Creation and characterization of T cell-derived iPS Cell Banks Derived Using Reagents and Workflows Optimized for Cell Therapy Manufacturing

Chad C. MacArthur, Stephanie Switalski, Suman Pradhan, Mark Landon, and Uma Lakshmipathy
Cell Biology, Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92011

ABSTRACT

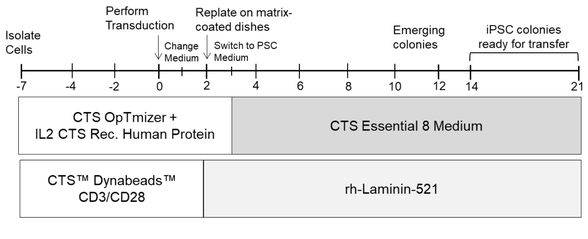
Advances in induced pluripotent stem cell (iPSC) research is moving the field towards clinical and translational applications. iPSC derived from T cells may be particularly useful in immune oncology applications; with proper differentiation T-iPSC could provide an indefinite source of therapeutic T cells. Cells intended for therapeutic applications require robust and consistent iPSC generation workflows that utilize high quality reagents, preferably xeno-free. In addition, there is an imperative need for accurate and high throughput characterization methods that qualify the identity, pluripotency, and genomic integrity of cells. Methods that enhance consistency and thorough characterization will minimize extra effort and costs associated with clones that fail to expand, or do not meet quality standards for downstream use.
To streamline iPSC generation from T cells, and ensure the consistent creation of high quality iPSCs, xeno-free workflows were optimized to minimize the variability in reprogramming efficiency observed between donors. Prior to reprogramming, T cells were phenotyped, and a combination of conditions were tested including hypoxia, matrix, and seeding density. Optimization yielded consistent iPSC generation from potentially difficult to reprogram donor cells, with varying efficiencies. The resulting iPSC lines were also confirmed to be foot-print free, and were further subjected to comprehensive characterization methods to assess the quality and safety profile. Pluripotency and differentiation potential of iPSC clones was confirmed using ScoreCard, a focused qPCR panel, and PluriTest, an array-based global gene expression platform. In addition, qualified clones were thoroughly investigated for genomic stability. Each of the clones tested showed a normal karyotype using both traditional G-banding as well as array-based methods KaryoStat and KaryoStat HD. The adoption of defined xeno-free workflows with qualified reagents, in combination with comprehensive and predictive characterization assays aids in easy transition of early investigational work towards translational and clinical research.

INTRODUCTION

For iPSC to be used as starting material for clinical research, it is important that the iPSC are created using relevant workflows which includes xeno-free workflows. We recently reported CTS™ CytoTune™-iPS 2.1, the first commercial reprogramming kit specifically developed for clinical and translational research. Given these changes, the new reprogramming kit was used to reprogram blood-derived cells in xeno-free workflows, and both reprogramming efficiency and quality of the resulting iPSC clones were examined. T-cell reprogramming was further optimized for the consistent derivation of iPSC.
iPSC generated from T-cells under completely xeno-free conditions were further characterized for pluripotency and genetic integrity using comprehensive characterization tools that relies on reference standards and hence serves as reliable qualification tools.

MATERIALS AND METHODS

All materials were from Thermo Fisher Scientific unless otherwise specified. T-cells were isolated from PBMCs using either negative selection (Dynabeads™ Untouched™ Human T Cells Kit) or positive selection (Dynabeads Human T-Expander CD3/CD28) , after which they were reprogrammed under xeno-free conditions according to the workflow below:



RESULTS

Figure 1. Optimization of T-cell Reprogramming with CTS CytoTune 2.1

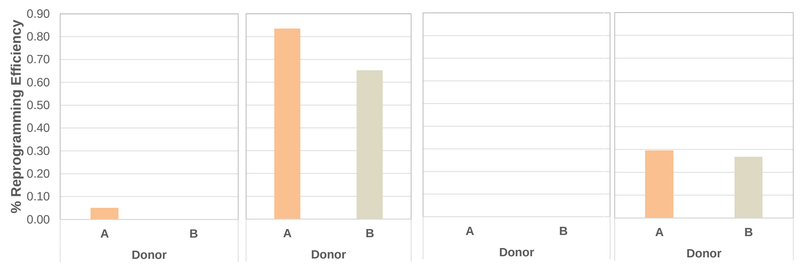
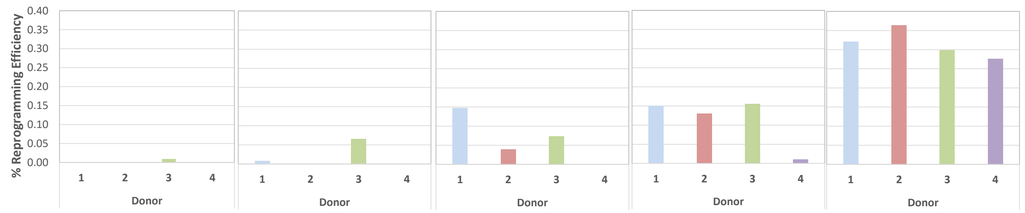


