



Reimagined
2020 
NOVEMBER 9-14 



Society for Immunotherapy of Cancer

PARKER INSTITUTE
for CANCER IMMUNOTHERAPY



Penn Medicine
Center for Cellular Immunotherapies

Corona madness: flattening the curve with CAR T

Carl June

University of Pennsylvania

November 9, 2020



Society for Immunotherapy of Cancer

#SITC2020

Conflict of Interest Statement

Declaration of financial interest due to intellectual property and patents in the field of cell and gene therapy:

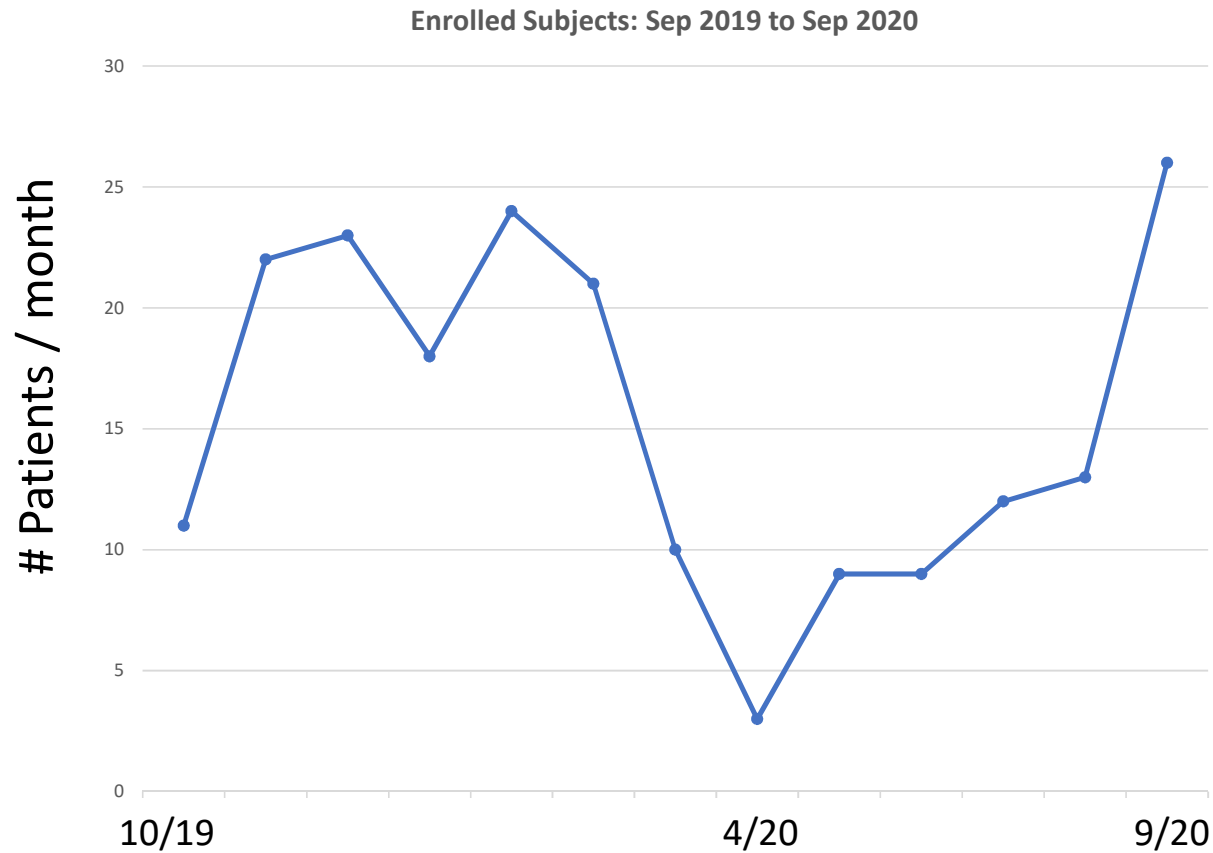
- Royalties and IPR: Novartis
- Scientific Advisory Board for Celldex, Cabaletta, Carisma, Kiadis, Viracta and WIRB-Copernicus Group
- Scientific Founder and equity: Tmunity Therapeutics, DeCart Therapeutics

Conflict of interest is managed in accordance with The University of Pennsylvania policies and oversight

Overview

- Cell therapies during COVID
- CAR T cell dysfunction
- Allogeneic “off the shelf” CAR T cells

Clinical Updates: Effect of COVID19 on Experimental Cell Therapy at Penn



Completed and Ongoing Cell Therapies for COVID-19 *

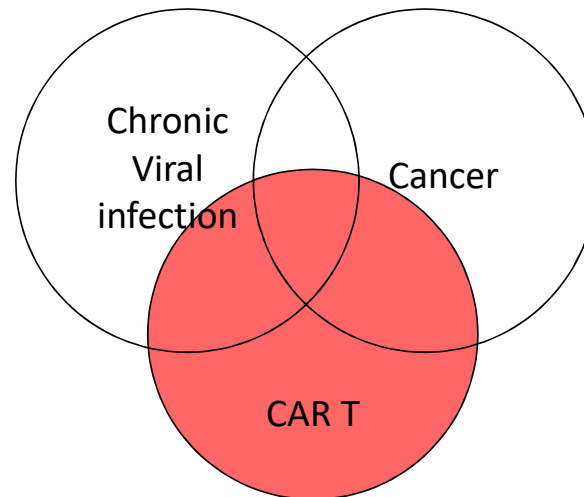
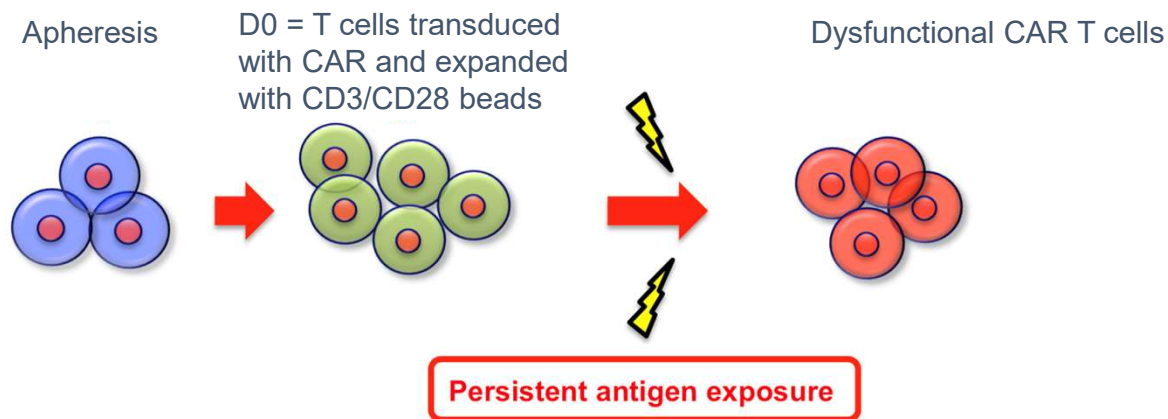
Cell Type	Title	Location	Status
NHPBSC	<u>Study Evaluating the Safety and Efficacy of Autologous Non-Hematopoietic Peripheral Blood Stem Cells in COVID-19</u>	Abu Dhabi	Completed
MSC	<u>Mesenchymal Stem Cells for the Treatment of COVID-19</u>	California	Completed
MSC	<u>Treatment With Human Umbilical Cord-derived Mesenchymal Stem Cells for Severe Corona Virus Disease 2019 (COVID-19)</u>	Wuhan, China	Completed
Treg	<u>REGulatory T Cell infuSion fOr Lung Injury Due to COVID-19 PnEumonia</u>	Hopkins, Columbia, UNC	Ongoing
DC / CTL	<u>Immunity and Safety of Covid-19 Synthetic Minigene Vaccine / CTLs</u>	Shenzhen, China	Ongoing
Allo NK	<u>Phase I / II Clinical Study of Immunotherapy Based on Adoptive Cell Transfer as a Therapeutic Alternative for Patients With COVID-19 in Colombia</u>	Bogata, Columba	Pending

