Personalized Medicine

Update Discussion of Recent Results on Protocol 793 titled: Pilot Study of Redirected Autologous T Cells Engineered to Contain Anti-CD19 Attached to TCRζ and 4-1BB Signaling Domains in Patients withChemotherapy Resistant or Refractory CD19+ Leukemia and Lymphoma

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December 14, 2011

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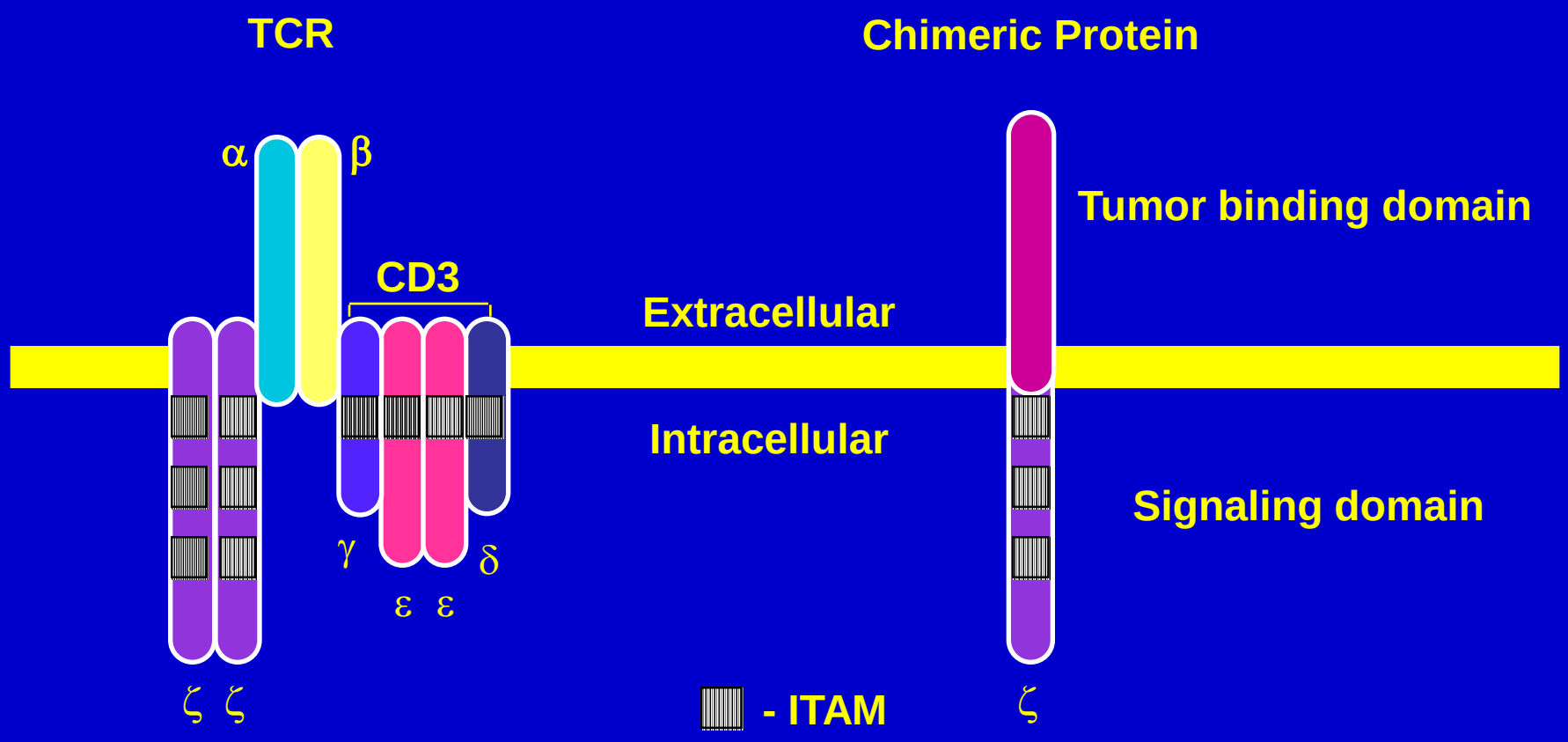
Two Basic Approaches to Overcome Tolerance
Creation of Bi-specific T cells

TCR heterodimer approach "CAR" or T body approach



scFv Chimeric Antigen Receptors (CARs) Background

- 1989 Redirected T cell concept pioneered in vitro by Eshhar and colleagues: (Gross et al, PNAS 86: 10024, 1989). First CAR trial: Walker et al. Blood. 2000;96(2):467
- Despite strong pre-clinical rationale, technical difficulties have prevented clinical translation until recently:
  - Efficient T cell culture systems
  - Efficient gene transfer systems
- First clinical experiences in cancer:
  - Kershaw et al. *Clin Cancer Res,* 2006
  - Pule et al. *Nat Med*, 2008
  - > Trials failed due to poor T cell engraftment?