Table 1. Donor Information

Donor	Blood Type	Gender	Age	Weight (lbs)	Height (in.)
A	O+	M	27	212	72
B	AB-	M	37	280	75
1	A+	M	39	241	71
2	A+	F	26	151	63
3	O+	F	50	142	68
4	A+	M	44	No info	No info

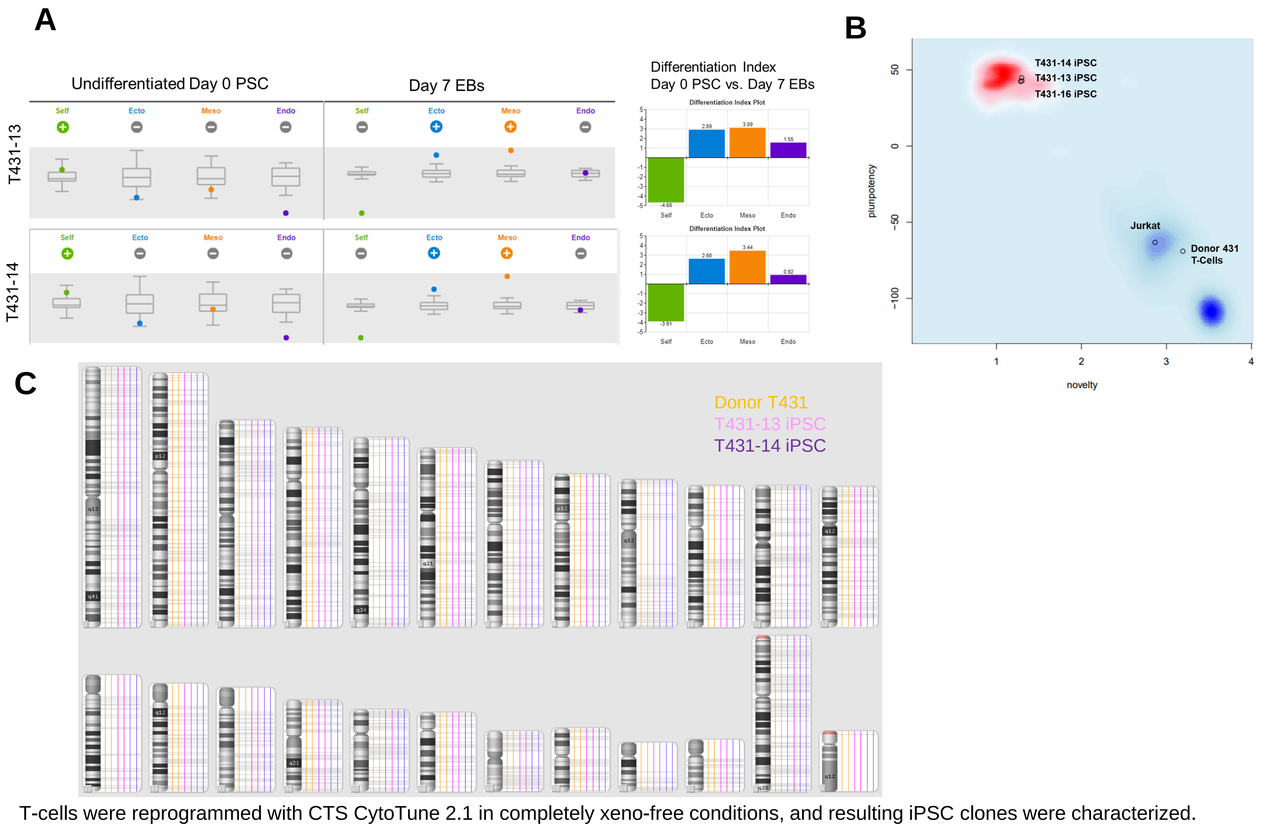
Reprogramming Kit	CytoTune 2.0		CTS CytoTune 2.1	
PSC Matrix	VTN-N	Laminin-521	VTN-N	Laminin-521



