome): For TiPSC Lines

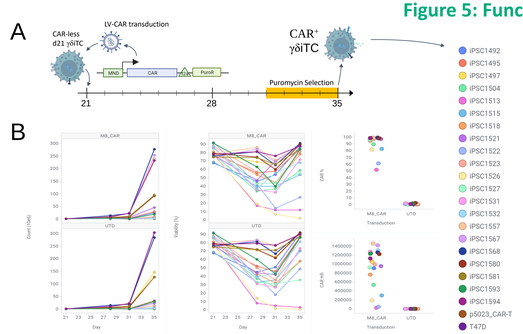


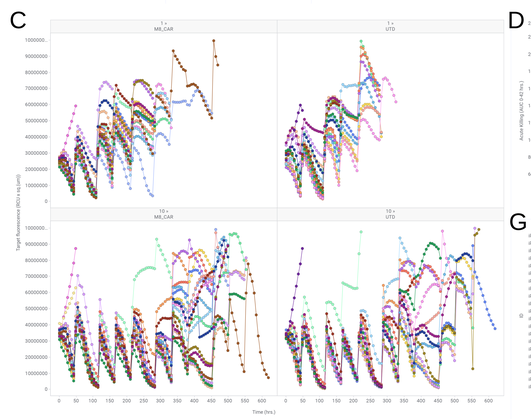
TiPSC lines were differentiated into iT cells by using our current best protocols (CBPs) for hematopoietic progenitor cells (HPCs) and T-lineage (iT) cells. 8 days process of aggregation-mediated method allows us to obtain high pure CD34+/CD43+ HPCs. Downstream 21-day iT cell induction were supported by the Notch ligand. iT cells express typical T-lineage markers such as CD7, CD5, CD3, TCRgd.



(**A**) PCA of day 21 iT cell bulk RNA-seq. (**B**, **C**) CD5 and CD56 are the reliable markers that separate T-like cells (CD5+) from NK-like cells (CD56+) at this phase of in vitro differentiation. Among TiPSC lines, and counter-correlation was observed in CD5 and CD56 expression. Heatmap displays of CD5 and CD56 expression in (**B**) RNA-Seq and (**C**) flow cytometric analyses.

Figure 5: Functional Screening





(**A**) d21 cells were transduced with a CAR targeting Nectin-4 (M8_CAR) or un-transduced (UTD). Cells continued differentiation to d35 with puromycin to select for CAR+ cells as appropriate. A total of 21 founder lines were generated for screening alongside a primary T cell line expressing the same CAR (p5023_CAR-T) and target cells (T47D). (**B**) Expansion (left) and viability (middle) of CAR-transduced (top) and un-transduced (bottom) is shown for the d21-35 differentiation. CAR expression (right) is shown as percentage (top) and mfi (bottom). (**C**) M8_CAR (left) and UTD (right) founders were screened in a serial killing assay against T47D target cells in the presence of 1 (top) or 10 (bottom) ng/ml of IL-15. Acute killing of targets was assessed as AUC for the first 42 hrs (**D**). Serial killing performance was assessed as target escape time (**E**). Persistence was assessed as d7 fold-expansion (**F**). The functional impact of CAR expression over innate killing was assessed as the delta (M8_CAR minus UTD) for acute (**G**) and serial (**H**) killing.

- The T47D target line was susceptible to innate killing (**C**, right)
- Lines with stronger functional impact of CAR for acute killing had lower $\Delta AUC0{:}42_{\text{CAR-UTD}}$ (**G**)
- Lines with stronger functional impact of CAR for serial killing had higher $\Delta EscapeTime_{\text{CAR-UTD}}$ (**H**)

Summary and Conclusions

- PBMC derived (PiPSC) and g/d T Cell derived (TiPSC) IPSC cell lines were successfully reprogrammed from clinical grade donor material
- IPSC lines were analyzed by Century's Genomic Characterization Pipeline and Tiered based on potential genetic liabilities. Multiple lines were identified as being suitable for further clinical development
- PiPSC and TiPSC (data shown) were successfully differentiated to immune effector cells.
- d35 iT cells exhibited diverse phenotypes, yields, and function. Lines with specific attributes may be advanced per the requirements of specific programs.