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Molecular Characterization of T Cell Receptor and HLA in T cell Derived iPSCs

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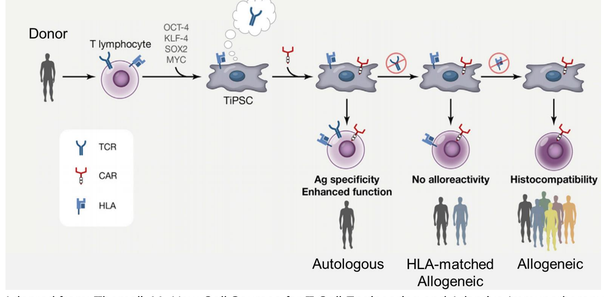
ABSTRACT

As the immunotherapy market continues to grow, significant progress has been made in treating diseases and cancer by leveraging the potential of immune cells, particularly T cells. Autologous CAR T cell therapy has shown high levels of complete remission in treatment of blood-based cancers, but the personalized medicine is hard to scale. Off-the-self, allogeneic CAR T treatments derived from donor T cells are being explored as a potential to address issues with the autologous treatments. CAR T cell therapies could be advanced through the use of induced pluripotent stem cells (iPSC). iPSCs derived from T cells offer advantages over traditional somatic cell iPSCs by conservation of T cell receptor (TCR) through reprogramming. Additionally, the ability to engineer chimeric antigen receptors (CAR) into iPSCs, which can then be expanded and differentiated back to T cells, provides a cost-effective method to generate large number of allogeneic CAR-T cells. The therapeutic success of such allogeneic T cell therapies can be further increased with high-resolution HLA typing to reduce the risk of any post-transplant complications.

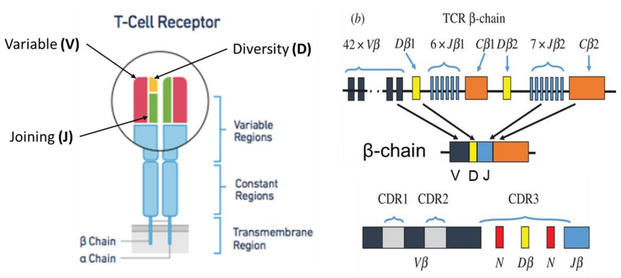
In this study, qPCR and next generation sequencing tools are utilized for the comparison and thorough characterization of TCR and HLA in iPSCs derived from Fibroblast, PBMC and T cells. The parental lines and iPSC clones that were subjected to comprehensive characterization to confirm pluripotency were used for downstream molecular characterization. Since reprogramming is carried out from a heterogeneous population of T cells, it is important to understand the parental cell type. Next Generation Sequencing-based Immune Repertoire was used to generate high-throughput sequencing data of TCRβ populations. These assays revealed the diversity, or the number of unique TCR sequences, present in the starting T cells and the resulting iPSC clones. Further, HLA typing was carried out on the parental cells and iPSC clones to track cell lineages and qualify the cells for use in immune therapy applications. The development a robust workflow to characterize the starting immune cell population and the iPSC lines will benefit the development of translational therapies.

INTRODUCTION



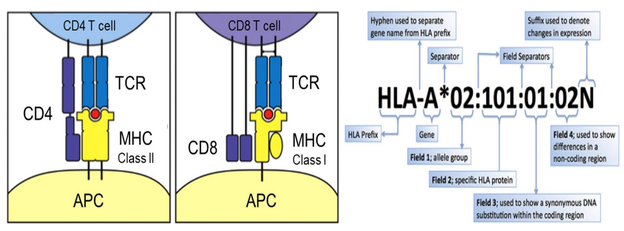
Adapted from: Themeli, M. *New Cell Sources for T Cell Engineering and Adoptive Immunotherapy.*

Induced Pluripotent Stem Cells created from T cells (TiPSCs) have the potential to move the field from autologous to allogeneic therapies. TCRs are created through permanent genomic rearrangements that are unaffected by reprogramming. The unique CDR3 sequence can act like a barcode for any TCR and is often the focus of TCR sequencing. Immune Repertoire refers to the total T cell population.



Adapted from: Murphy, K. *Janeway's immunobiology*.

HLA typing is essential for all allogeneic cell therapies. Human Leukocyte Antigens (HLA) are responsible for presenting antigens to T cells for recognition as self or foreign. There are 2 classes of proteins and 10 total HLA genes of interest. Low resolution HLA typing provides the allele groups and is easily available though methods like qPCR.