Cell Plating Density	7.5e3 cells/cm^2	1.0e4 cells/cm^2	1.0e4 cells/cm^2	1.0e4 cells/cm^2	1.0e4 cells/cm^2
PSC Matrix	VTN-N	VTN-N	VTN-N	Laminin-521	Laminin-521
Oxygen %	Normal	Normal	Hypoxia (5%)	Hypoxia (5%)	Hypoxia (5%)

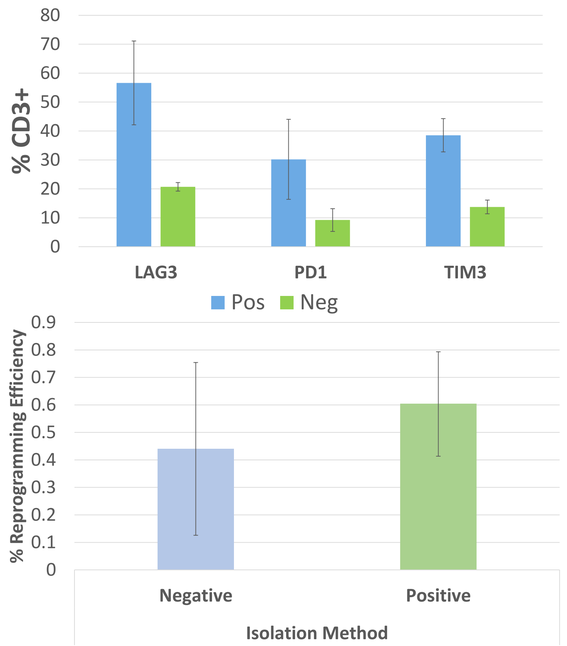
T-cells were reprogrammed according to the workflow in the lower left. T-cells from two different donors with either CytoTune 2.0 or CTS CytoTune 2.1 and plated onto either VTN-N or Laminin-521 **(A)**. T-cells from four different donors were reprogrammed with CTS CytoTune 2.1 using various protocol optimizations including oxygen concentration: normal (20% O_2) or hypoxic (5% O_2); cell culture matrix for plating step on Day 2: recombinant human vitronectin (VTN-N) or recombinant human laminin-521; and cell plating density: 7.5e3 or 1.0 e3 cells per cm^2 **(B)**. At 16 days after transduction, cells were stained for AP, and reprogramming efficiency was calculated as the number of AP positive colonies, relative to the number of cells replated.

Figure 2. T-cell derived iPSCs generated in a xeno-free workflow have a normal karyotype, expression of self-renewal markers, and display functional pluripotency



T-cells were reprogrammed with CTS CytoTune 2.1 in completely xeno-free conditions, and resulting iPSC clones were characterized. Trilineage differentiation potential was assessed using hPSC TaqMan™ ScoreCard™, a focused gene expression array that confirms gene expression profiles by comparing data against a reference dataset **(A)**. Results indicate both iPSC clones have trilineage differentiation potential. iPSC were also analyzed using PluriTest™, an algorithm that compares profiles to an extensive reference set. Results confirm high pluripotency and low novelty score for two iPSC clones **(B)**. Parental T-cells and two derived iPSC clones were analyzed for genomic stability using KaryoStat™, which revealed no genetic abnormalities at a resolution of 1-2 Mb **(C)**.

Figure 3. Comparison of T-cell Isolation Method on Phenotype and Reprogramming Efficiency



On the day of transduction, T-cells from different donors, which had been isolated from PBMC with either negative or positive selection, were stained with antibodies for the exhaustion markers LAG3, PD1, and TIM3 **(A)**. These cells were reprogrammed, and analyzed for reprogramming efficiency. No significant difference in reprogramming efficiency was observed between the two isolation methods **(B)**.

CONCLUSIONS

- T-Cell reprogramming was optimized by using LN521 as the cell culture matrix, hypoxic culture conditions, and an increased seeding density.
- Though T-cells isolated using positive selection displayed a more exhausted phenotype, no significant difference in reprogramming efficiency was observed between the two methods.
- iPSC generated from T-cells in xeno-free conditions with CTS CytoTune 2.1 were pluripotent and displayed a normal karyotype as determined using comprehensive characterization methods
  - PluriTest identifies pluripotent cells by comparing global gene expression profile with an extensive reference set of over 450 cell types including 223 hESC and 42 iPSC lines among others.
  - hPSC Scorecard Panel assesses pluripotency and trilineage differentiation potential using real-time qPCR assays and intuitive data analysis software.
  - KaryoStat assay enables the detection of chromosomal gain and loss, aneuploidies, mosaic events, loss of heterozygosity and copy neutral events

TRADEMARKS/LICENSING