Trials Testing CD19 and CD20 CARs for B Cell Malignancies in US (Circa August 2010)

	Antigen	Tumor target	Viral-specific T cell	Lympho-depletion	CAR generation	ClinicalTrial.gov identifier	Enrolling	SAE	Gene transfer
1	Kappa light chain	B-NHL and B-CLL	No	Yes	First and second	NCT00881920	Yes	TBM	Virus
2	CD19	Lymphoma/leukemia (B-NHL) and CLL	No	No	First and second	NCT00586391	Yes	TBM	Virus
3	CD19	Advanced B-NHL/CLL	Yes	No	First and second	NCT00709033	Yes	TBM	Virus
4	CD19	Lymphoma and leukemia	No	Yes	Second	NCT00924326*	Yes	TBM	Virus
5	CD19	ALL (post-HSCT)	Yes	No	Second	NCT00840853	Yes	TBM	Virus
6	CD19	Follicular NHL	No	Yes	First	NCT00182650	No	No	Electroporation
7	CD19	CLL	No	Yes/no	Second	NCT00466531*	Yes	Yes (1 Death)	Virus
8	CD19	B-NHL/leukemia	No	Yes	First and second	NCT00891215	Yes	TBM	Virus
9	CD19	B-cell leukemia, CLL and B-NHL	No	No	Second	NCT01087294	Yes	TBM	Virus
10	CD19	B-ALL	No	Yes	Second	NCT01044069	Yes	TBM	Virus
11	CD19	B-lymphoid malignancies	No	Yes	Second	NCT00968760	No	TBM	Electroporation (SB system)
12	CD20	Relapsed/refractory B-NHL	No	Yes	First	NCT00012207*	No	No	Electroporation
13	CD20	Mantle cell lymphoma or indolent B-NHL	No	Yes	Third	NCT00621452	Yes	TBM	Electroporation

- All CAR development has been in academic setting, with the exception of the first CAR trials that were conducted testing CD4zeta CARs.

Jena B, Dotti G, Cooper L. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010;116(7):1035-44.

Review

Cancer Research

Considerations for the Clinical Application of Chimeric Antigen Receptor T Cells: Observations from a *Recombinant DNA Advisory Committee Symposium* Held June 15, 2010

Hildegund C.J. Ertl[1], John Zaia[3], Steven A. Rosenberg[4], Carl H. June[2], Gianpietro Dotti[7], Jeffrey Kahn[9], Laurence J.N. Cooper[8], Jacqueline Corrigan-Curay[5], and Scott E. Strome[6]

Abstract

T cells that are genetically modified to express single-chain chimeric antigen receptors (CAR) have shown promise in early cancer immunotherapy clinical trials. Unfortunately, 2 recent deaths in cancer patients treated with CAR T cells have created some uncertainty on how to best mitigate patient risk, while continuing to advance this very promising therapeutic avenue. In order to address these concerns, the Recombinant DNA Advisory Committee (RAC) held a symposium, the objectives of which were to first review the reported treatment-associated toxicities and, second, to discuss methods for improving safety and efficacy. This report highlights the issues raised as part of this discussion, with a specific focus on protocols infusing CAR T cells. Because this was not a consensus conference, the opinions described should not be construed to represent those of any individual RAC member, the RAC as a body, conference participants, the National Institutes of Health, or the U.S. Food and Drug Administration. *Cancer Res; 71(9); 3175–81. ©2011 AACR.*

Principles Applicable to Clinical Trials Using 2nd and 3rd Generation CAR-T cells (Ertl et al)

Caveats:

• Suggested dosing is based on transfer of unselected CAR-T cells. Prior subset selection may affect toxicity.

Governing Principles:

• 2nd and 3rd generation CAR-T cells have the potential for proliferation and long term engraftment.

• Survival and engraftment are likely enhanced by preconditioning.

• Cytokine support of transferred cells may enhance initial cell proliferation and survival and should be investigated in more depth.

• Initial toxicity might be avoided by reducing cell dose.

• Split dose infusion strategies to give infusions over several days may mitigate the risk of initial toxicity.

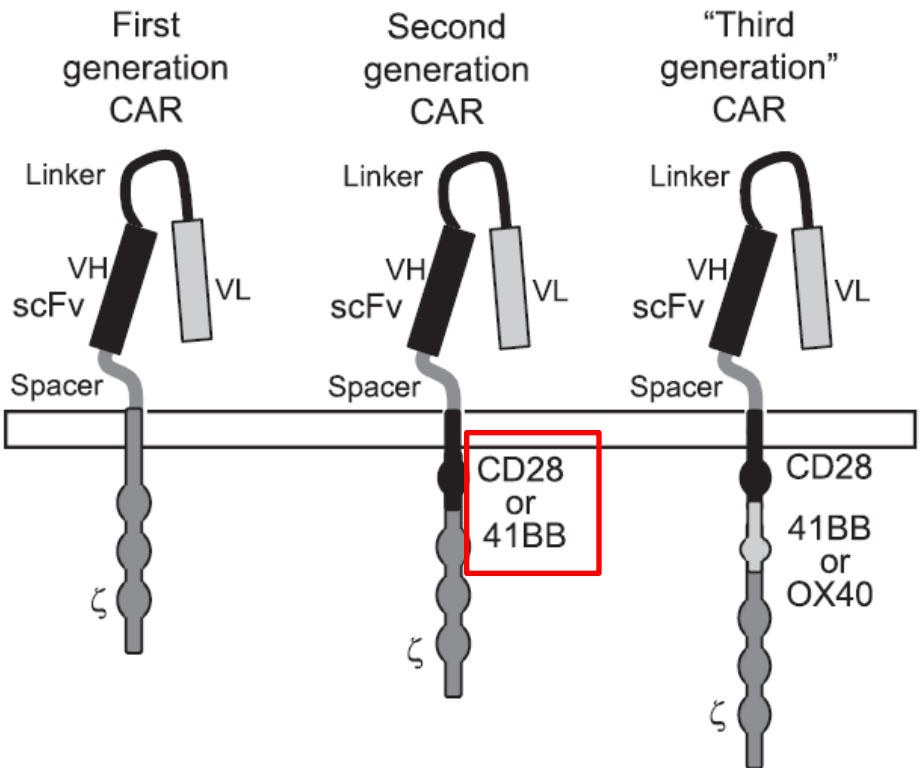
Strategies for use:

• Based on data from trials to date a moderate initial cell dose (e.g., 3 x 105 cells/kg) might be appropriate if supported by other trials with CAR-T cells (including 1st generation CARs) having similar targets; for CAR-T cells targeting novel targets a lower dose (e.g., 104 cells/kg) should be considered.