* Clinicaltrials.gov, accessed Nov 7, 2020

Cell Therapies for COVID-19: Crazy or Not?

- Cell therapies for advanced O₂-dependent patients in ICU?
 - It is possible that Tregs or MSC infusions could accelerate repair of pulmonary damage
 - These studies are very difficult to conduct when the standard of care already includes high dose corticosteroid therapy and patients with many comorbidities
- Prophylactic therapies to prevent COVID?
 - Infusions of CTLs are effective in many viral infections, including respiratory infections:
 - =>Walter EA et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *NEnglJMed*. 1995;333(16):1038-44.
 - =>Leen AM. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood*. 2009;114(19):4283-92.

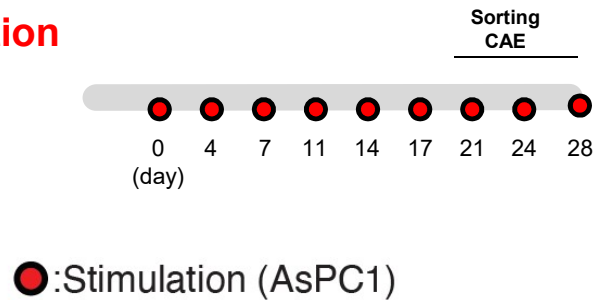
In vitro and In vivo Mechanisms of CAR T Dysfunction



- Will hallmarks of CAR T cell dysfunction overlap with identified signatures of T cell exhaustion in chronic viral infection and cancer?
- Can we use this model to identify novel hallmarks of CAR T cell dysfunction?

In Vitro Model of CAR T Cell Dysfunction

Continuous Stimulation



Functional characteristics

Reduced cytotoxicity

Reduced production of cytokines
- IL-2 - TNF- α - IFN- γ

Reduced proliferation

Phenotypical characteristics

Maintain expression of IRs
- PD-1 -CTLA4

Distinct transcriptional profiling with established markers of exhaustion

Cell killing assay



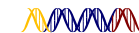
Cytokine production assay



Flowcytometry



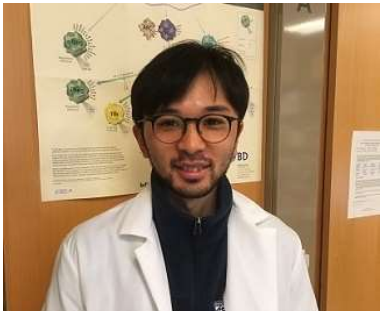
RNA-seq/ATAC-seq



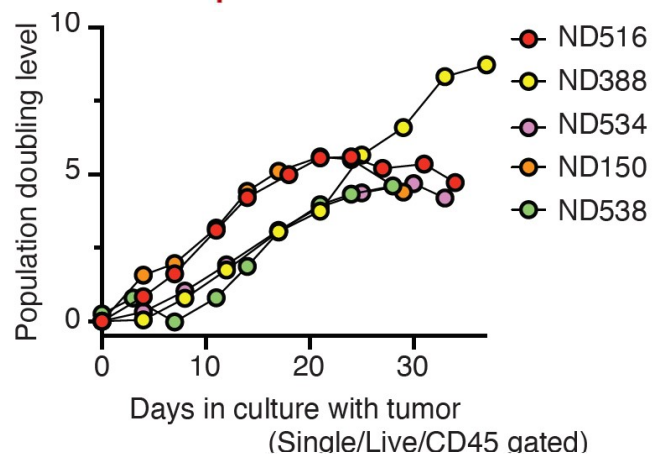
Points

1. anti-mesothelin CAR T cells
AsPC1 pancreatic cancer cell line
2. Stimulation : every 3-4 days
3. Keep in low E:T ratio
4. Use conditioned media
(50% fresh media + 50% conditioned media)

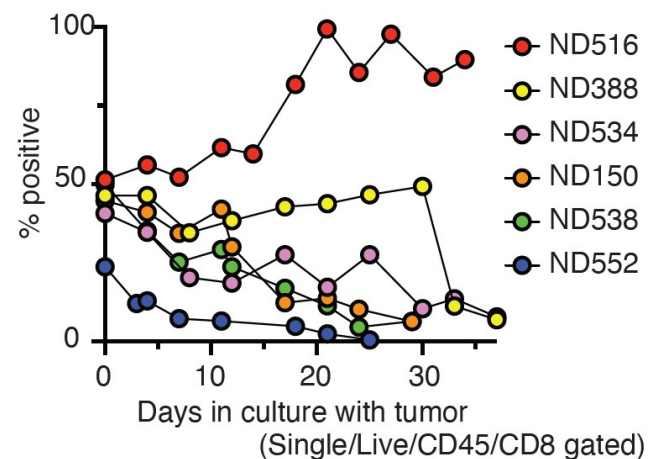
Shunichiro Kuramitsu MD PhD Charly Good PhD



