Variability in the Development of Cellular Therapies: A Case Study on Manufacturing CD19- and CD22-CAR T Cells for the Treatment of Acute Lymphocytic Leukemia

October 18, 2018

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NIH Center for Cellular Engineering

Products Manufactured

Cancer Immunotherapy

- Dendritic cells
- NK cells
- Cytokine treated monocytes and lymphocytes
- CAR T cells

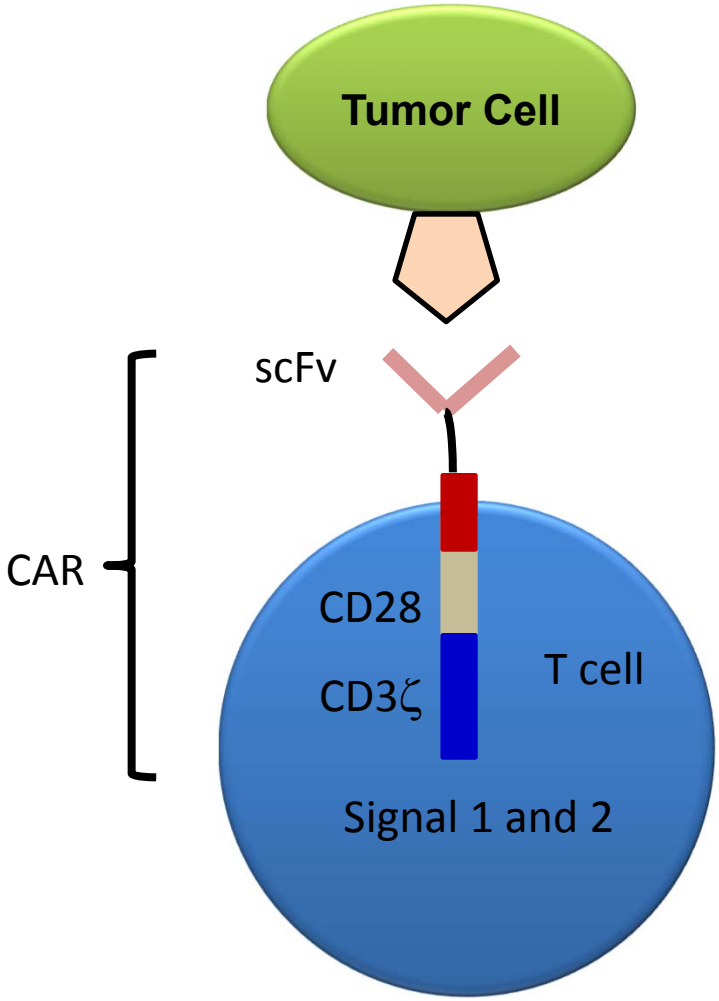
Regenerative Medicine

- Induced pluripotent stem cells (iPS) cells
- Mesenchymal Stromal Cells (MSCs)

Gene Therapy

- Chronic Granulomatous Disease (CGD)
- Severe Combined Immune Deficiency (SCID)