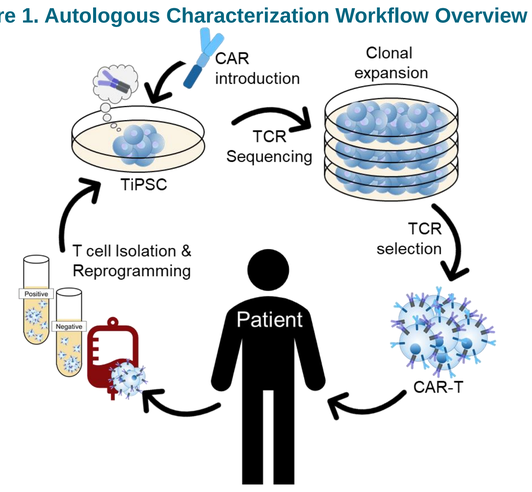
Adapted from: Murphy, K. *Janeway's immunobiology*.
Choo, S. *The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications*.

MATERIALS AND METHODS

All materials were from Thermo Fisher Scientific unless otherwise stated . Procedures were carried out as per manufacturer's instructions.

RESULTS

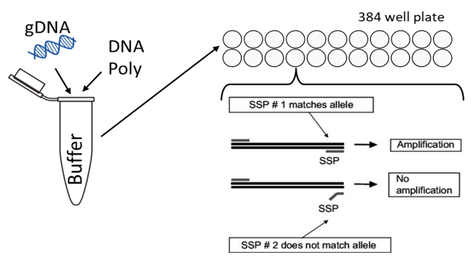
Figure 1. Autologous Characterization Workflow Overview



Donor T cells were isolated by either Positive or Negative isolation prior to reprogramming with CTS™ CytoTune™-iPS 2.1 Sendai Reprogramming Kit. Positive selection binds T cells to magnetic beads to remove them from solution. Negative selection binds all other cell types, leaving only T cells in suspension. All donors under both methods showed high purity isolations. These T cells were reprogrammed and characterized to show pluripotency, as well as immune repertoire and HLA typing.

Figure 2. HLA Typing

A



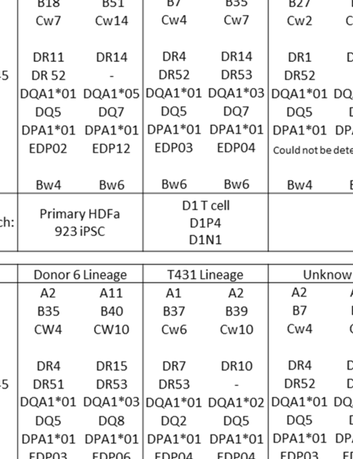
B

	HDFa Lineage		Donor 1 Lineage		Donor 3	
HLA-A	A2	A31	A2	A11	A2	A68
HLA-B	B18	B51	B7	B35	B27	B60
HLA-C	Cw7	Cw14	Cw4	Cw7	Cw2	Cw10
HLA-DRB1	DR11	DR14	DR4	DR14	DR1	DR13
HLA-DRB345	DR 52	-	DR52	DR53	DR52	-
HLA-DQA1	DQA1*01	DQA1*05	DQA1*01	DQA1*03	DQA1*01	DQA1*01
HLA-DQB1	DQ5	DQ7	DQ5	DQ7	DQ5	DQ6
HLA-DPA1	DPA1*01	DPA1*01	DPA1*01	DPA1*01	DPA1*01	DPA1*01
HLA-DPB1	EDP02	EDP12	EDP03	EDP04	Could not be determined	
Bw	Bw4	Bw6	Bw6	Bw6	Bw4	Bw6
10/10 Match:	Primary HDFa 923 iPSC		D1 T cell D1P4 D1N1			

	Donor 6 Lineage		T431 Lineage		Unknown	
HLA-A	A2	A11	A1	A2	A2	A11
HLA-B	B35	B40	B37	B39	B7	B35
HLA-C	CW4	CW10	Cw6	Cw10	Cw4	Cw7
HLA-DRB1	DR4	DR15	DR7	DR10	DR4	DR14
HLA-DRB345	DR51	DR53	DR53	-	DR52	DR53
HLA-DQA1	DQA1*01	DQA1*03	DQA1*01	DQA1*02	DQA1*01	DQA1*03
HLA-DQB1	DQ5	DQ8	DQ2	DQ5	DQ5	DQ7
HLA-DPA1	DPA1*01	DPA1*01	DPA1*01	DPA1*01	DPA1*01	DPA1*01
HLA-DPB1	EDP03	EDP06	EDP04	EDP04	EDP03	EDP04
Bw	Bw6	Bw6	Bw4	Bw6	Bw6	Bw6
10/10 Match:	D6 T cell D6N2		T431-13 T431-14 T431-16		Sample "D3N1" identified as Donor 1	