Reduced proliferation

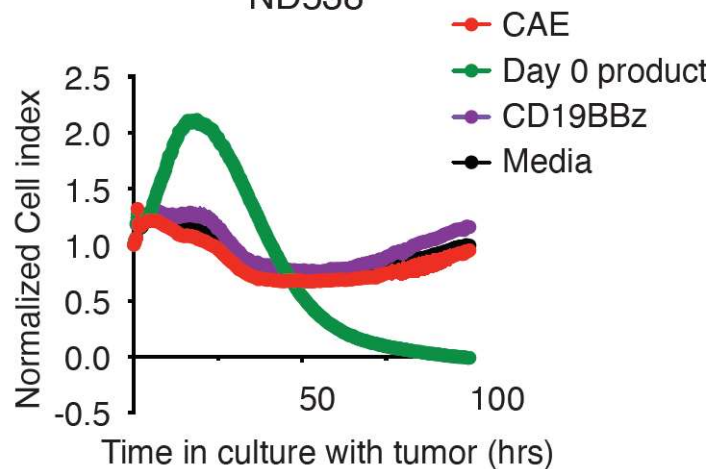


Surface CAR Expression

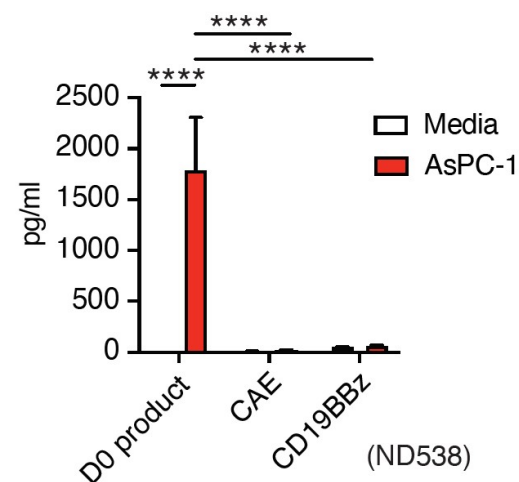


Reduced cytotoxicity

ND538

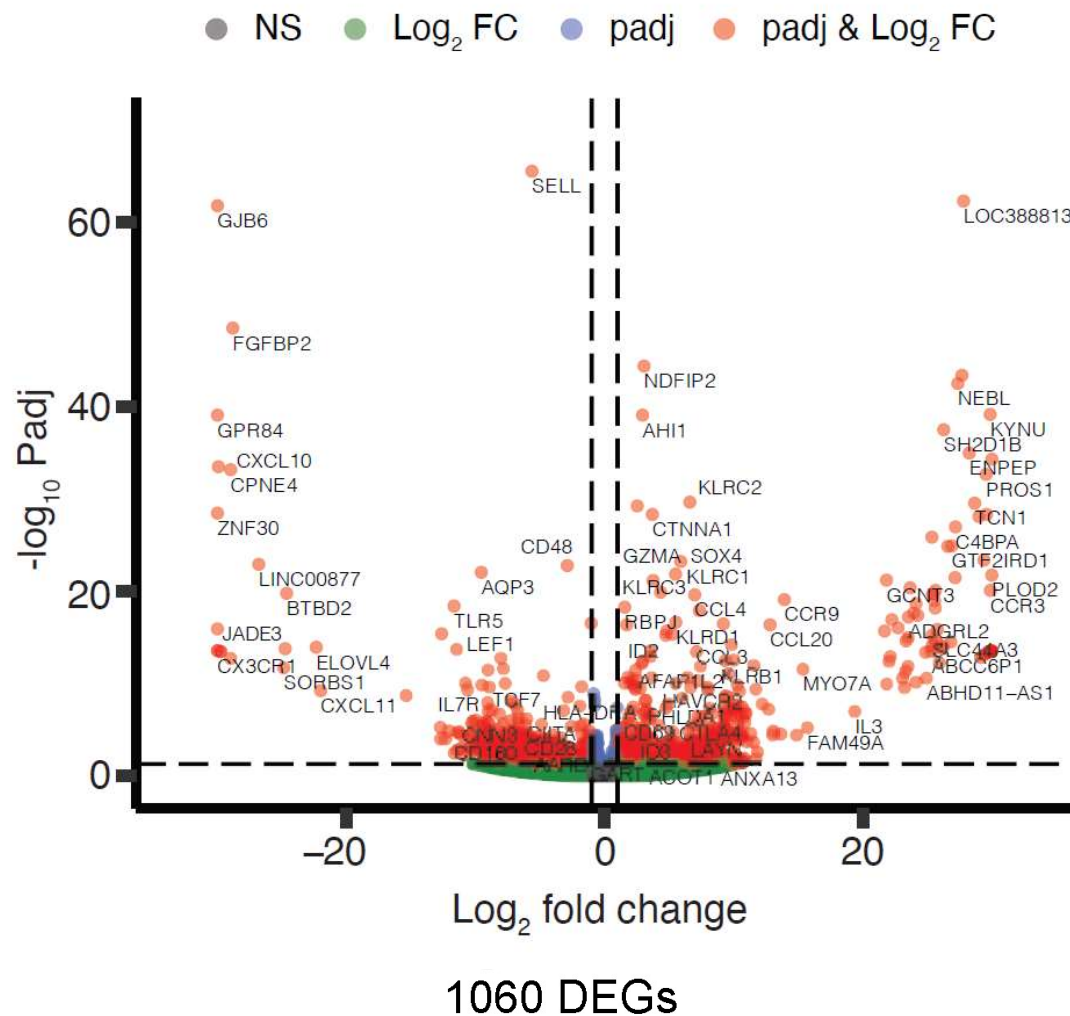


TNF- α

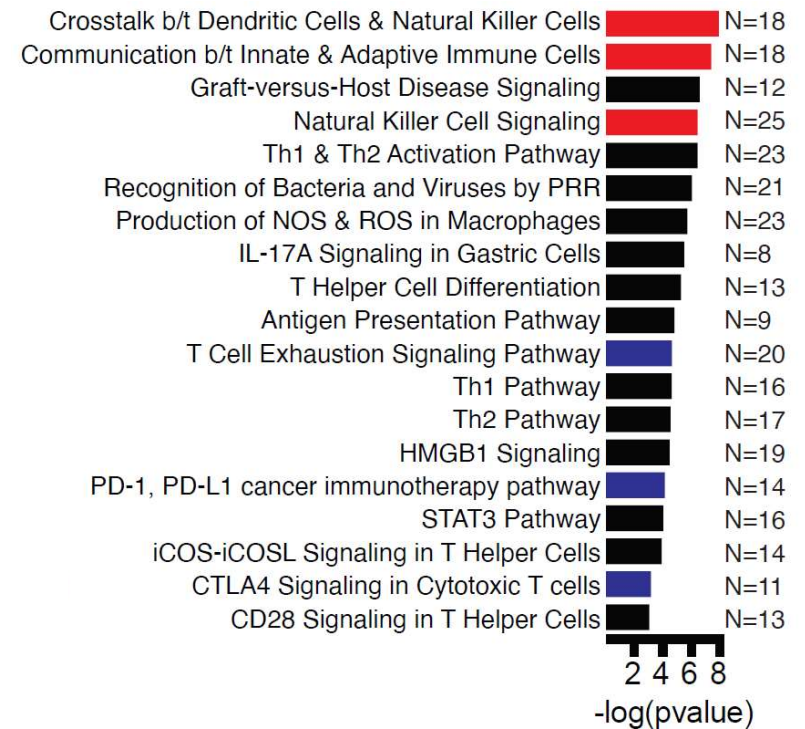