Chimeric Antigen Receptor (CAR) T Cells



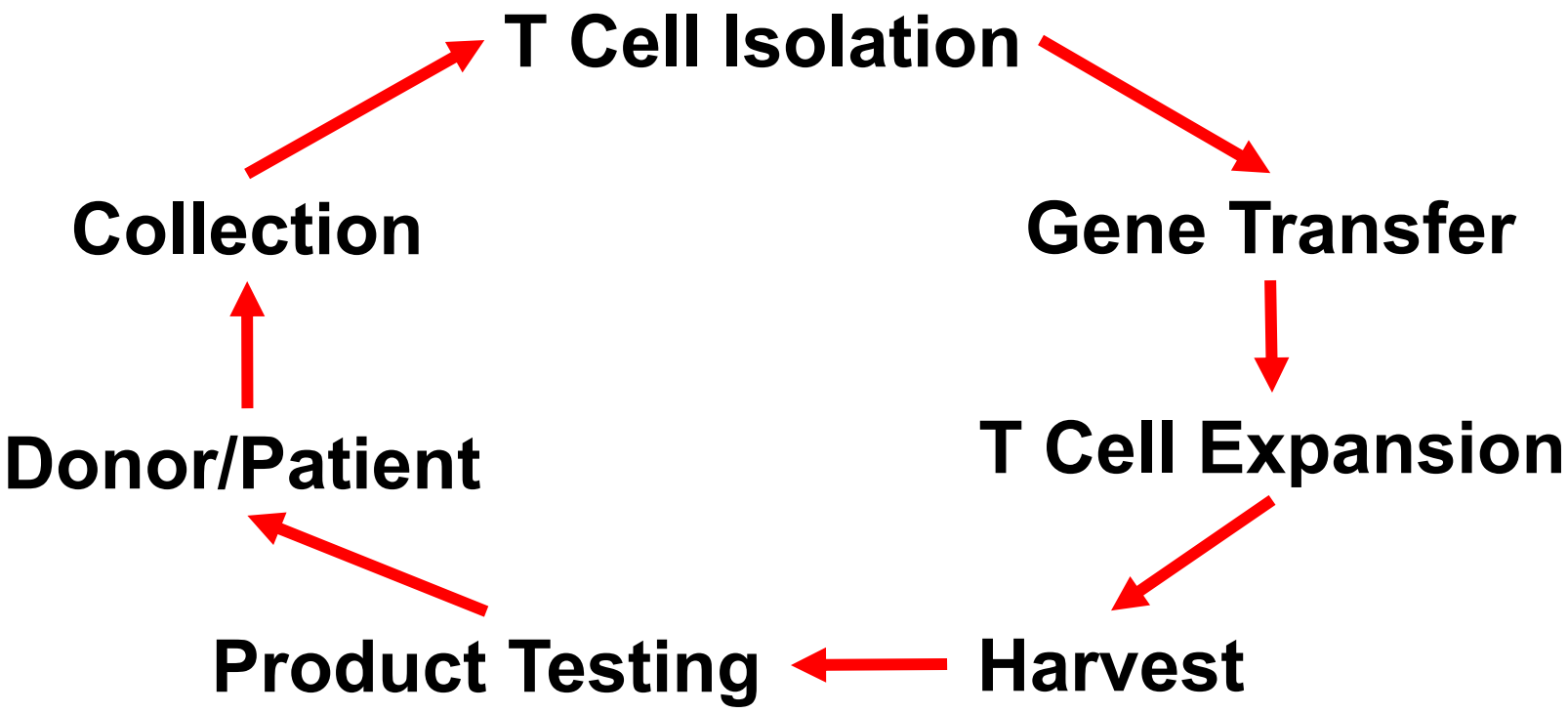
Key Components

- Antigen-binding (scFv)
- CD3-zeta → Signal 1
- Costimulatory → Signal 2
  - (CD28, CD137 (41BB))

Advantages Over T-Cell Receptor Target Recognition

- Specific for a surface antigen
- Free of MHC restriction
- Signals for full activation are self-contained

CAR T Cell Manufacturing



Manufacturing Challenges

- T cell expansion
- T cell transduction

Composition of Peripheral Blood Mononuclear Cell (PBMC) Concentrates

PBMC Concentrates

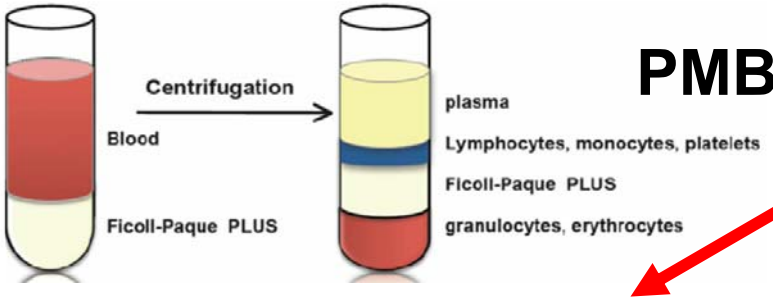
- Lymphocytes
- Monocytes
- Granulocytes
- Natural Killer cells
- Red blood cells
- Platelets

Composition of PBMC Concentrates from Healthy subjects (n = 41)

	Mean ± 1SD	Range
Lymphocyte (%)	68.4 ± 9.8	42 to 83
Monocytes (%)	18.8 ± 6.1	1 to 32
Granulocytes (%)	9.54 ± 10.1	0 to 42