• Preconditioning is indicated in most circumstances.

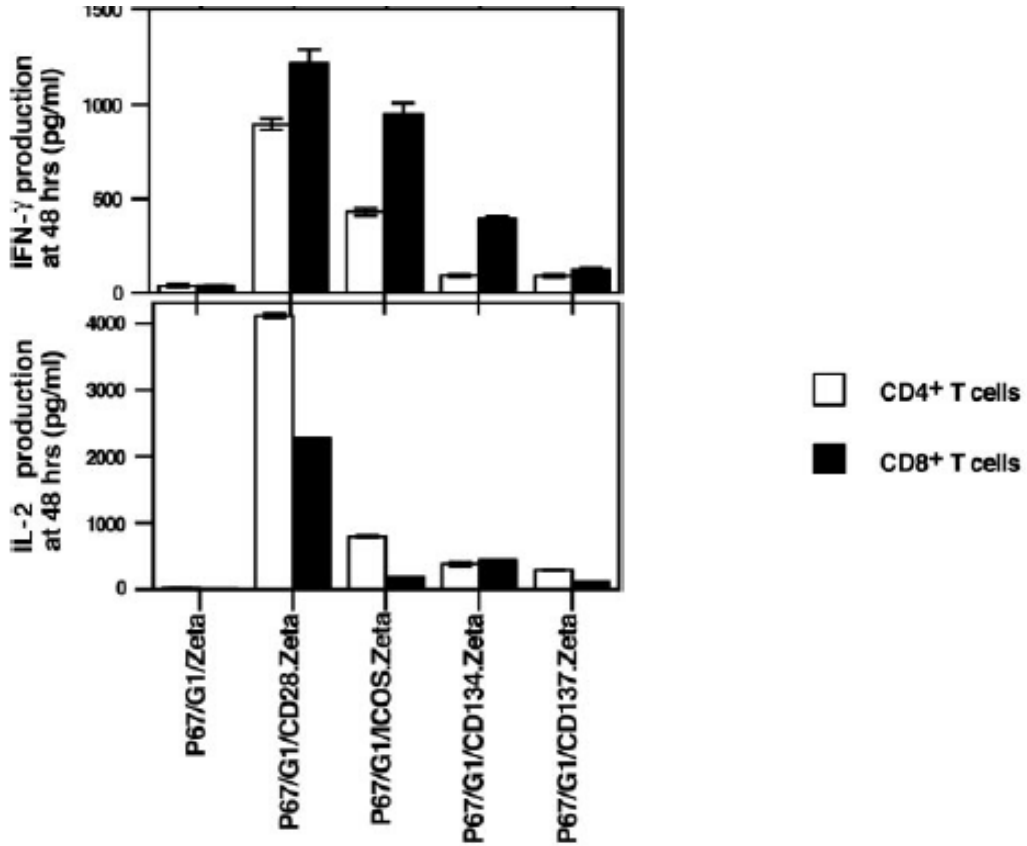
• Potential benefits of cytokine support should be explored in more depth.