a) LinkSēq™ HLA ABCDRDQDP SABR 384 kit is a qPCR-based HLA Typing kit that determines HLA type to a low resolution.
b) All donor and TiPSC samples had a perfect 10/10 HLA match to each other. There was a clear difference between samples of different sources. Prior to typing, TiPSC sample D3N1 was discovered to not be a Donor 3 clone. Using this method, the Unknown sample was identified as Donor 1 lineage.

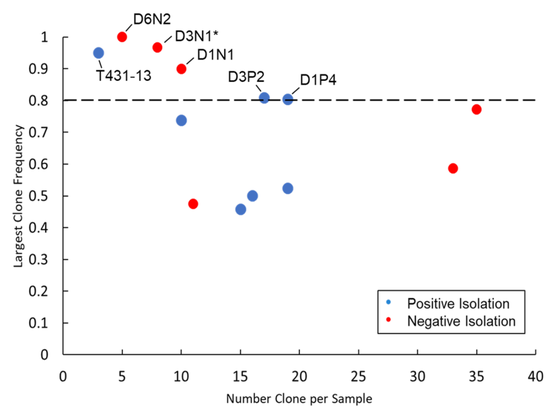
Figure 3. Immune Repertoire



The Oncomine TCR Beta-LR Assay uses a TCRβ primer panel to amplify the TCRβ loci prior to Next Generation Sequencing (NGS). This assay was performed using gDNA isolated from TiPSC colonies.

Figure 4. TCR Diversity in TiPSC Colonies

A



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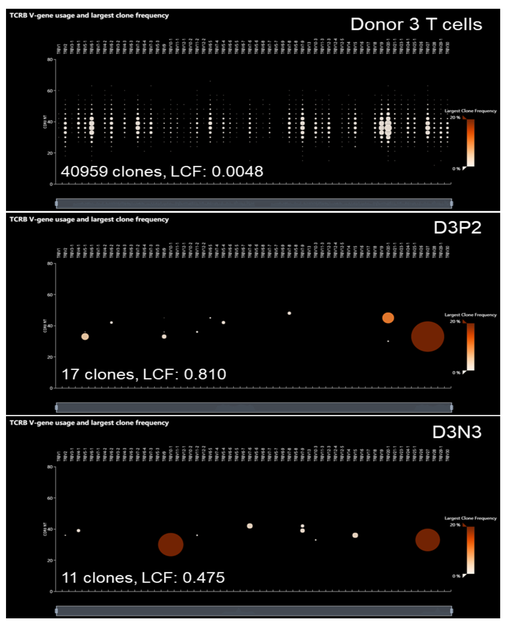


a) Graph showing the number of unique TCR sequences and the TCR sequence with the most number of reads (Largest Clone Frequency, LCF) for each TiPSC clone. Clones with LCF ≥ 80% were selected for further characterization methods. No iPSC clone showed only having a single TCR sequence.
b) Spectratyping Plots generated by IonReporter displaying the diversity within each sample. The T cell donor shows high diversity, while the TiPSC samples show lower levels of diversity.

Figure 5. T cell lineage in TiPSC Colonies

A

Sample	Number of Clones	Largest Clone Frequency	%Productive Reads	Number Productive Reads
D1P2	16	0.501	16	132234
D1P3	19	0.525	53	467144
D1P4	19	0.804	68	697652
D3P1	15	0.459	52	362828
D3P2	17	0.810	89	831375
D5P5	10	0.739	52	388925
D1N1	10	0.900	87	742157
D1N3	35	0.772	86	779622
D1N5	33	0.586	77	874861
D3N1*	8	0.967	86	795594
D3N3	11	0.475	81	594308
D6N2	5	1.000	55	401067
T431-13	3	0.951	35	276208
T431-14	-	-	0	10
T431-16	-	-	0	1221
D1P1	-	-	0	0
D5P2	-	-	25	167
D5P3	-	-	9	260