T Cell Isolation and Expansion

Apheresis Collection



PMBC Concentrate

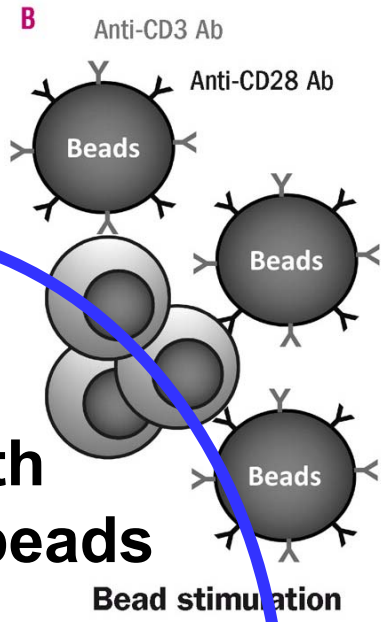
Density Gradient Separation

Stimulation with anti-CD3 + IL-2

Transduction

Expansion

Selection with anti-CD3/CD28 beads



anti-CD3/CD28 bead stimulation + IL-2

Transduction

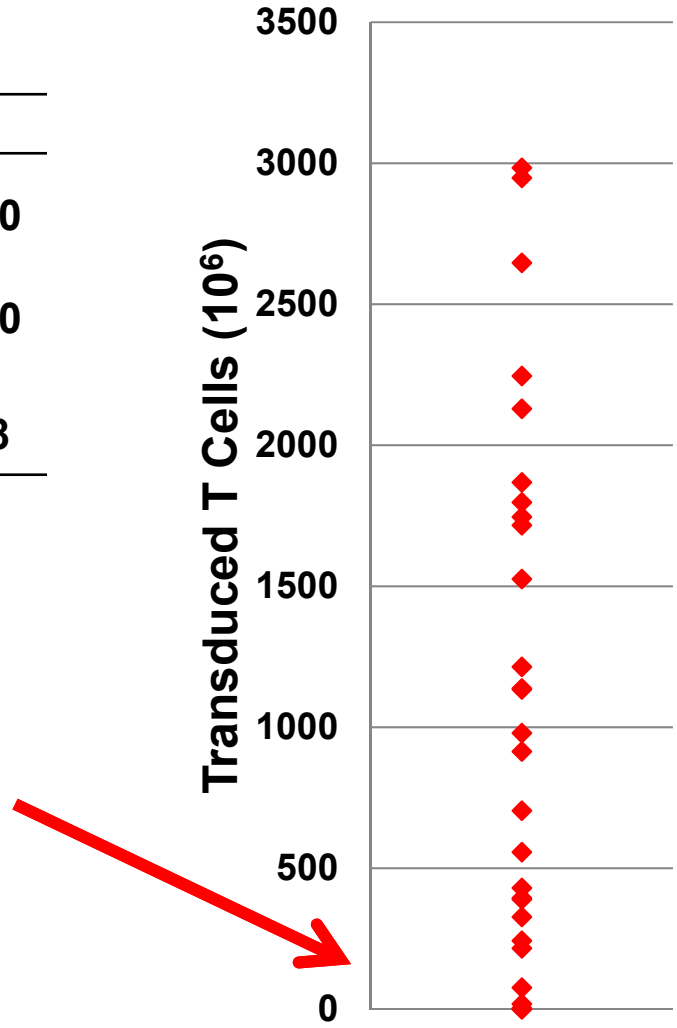
Expansion

First 28 CD19-CAR T Cell Products: Transduced T Cell Yield

	Cells in Final Product	
	Mean ± 1SD	Range
T Cells ($x10^6$)	1,362 ± 1167	4.61 to 3,800
Transduced T Cells ($x10^6$)	1,084 ± 920	2.36 to 2,990
Transduced T Cells (%)	68.3 ± 23.9	18.3 to 96.8

Four Products Failed to Meet Dose

- Patient 2 (3.9 $x10^6$ transduced T cells)
- Patient 5 (19.4$x10^6$ transduced T cells)
- Patient 22 (0.0 transduced T cells)
- Patient 26 (2.4$x10^6$ transduced T cells)



Comparison of PBMC Concentrates that Resulted High and Low CD19-CAR T Cell Yields

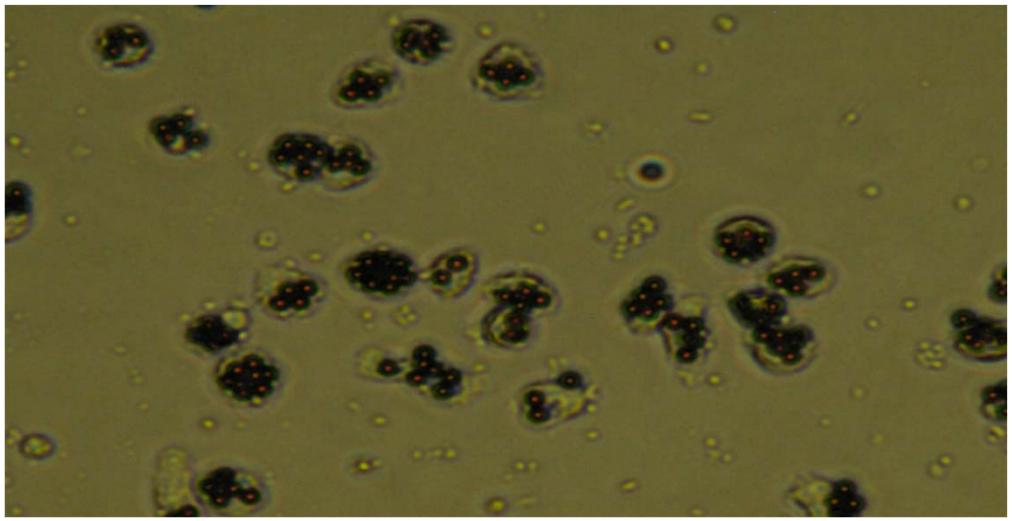
	Met Dose Requirements (n=24)	Did Not Meet Dose Requirements (n=4)	p
Lymphocytes	75.3 ± 14.1%	42.3 ± 8.4%	0.00018
Monocytes	15.3 ± 10.8%	39.8 ± 12.9%	0.0014
Granulocytes	6.9 ± 8.6%	16.3 ± 12.2%	0.083