Reduced cytokine production

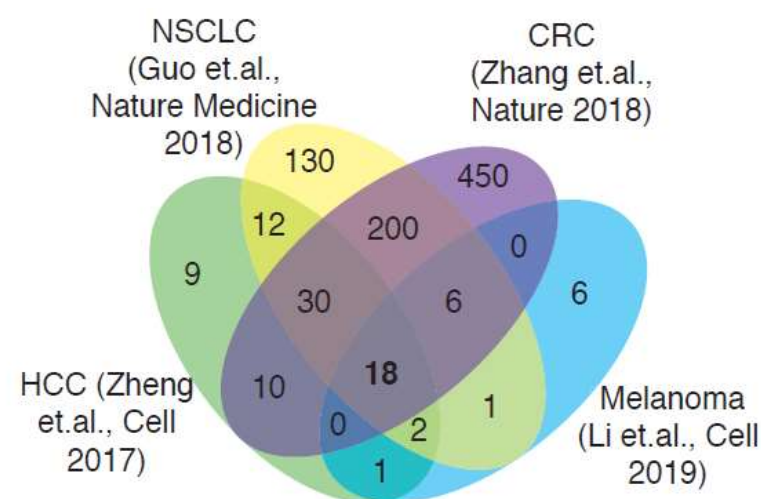
Exhaustion Genes and NK Receptors are Upregulated in Dysfunctional CAR T Cells



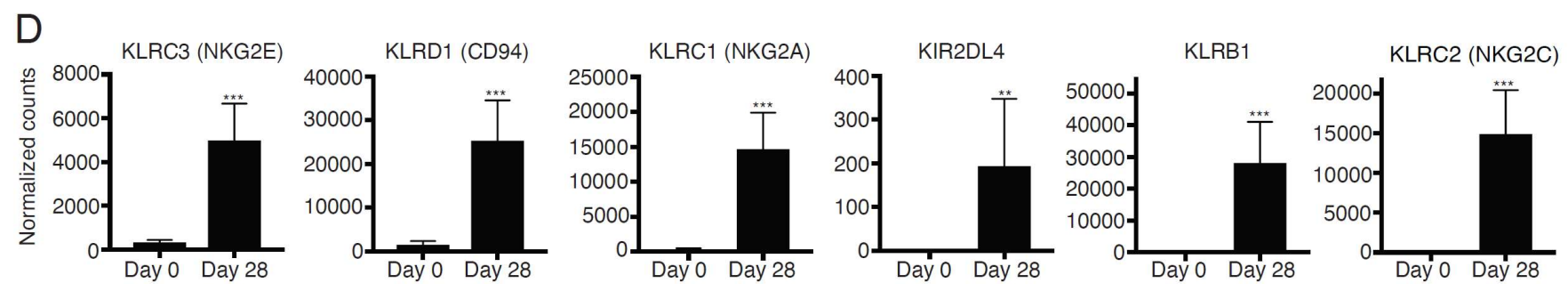
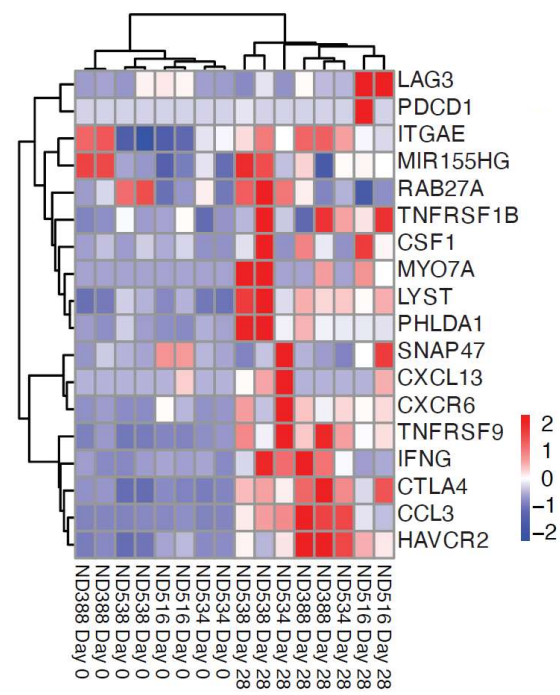
Ingenuity pathway analysis