Evolution of CARs with additional costimulatory domains



Dotti et al. 2009

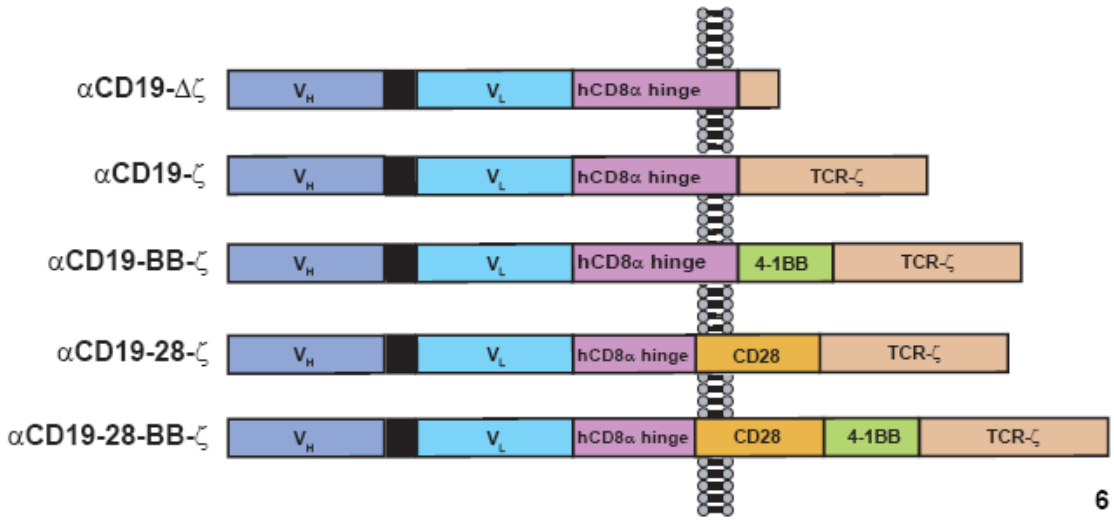
Co-signaling: CAR costimulatory domains

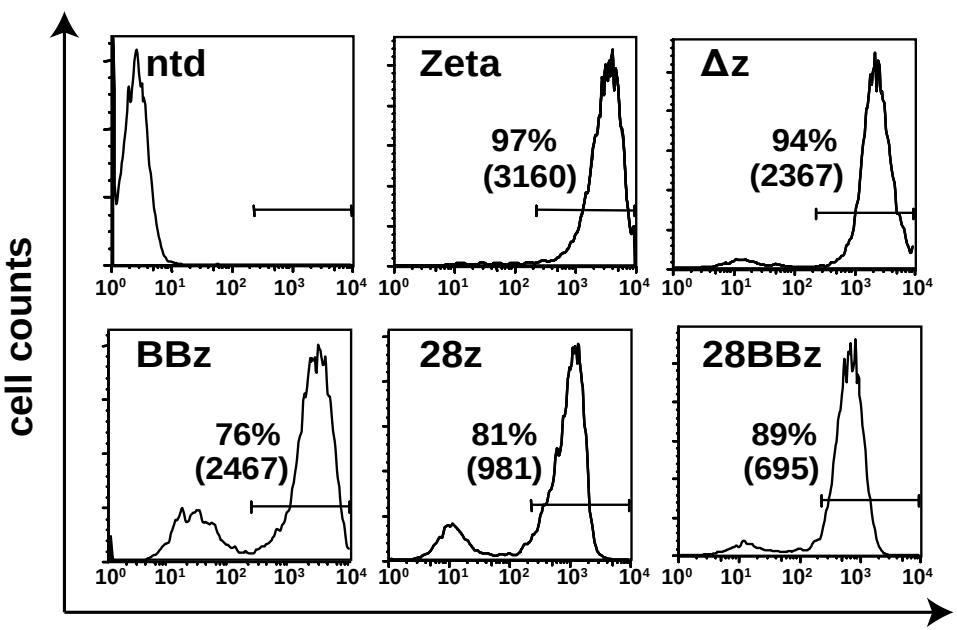


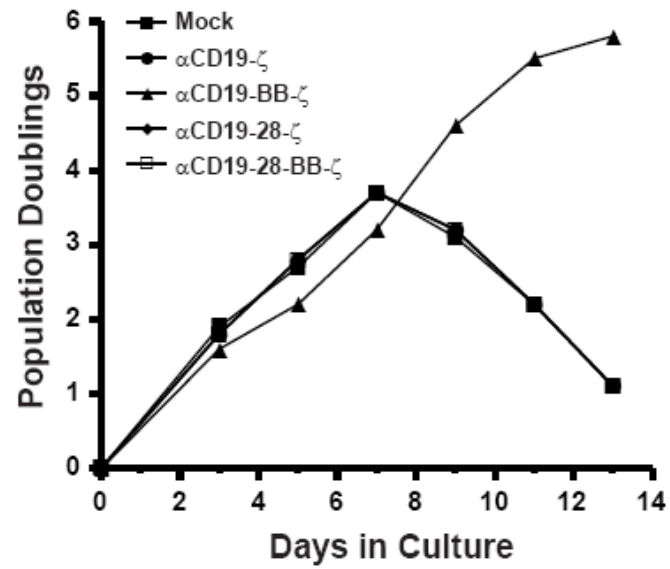
Short term in vitro comparison of zeta, CD28, ICOS, Ox40 and 4-1BB CARs

Finney HM, Akbar AN, Lawson ADG. Activation of resting human primary T cells with chimeric receptors: Costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. Journal of Immunology. 2004;172(1):104-13.

4-1BB Signaling Domain Promotes CAR T Cell Proliferation



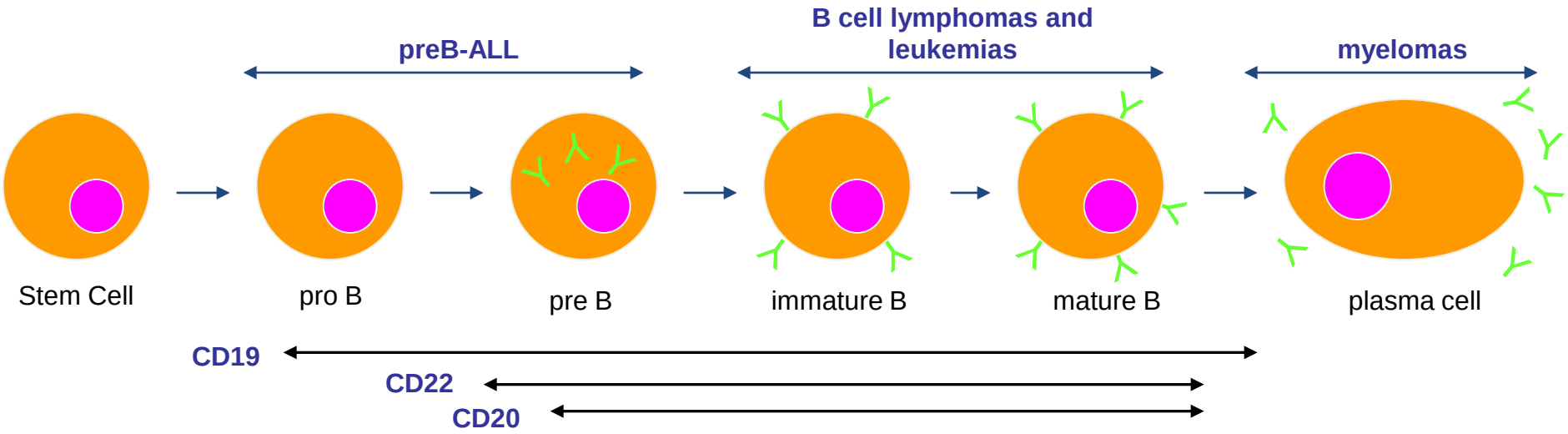




Imai et al. Leukemia 2004
Milone, et al. Mol Ther 2009
Carpenito, et al. PNAS 2009