Stroncek DF, Ren J, Lee DW et al. Cytotherapy. 2016 Jul;18(7):893-901.

Mechanism of Myeloid Cell Inhibition of T Cell Expansion

Monocytes and/or granulocytes bind to anti-CD3/CD28 beads and are carried into the T cell culture

- **Myeloid cells release factors that inhibit expansion**
- **Myeloid cells prevent T cell binding to anti-CD3/CD28 beads**

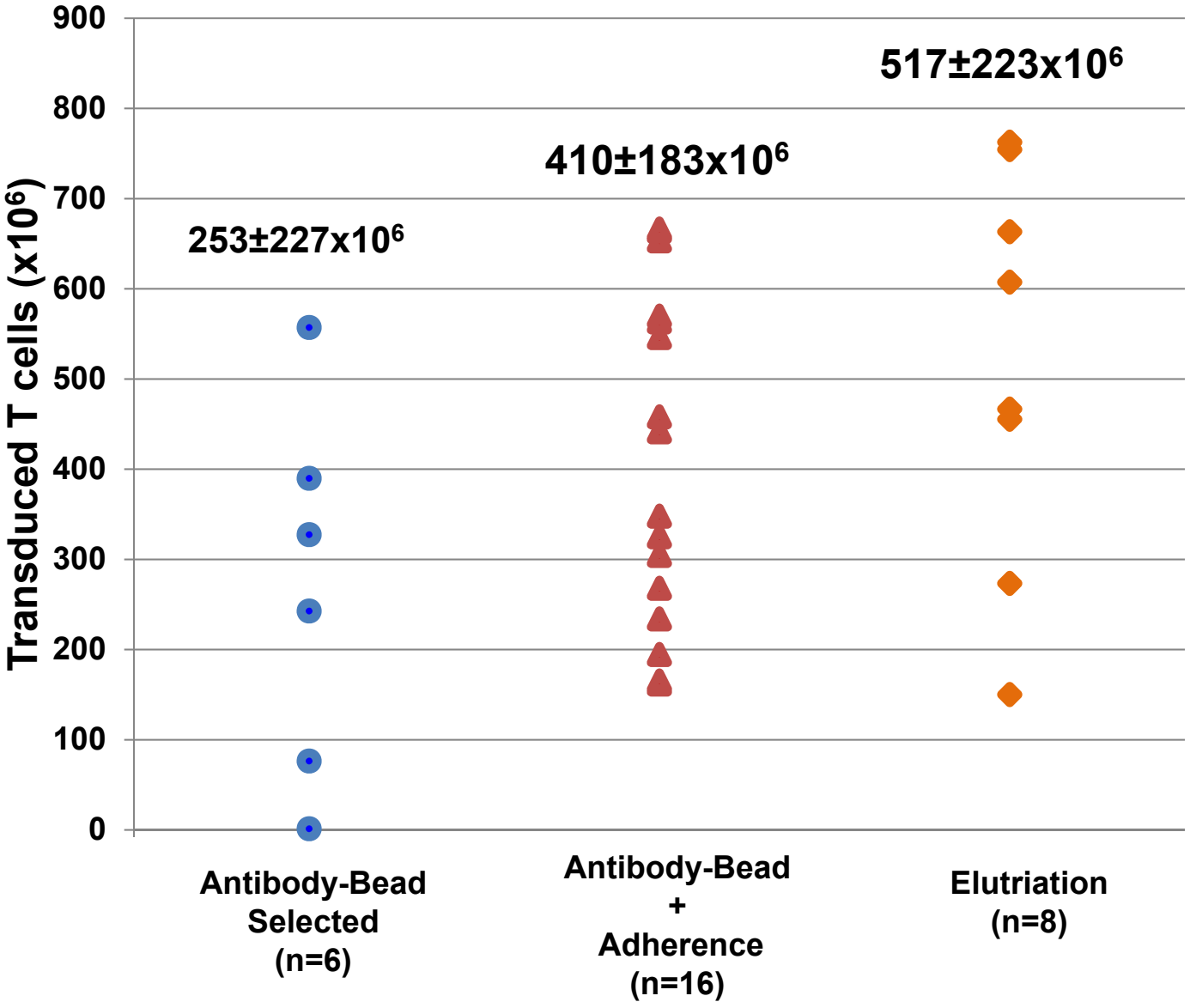


Better T Cell Isolation

- Plastic adherence to remove monocytes