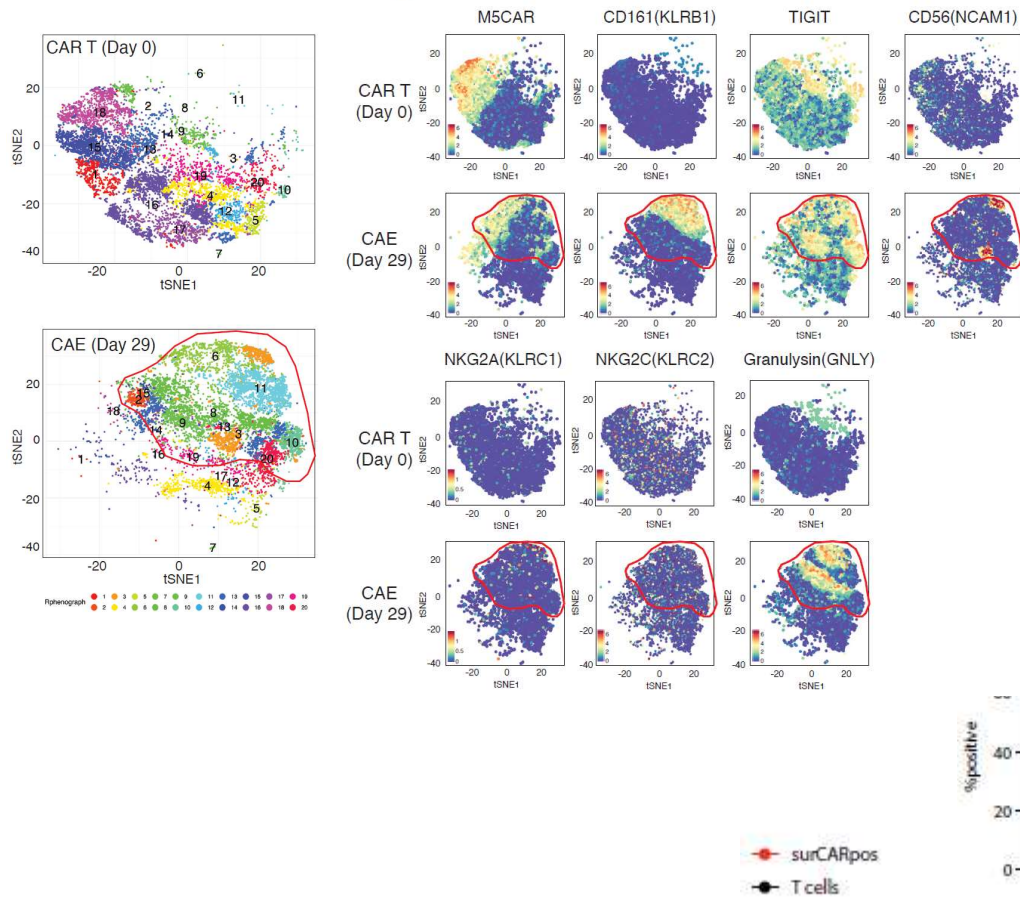
Exhaustion Genes and NK Receptors are Upregulated in Dysfunctional CAR T Cells



To obtain a more stringent data set we overlapped the data sets from the four cancer types and found a common group of 18 TIL marker genes

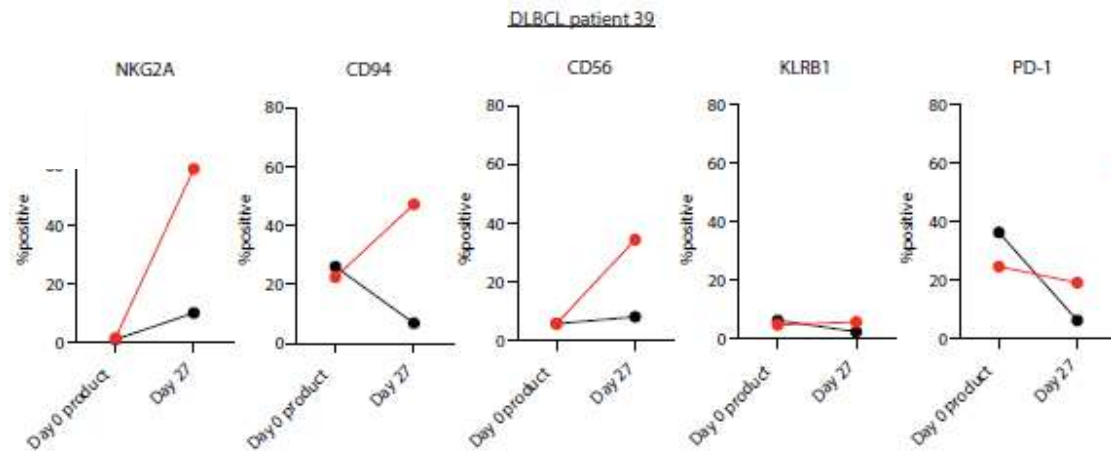
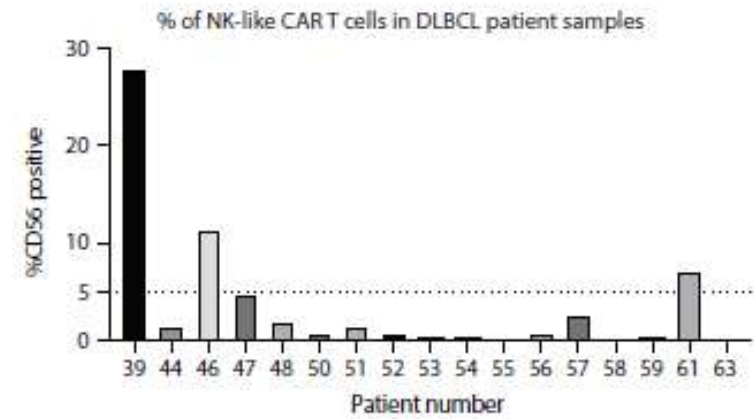