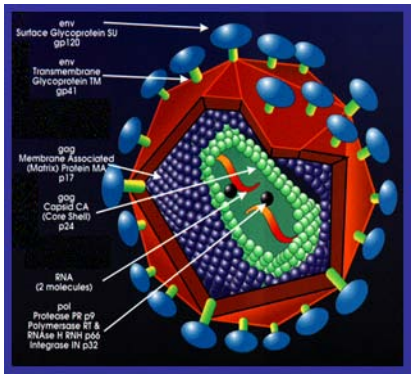
CD19: The first ideal CAR Target

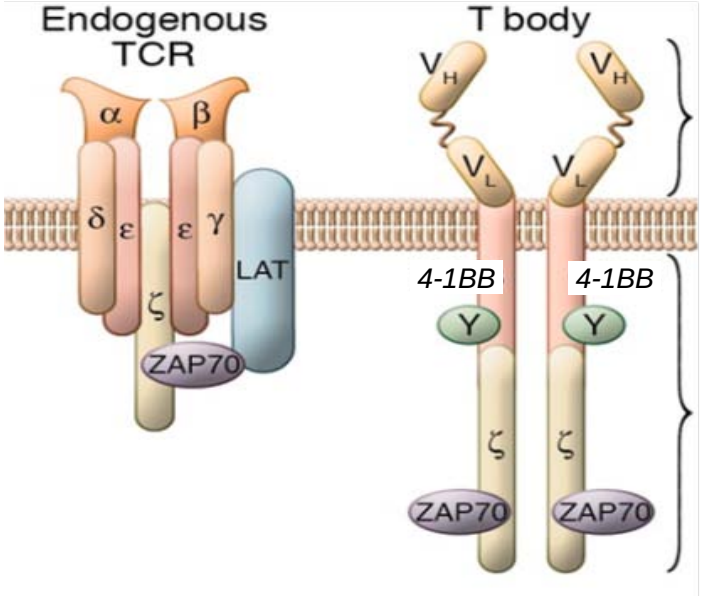
- CD19 expression is restricted to B cells and possibly follicular dendritic cells
- CD19 is <u>not</u> expressed on pluripotent bone marrow stem cells
- CD19 is expressed on the surface of most B cell malignancies
- Antibodies against CD19 inhibit growth of tumor cells

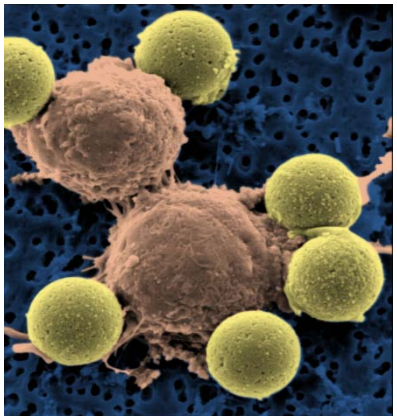


Renier Brentjens

Autologous T Cells Transduced w/ Anti-CD19 mAb spliced to with TCR-ζ and 4-1BB Signaling Domains







Lentiviral vector to deliver construct

CD3-ζ and 4-1BB signaling domains

Anti-CD3/anti-CD28 mab coated bead stimulation

Protocol Designations:
UPCC 04409
IRB#805313
RAC 2007-793
Clinicaltrials.gov NCT01029366

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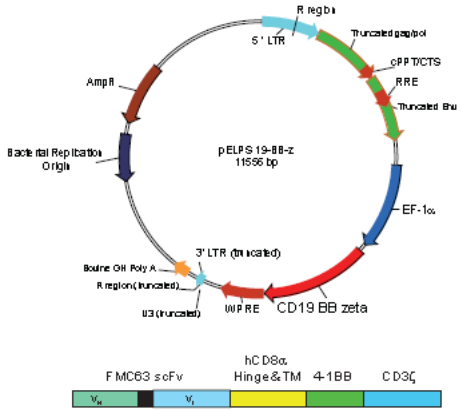
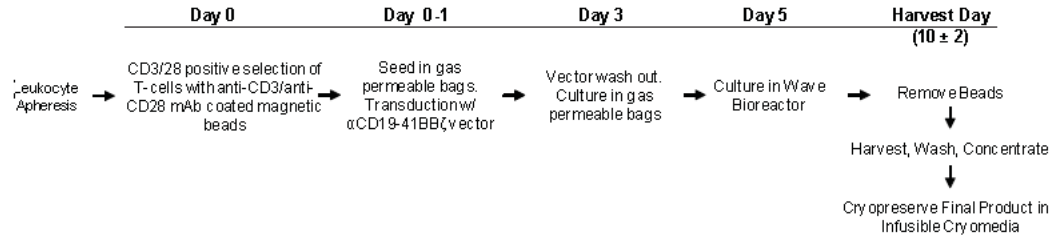
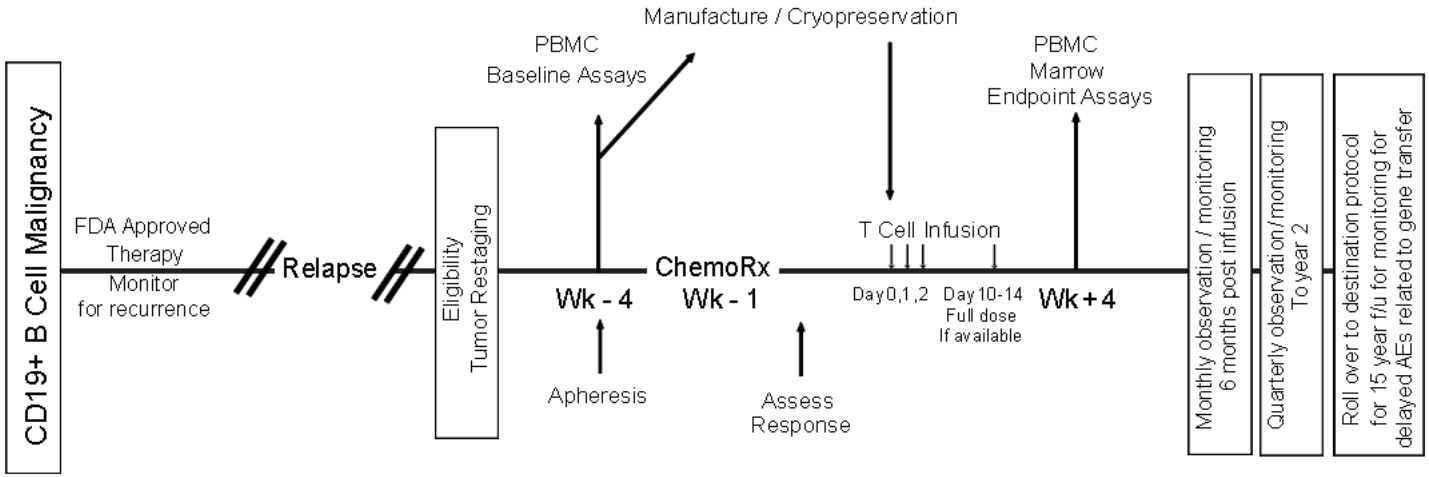
Study Overview- ClinicalTrials.gov #NCT01029366