- Counter-flow elutriation
- Antibody selection: antibodies and paramagnetic particles

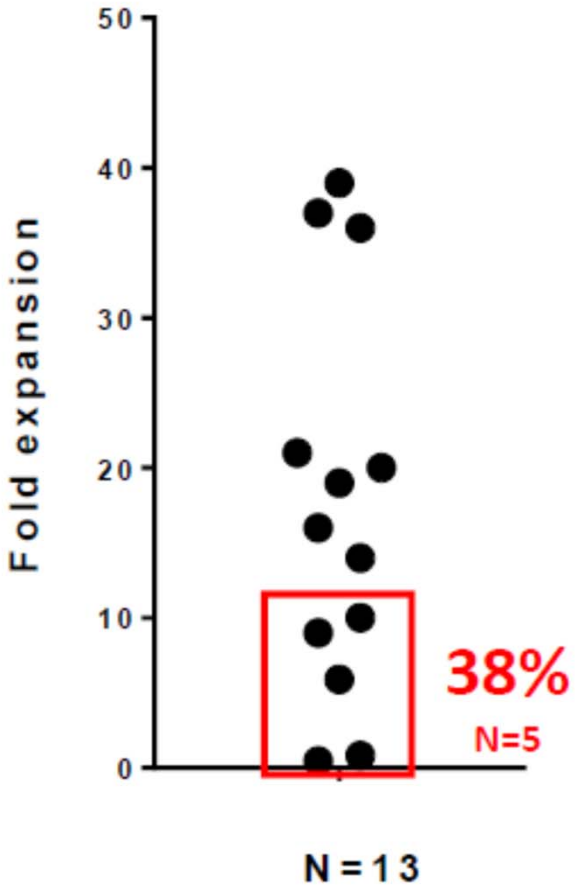
Plastic Adherence

Yields of CD19-CAR T Cells Manufactured from PBMC Concentrates Enriched with Anti-CD3/CD28 Beads, Anti-CD3/CD28 Beads plus Adherence, and Elutriation

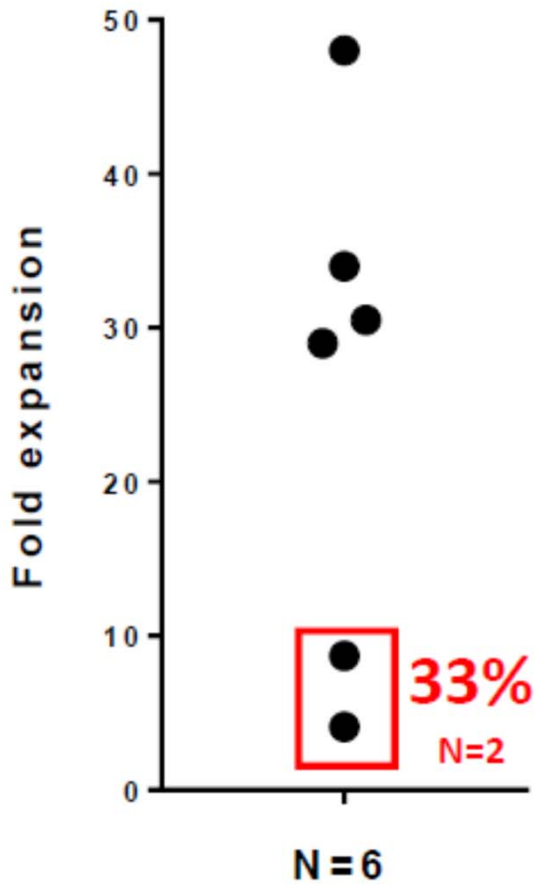


CD22-CAR T Cell Manufacturing: Enrichment and Elutriation Does Not Always Rescue Expansion