Mass Cytometry Day 0 and Day 29

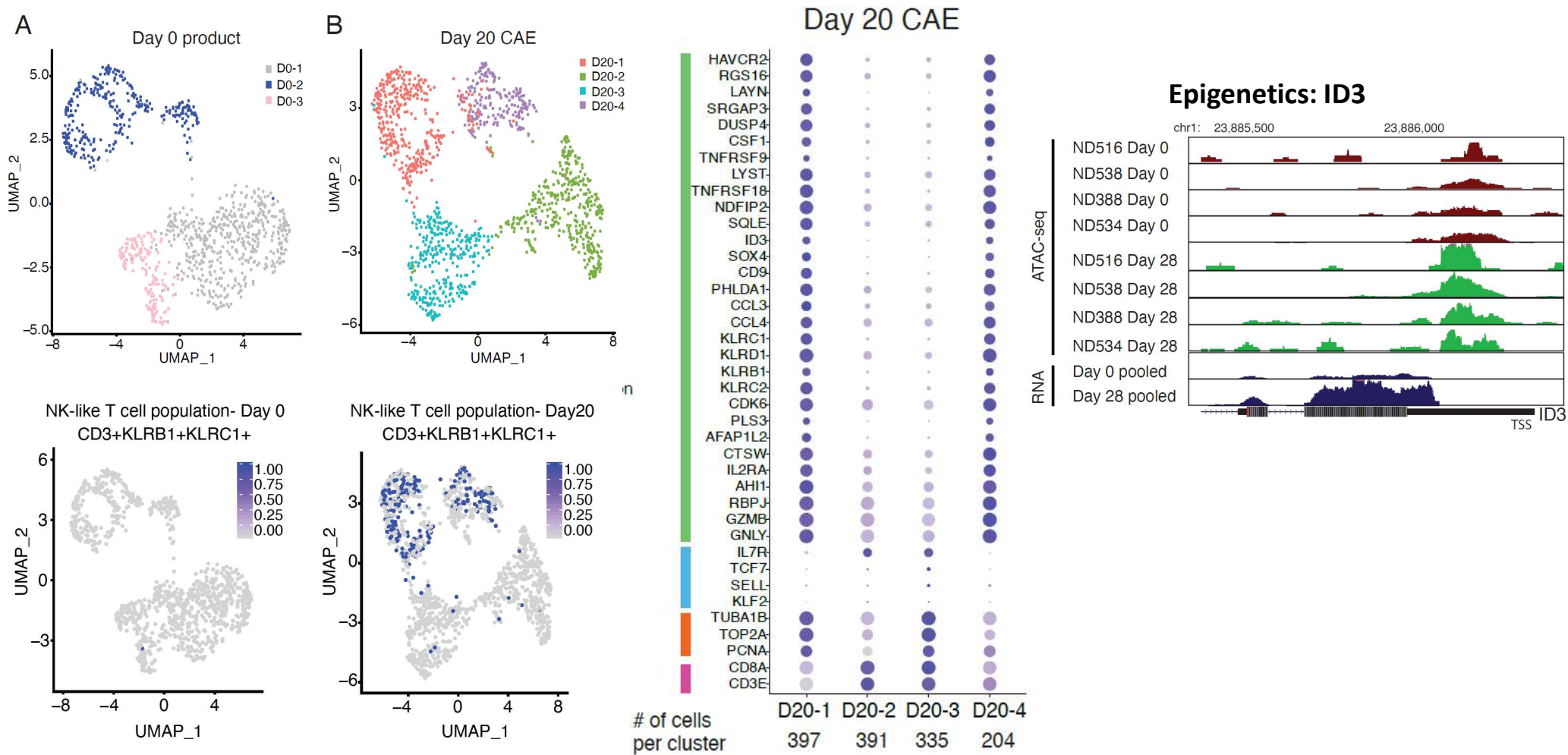


Lanier Lab, UCSF

Non-responding CD19 CAR T Patient With DLBCL has NK-like CAR T

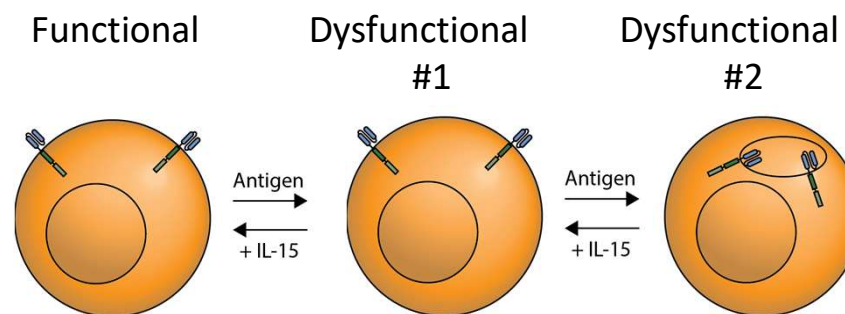


Emergence of Two Dysfunctional CAR T Cell Clusters



CAR T Cell Dysfunction: Summary

- Identified novel hallmarks of CD8+ CAR T dysfunction
 - Loss of surface CAR expression
 - Surface CAR T cells also acquire dysfunctional phenotype
 - Acquisition of a post-thymic NK-like T cell fate
 - CAR/ TCR dysfunctional gene signature
- Demonstration of loss of surface CAR and presence of NK-like T cells in patient samples from CAR T clinical trials.
- Identified transcription factors potentially regulating the exhaustion signature and NK-like T cell transition



Multiplex Human Genome Engineering to Extend the Reach of CAR T Cells

RESEARCH

RESEARCH ARTICLE SUMMARY

CLINICAL TRIALS

CRISPR-engineered T cells in patients with refractory cancer

Edward A. Stadtmauer^{*†}, Joseph A. Fraietta^{*}, Megan M. Davis, Adam D. Cohen, Kristy L. Weber, Eric Lancaster, Patricia A. Mangan, Irina Kulikovskaya, Minnal Gupta, Fang Chen, Lifeng Tian, Vanessa E. Gonzalez, Jun Xu, In-young Jung, J. Joseph Melenhorst, Gabriela Plesa, Joanne Shea, Tina Matlawski, Amanda Cervini, Avery L. Gaymon, Stephanie Desjardins, Anne Lamontagne, January Salas-McKee, Andrew Fesnak, Donald L. Siegel, Bruce L. Levine, Julie K. Jadowsky, Regina M. Young, Anne Chew, Wei-Ting Hwang, Elizabeth O. Hexner, Beatriz M. Carreno, Christopher L. Nobles, Frederic D. Bushman, Kevin R. Parker, Yanyan Qi, Ansuman T. Satpathy, Howard Y. Chang, Yangbing Zhao, Simon F. Lacey^{*}, Carl H. June^{*†}

PI: Edward Stadtmauer, MD

IND 17297 and Clinicaltrials.gov NCT03399448

Sponsor: Tmunity and Parker Institute for Cancer Immunotherapy

Follow on CRISPR trial

PACE CAR: Programmed Allogeneic CRISPR Editd
CD19 CAR T

35th Anniversary Annual Meeting & Pre-Conference Programs



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PACE CAR: Universal CAR T Project

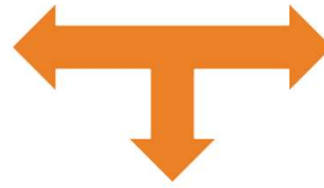
- Goal: Develop universal CAR T cells for leukemia and lymphoma
- Approach: Human genome engineering to overcome xenogeneic rejection mechanisms
- Translational output: Use dogs as a model system to test candidate human universal CAR T cells



Linette/Carreno and Posey labs (Penn Med) and Mason lab (Penn Vet)