PRIMARY OBJECTIVES:

I. Determine the safety and feasibility of the chimeric antigen receptor T cells transduced with the anti-CD19 lentiviral vector (referred to as CART-19 cells).

II. Determine duration of in vivo survival of CART-19 cells. RT-PCR analysis of whole blood will be used to detect and quantify survival of CART-19 TCR zeta:4-1BB and TCR zeta cells over time.

SECONDARY OBJECTIVES:

I. For patients with detectable disease, measure anti-tumor response due to CART-19 cell infusions.

II. To determine if the 4-1BB transgene is superior to the TCR zeta only transgene as measured by the relative engraftment levels of CART-19 TCR zeta:4-1BB and TCR zeta cells over time.

CLL Study Subjects Refractory and Relapsed: 2 with p53 deletions

Subject UPN	Age/sex Karyotype	Previous therapies
01	65/M normal	1. Fludarabine x 4 cycles (2002) 2. Rituximab/fludarabine x 4 cycles (2005) 3. Alemtuzumab x 12 wks (2006) 4. Rituximab (2 courses 2008-2009) 5. R-CVP x 2 cycles (2009) 6. Lenalidomide (2009) 7. PCR x 2 cycles (5/18-6/18/2010) 8. Bendamustine x 1 cycle (7/31-8/1/10) pre-CART19
02	77/M del(17)(p13) [p53 deficient]	1. Alemtuzumab x 16 wks (6/2007) 2. Alemtuzumab x 18 wks (3/2009) 3. Bendamustine/ Rituximab: 7/1/2010 (cycle 1), 7/28/2010, (cycle 2) 8/26/2010 (cycle 3) pre-CART19
03	64/M del(17)(p13) [p53 deficient]	1. R- Fludarabine x 2 cycles (2002) 2. R-Fludarabine x 4 cycles (10/06-1/07) 3. R- Bendamustine x1 cycle (2/09) 4. Bendamustine x 3 cycles (3-5/09) 5. Alemtuzumab x 11 wks (12/09-3/10) 6. Pentastatin/cyclophosphamide (9/10/10) pre-CART19

Science Translational Medicine Aug 10, 2011 3(95): 95ra73

CLL Incidence and Prognosis

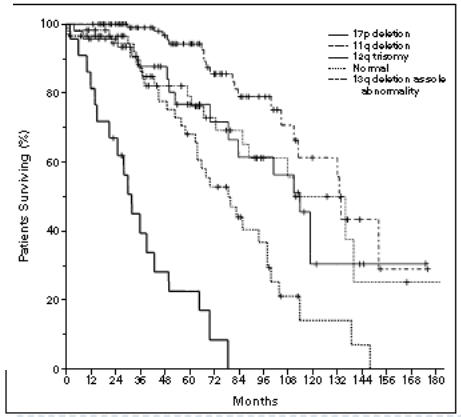
CLL Epidemiology:

- U.S. CLL population: **85,710**
- CLL represents **30%** of all leukemias

- **14,470** new cases of CLL in 2011
- **4,380** CLL fatalities expected

- 17p+: ~**10yr** survival from diagnosis
- 17p-: ~ **3yr** survival from diagnosis

Abnormality	Prognostic Ability	% of Patients with Defect	Median Survival Time (yrs.)
Normal Karyotype	N/A	N/A	9.25
17p deletion	Poor Prognosis	7%	2.5
11q deletion	Poor Prognosis	18%	6.6
Trisomy 12	N/A	16	9.5
13q deletion	Favorable Prognosis	55%	11.0