B

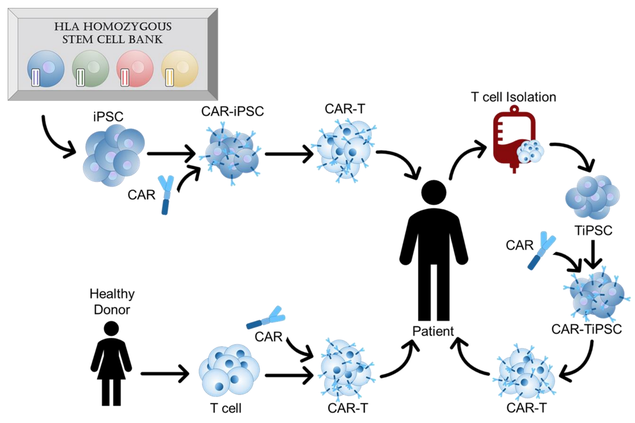
Sample	Number of Clones	Largest Clone Frequency	%Productive Reads	Number of Productive Reads
Donor 1	48821	0.00777	70.61	1908022
Donor 3	40959	0.00247	67.82	1687972
Donor 5	19971	0.01984	68.56	2473244
Donor 6	24177	0.09254	68.03	2864301

C

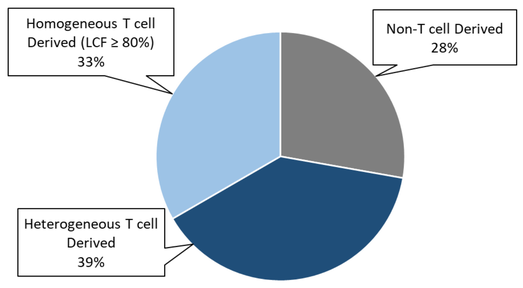
	Frequency	CDR3 NT	
T431-13 Round 1	0.96400037	AGTGCTAGTAGGGGTTACTCCTACGAGCAGTAC	A
	0.02218007	GCCAGCAGCTTAGCGCGGGATGGGCACGCTGAAGCTTTC	B
	0.00782366	GCCAGCAGCTTGGGAGGGGTTAATGAAAAACTGTTT	C
	0.00556308	GCCAGCAGCTCCCGTGGGGGTGACACTGAAGCTTTC	
	0.0004221	GCCAGCAGTTACCCGGGCAGCTCCTACAATGAGCAGTTC	
	0.00001072	GCCAGCAGCTGGGGGGCTCGCGGGGGCGGGGAGCTGTTT	
Round 2	0.95229928	AGTGCTAGTAGGGGTTACTCCTACGAGCAGTAC	A
	0.03082089	GCCAGCAGCTTGGGAGGGGTTAATGAAAAACTGTTT	C
	0.01687983	GCCAGCAGCTTAGCGCGGGATGGGCACGCTGAAGCTTTC	B

a) Table of TCRβ sequencing results from TiPSCs. 5 Clones did not show any productive αβ TCR rearrangements. %Productive reads and the number of productive TCR reads are important QC metrics. Highlighted samples contain LCF ≥ 80%.
b) Table of TCRβ sequencing results from Donor T cells. Donors have a high number of clones and relatively low largest clone frequencies.
c) Two rounds of sequencing were performed on T431-13 from different cultures. Although different sequences were present, the top 3 sequences remained the same.

CONCLUSIONS



- Introducing T cell derived iPSCs offers solutions to many of the current issues of CAR T cell therapies, such as T cell exhaustion and low CAR transduction efficiencies. TiPSCs also have the potential to create allogeneic therapies without the issues of lot-to-lot variation.
- TCR sequencing and HLA typing are necessary for autologous and allogeneic cell therapies. TCR sequencing requires methods like NGS or other high throughput sequencing methods. HLA typing may either be done by qPCR or NGS, however, qPCR is much faster and more cost-effective to perform.
- Immediately after T cell reprogramming, TCR sequencing should be the first method of characterization to confirm T cell lineage in TiPSC colonies.



  - Only 33% of TiPSC samples were considered homogeneous, containing a Largest Clone Frequency ≥ 80%.
  - 28% of samples did not have a recombined TCR sequence, indicating that the lines did not contain αβ parental cells.
- HLA typing is a useful alternative method of cell lineage tracking. The HLA type for every individual is unique, even at low resolution of typing. Many of the TiPSC clones contained unique HLA alleles that only occurred in their lineage.
  - Low resolution typing by easy-to-use qPCR kit is sensitive enough to identify mislabeled samples.
- There are multiple possible explanations for TiPSC samples containing multiple TCR sequences. One might be due to human error during manual picking. Another possibility is multiple reprogrammed cells form colonies which then merge to create heterogeneous colonies.
  - All possibilities highlight the need for single cell reprogramming and better clone picking techniques.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

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