Approach: Next Gen uCAR T Cells

1. Eliminate recognition by host T cells
Eliminate Graft vs Host disease
=> B2M, CIITA and TRAC knockouts



2. Eliminate recognition by host NK cells
Eliminate “missing self” and “stressed self”
=> HLA-E SCT expression

3. Test in large animal model
Xeno infusions of hCART19 B2M-TRAC-CIITA-HLA-E
=> Dogs with lymphoma

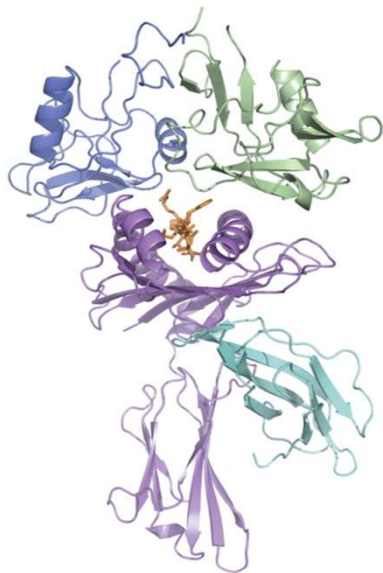


4. Test in patients w lymphoma
Allo infusions of hCART19 B2M-TRAC-CIITA-HLA-E
=> Humans with lymphoma

Linette/Carreno and Posey labs (Penn Med) and Mason lab (Penn Vet)

HLA-E as a decoy (inhibitory) ligand

Hypothesis: HLA-E*01:03 (over-)expression will enable donor TKO T cells to avoid recognition by host/recipient NK cells.

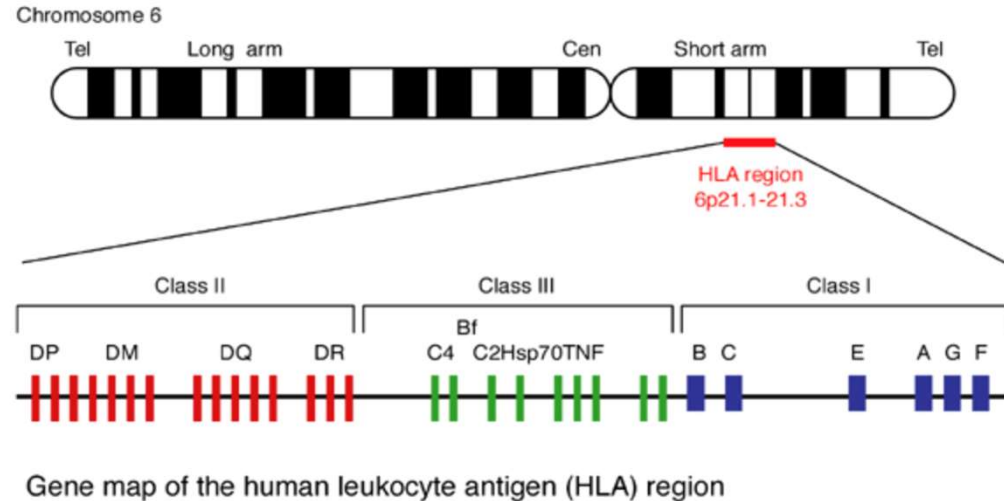


CD94-NKG2A - HLA-E
Green-blue-purple

HLA-E

- 2 alleles
 - E*01:01 R107
 - E*01:03 G107
- leader peptides MHC 1a
 - pathogens
- widely expressed
- inhibitory for NKG2A
- activating for NKG2C

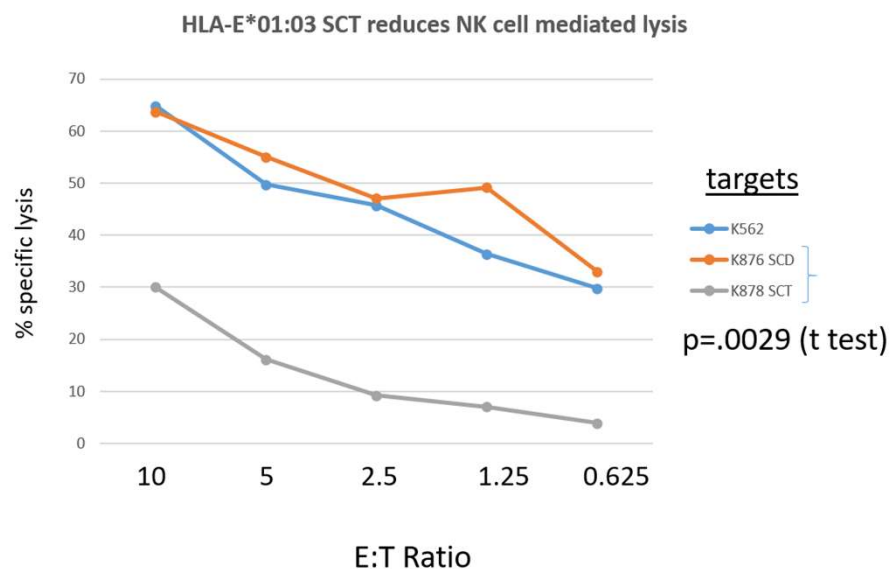
Braud et al. Nature 1998
Kaiser et al. PNAS 2008



Gene map of the human leukocyte antigen (HLA) region

Triple-Edited T Cells with HLA E Decoy Avoid T and NK Cell Mediated Lysis

HLA-E SCT reduces NK mediated lysis

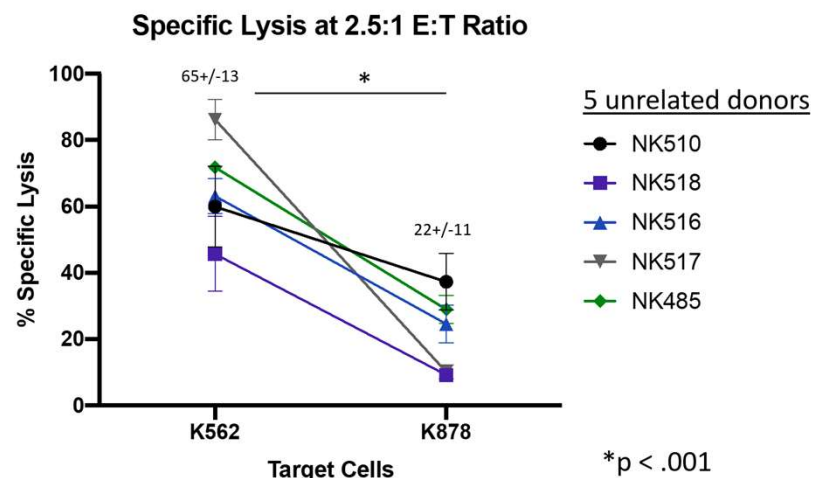


4h ⁵¹Cr release assay

Effectors: cytokine-activated donor NK518

Targets: K562 (wild type)
K876 SCD (no peptide)
K878 SCT (leader peptide)