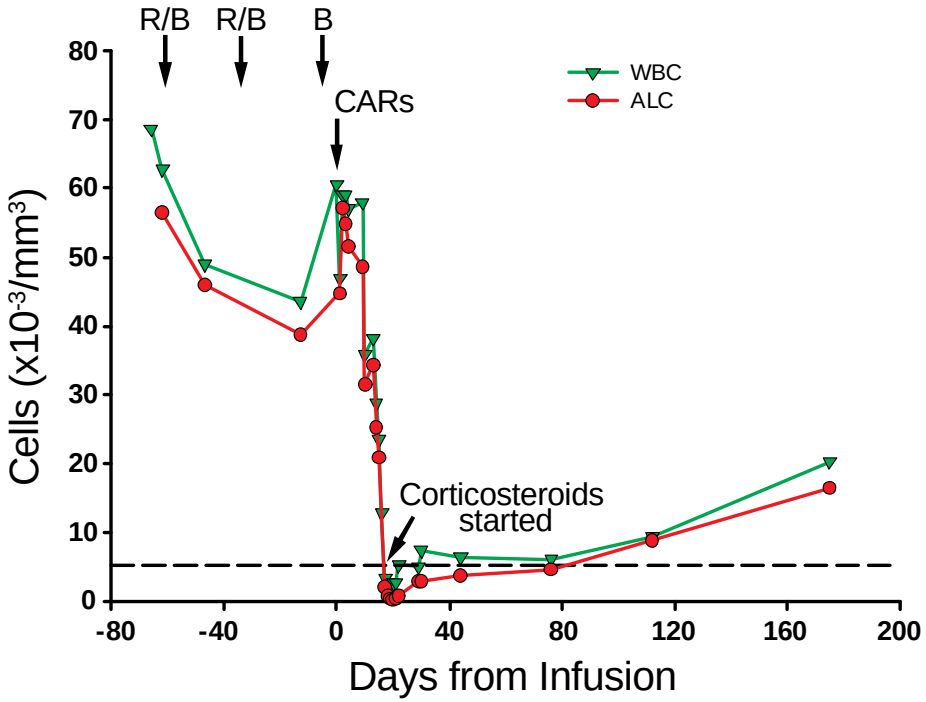


- 17p- patients show 3 year survival rate and are less responsive to current therapies
- 17p+ patients show 10 year survival rate and are well-responsive to current therapies

Rai, Kanti. Keating, Michael." Pathophysiology and cytogenetics of chronic lymphocytic leukemia, UpToDate, May 3rd, 2011.
Dohner, Hartmut. "Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia", The New England Journal of Medicine, December 28th, 2000

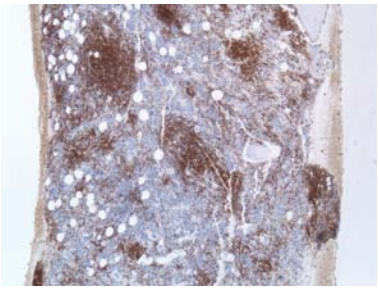
Clinical Responses: CD19 CAR Pilot Trial

UPN 02

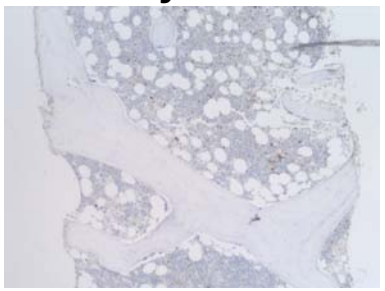


UPN 01

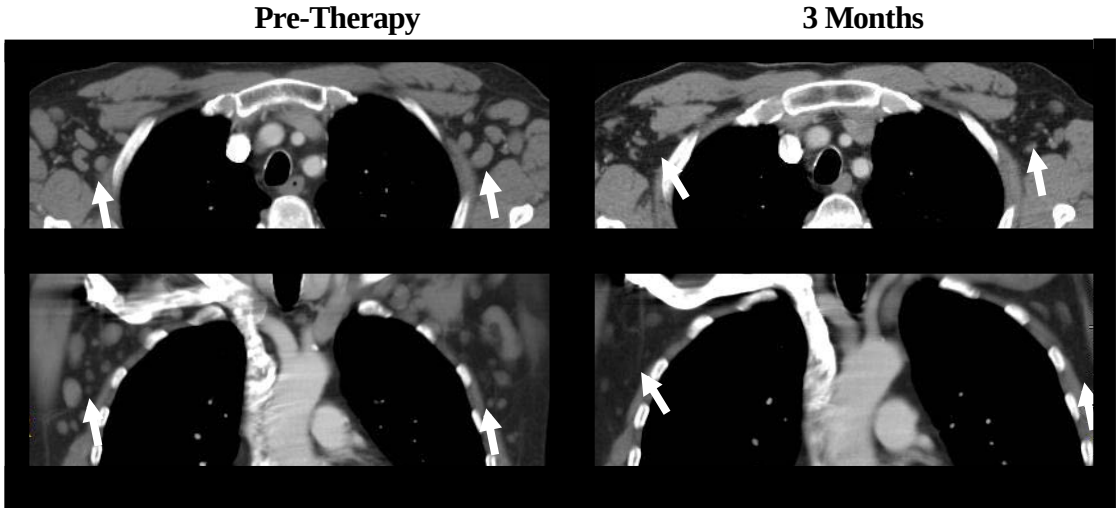
Day -21



Day 177



UPN 03



Clinical Responses:
Bulky Tumor Eradicated Following CART19 Infusion

Patient	Total Baseline Tumor Burden		Response
	# cells	tumor mass (pounds)	
UPN 01	2.51E+12	5.52	CR (+15 months)
UPN 02	3.48E+12	7.67	PR (12 months)
UPN 03	1.32E+12	2.90	CR (+13 months)

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Porter et al. New England Journal of Medicine. 2011;365(8):725-33.
Kalos et al. Science Translational Medicine. 2011 3(95): 95ra73