Anti-CD3/CD28 Enrichment + Adherence



Elutriation

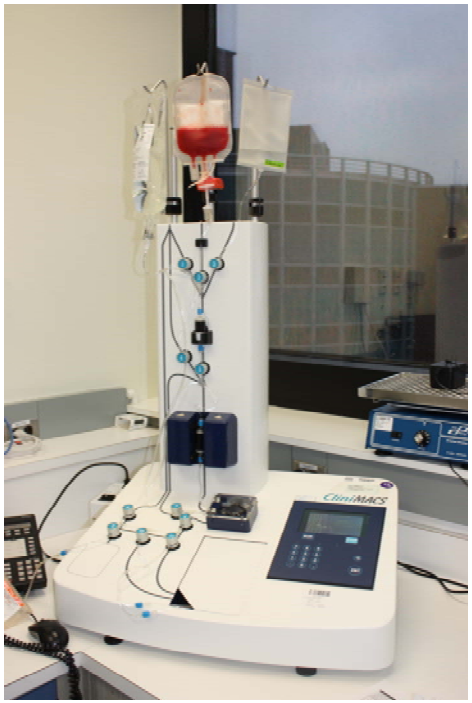


Highfill, Jin and Fellowes

Enriching PBMC Concentrates for T Cells by Antibody Selection

Selection of CD4+ and CD8+ cells using monoclonal antibodies conjugated to magnetic beads

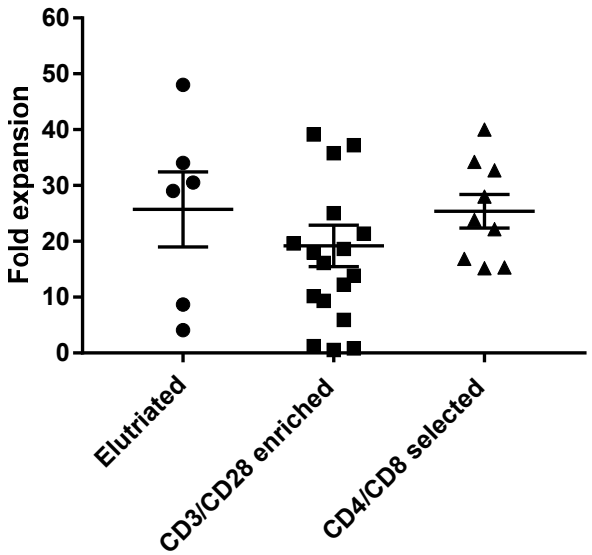
Miltenyi
CliniMACS Plus



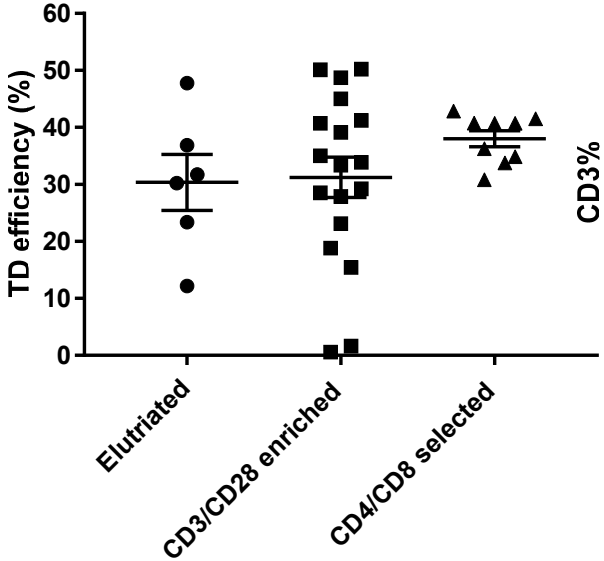
CD22-CAR T Cell Manufacturing Comparisons