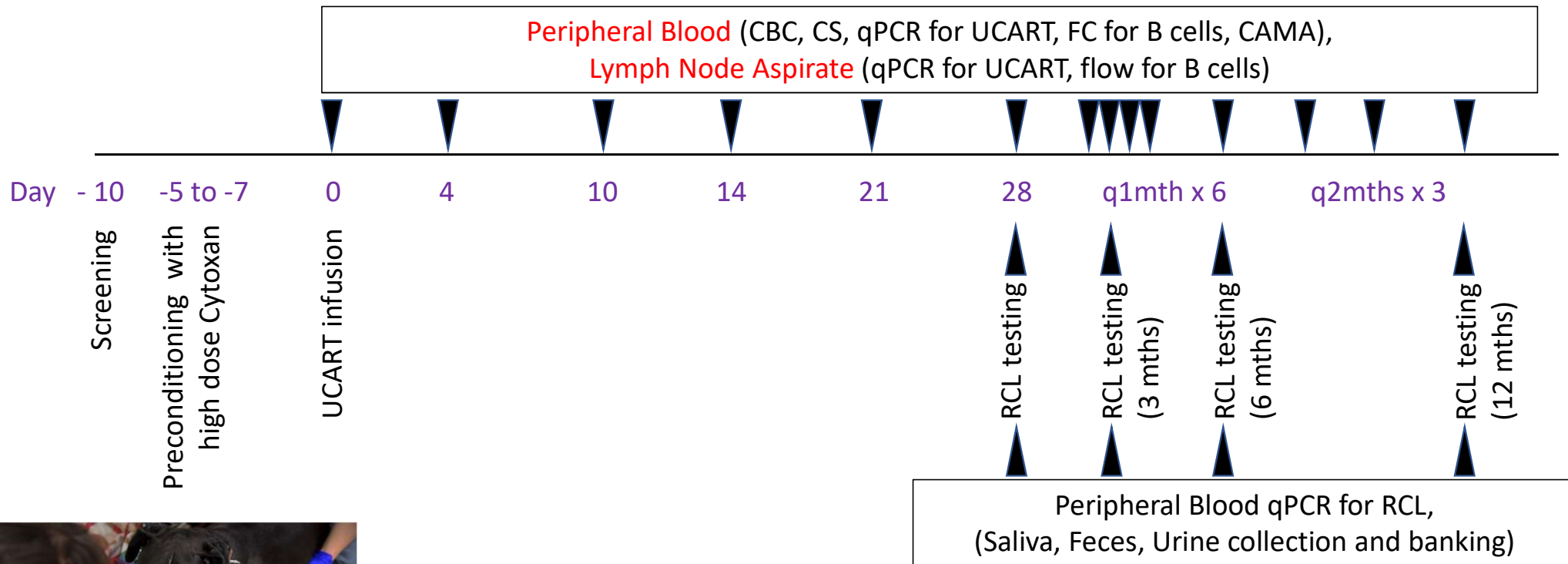
Consistent protection across normal donor NK cells



Linette lab, unpublished

Ongoing Trial: Test Human uCART TRAC-CIITA-B2M-HLA-E SCT in Canine Trial

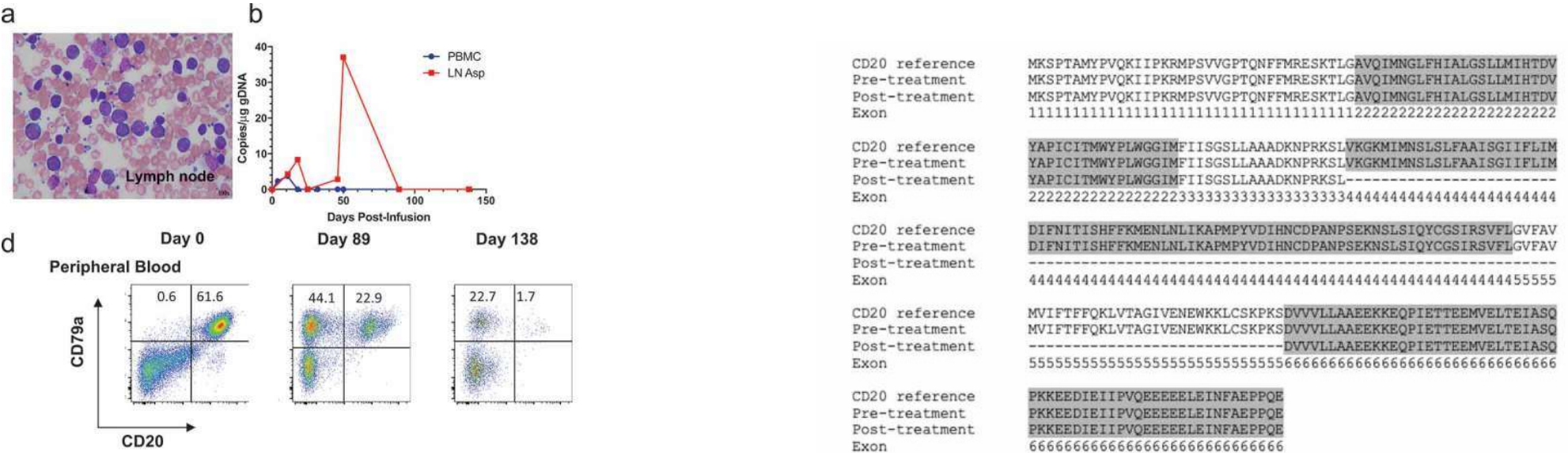
Anti-dog CD19 and CD20 CARs constructed



Nicola Mason, Penn Vet

Example: Treatment of Dogs with Autologous CD20 CAR T Induces Target Loss Similar to Human Patients

Anti-dog C20 CARs constructed



Panjwani MK, et al. Establishing a model system for evaluating CAR T cell therapy using dogs with spontaneous diffuse large B cell lymphoma. Oncoimmunology. 2019 Oct 23;9(1):1676615. doi:

Summary

- Cell therapies during COVID
- CAR T cell dysfunction and NK T transition occurs in vitro and in vivo
- Allogeneic “off the shelf” CAR T cells in dogs ongoing

Colleagues and Patients: Thank you!

Center for Cellular Immunotherapies

Anne Chew
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Penn Vet

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CHOP

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Shannon Maude

 NOVARTIS

T Cell Engineering

Yangbing Zhao
Jiangtao Ren
Chongyun Fang
Xiaojun Liu
Shuguang Jiang



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Families**

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