15-C-0029; N=35 patients

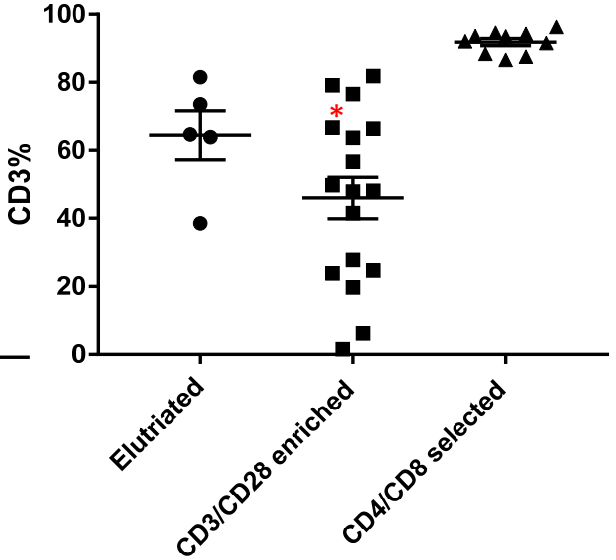
Fold Expansion



Transduction Efficiency



Purity

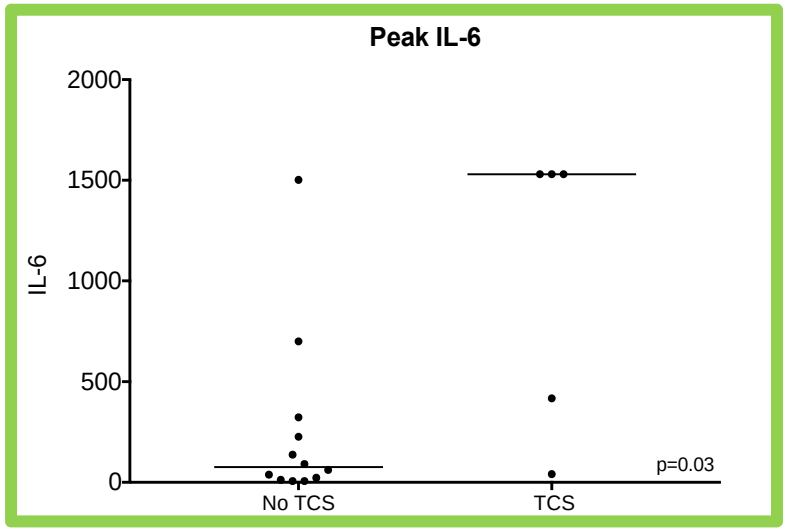


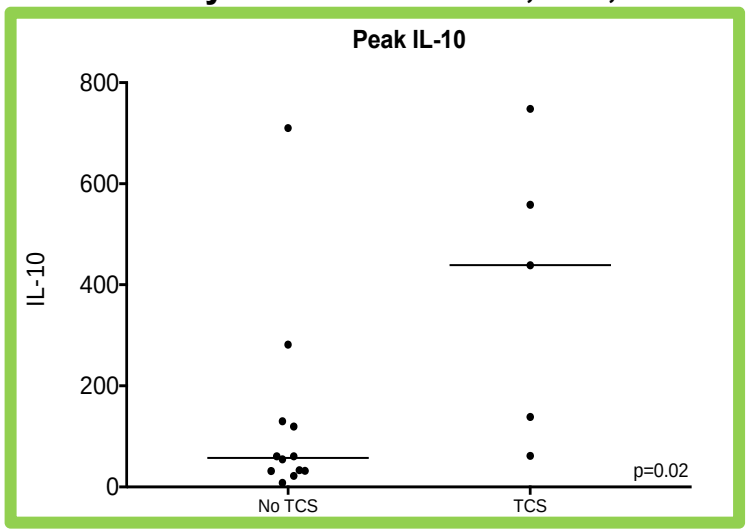
*Pre bead enrichment

Elutriated n=6; CD3/CD28 Enriched n=19; CD4/CD8 Selected n=10

Effect of T-Cell Selection on Cytokine Release Syndrome Following CD22-CAR T Cell Infusion

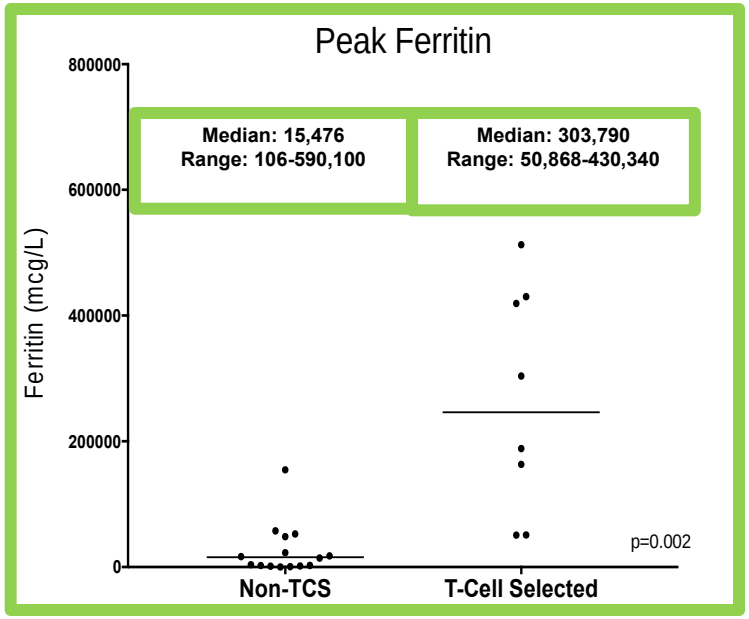
Courtesy of Dr. Nirali Shah, NCI, POB





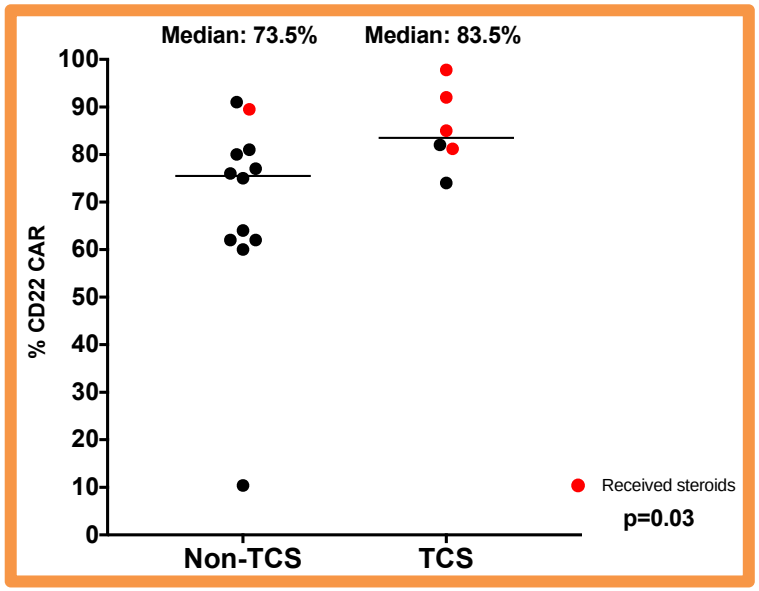
Following the Infusion of T Cell Selected (TSC) CD22-CAR T Cells:

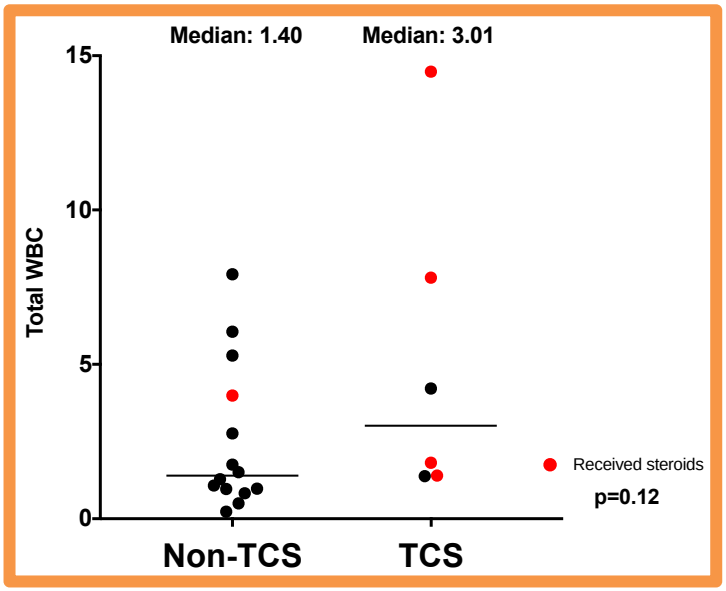
- More patients needed tocilizumab and steroids to prevent high grade CRS
- Cytokine levels and ferritin were consistently higher.



T Cell Selection (TCS) May Enhance *In Vivo* CAR T Cell Expansion

Courtesy of Dr. Nirali Shah, NCI, POB





Dose de-escalation to dose level 1: 3 x 10^5

- Previously limited efficacy (1/6 attained CR)
- 3 of 3 patients with CAR expansion
  - All 3 patients achieved a complete response (CR)

CAR T Cell Manufacturing Summary

- **Variability in leukocyte concentrates collected by apheresis and used as starting material for CAR T cell manufacturing can lead to variability in T cell expansion.**
- **Changes in the method used for the enrichment of leukocyte concentrates for T cells expansion change CAR T cell potency.**

Acknowledgements

NIH, Cell Process Section

Minh Tran
Sue Ellen Frodigh
Jiaqiang Ren
Ping Jin
Hui Liu
Vicki Fellowes
Jianjian Jin
Sandhya Panch
Hanh Khuu
Virginia DavidOcampo
Marianna Sabatino
Steven Highfill

NCI, Pediatric Oncology Branch

Trey Lee
Terry Fry
Crystal Mackall
Nirali Shah

NCI, ETIB

James Kochenderfer

DTM

Cathy Cantilena
Kamille West
Bill Ward
Harvey Klein