CART19 Cells: In Vivo Effector to Target Ratio "Serial Killer" T Cells

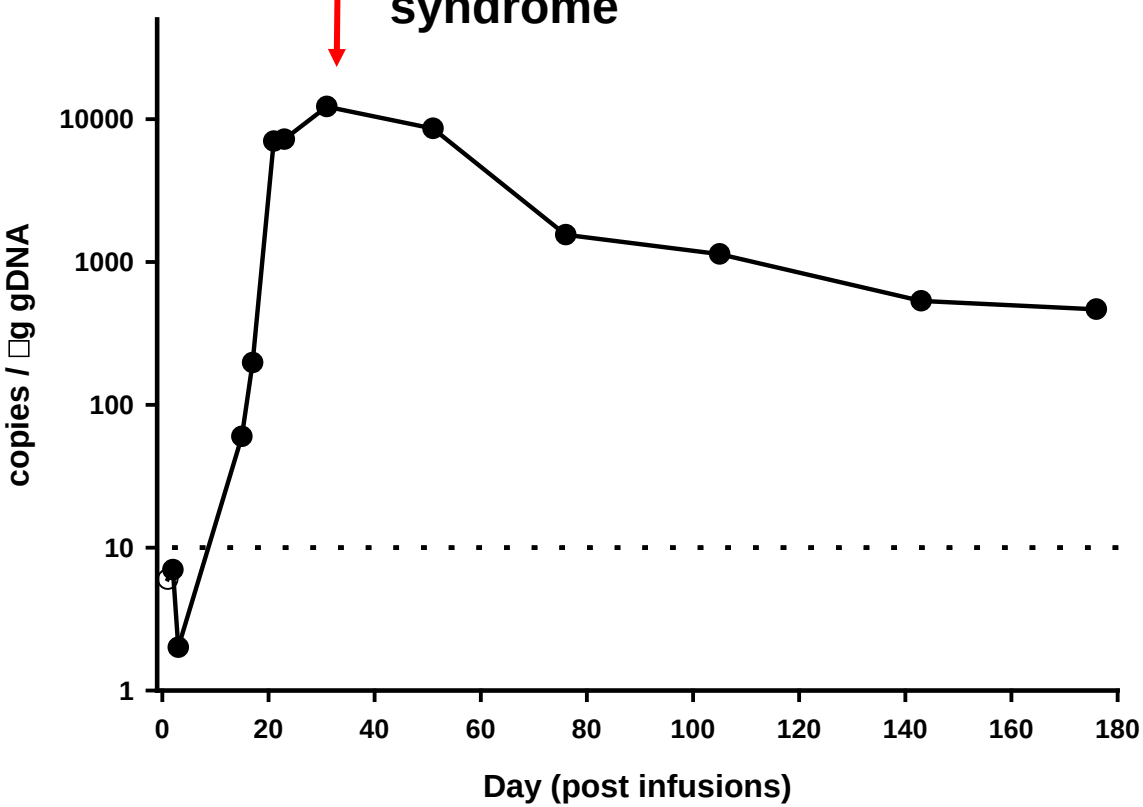
Patient	Tumor Burden (Baseline and Delta)				CART19^{+} cells Infused	In Vivo E:T
	Bone marrow Baseline	Blood Baseline	Nodes/Spleen[1] Baseline	Total Change in CLL Burden		
UPN 01	1.70E+12	N/A	8.1E+11	2.51E+12	1.13E+09	1:2200
UPN 02	3.20E+12	2.75E+11	1.6E+12	5.81E+11[2]	5.80E+08	1:1000
UPN 03	8.80E+11	N/A	4.4E+11	1.32E+12	1.42E+07	1:93,000
					Range	1000 - 93,000

CAR Persistence and Expansion in PBMC: subject #3

CART-19 persistence and trafficking blood:

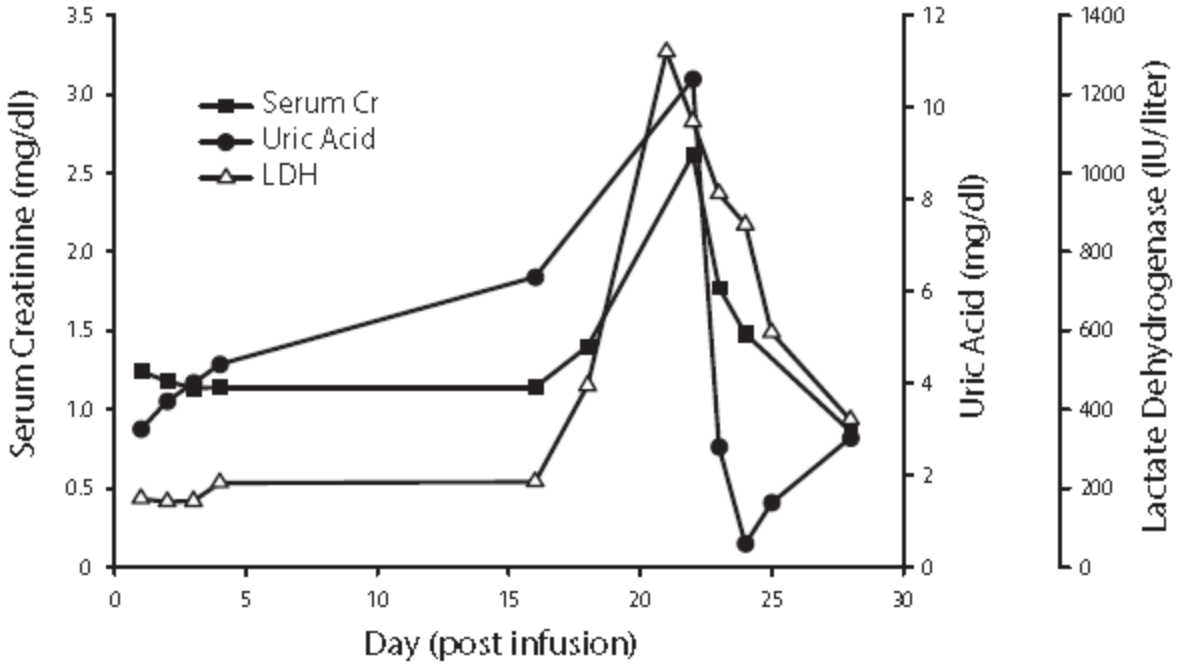
Hospitalized for tumor lysis syndrome

=> First case of delayed tumor lysis syndrome



Timepoint	% marking
D-1	0.00
D0 post infusion	Below limit****
D+1	Below limit****
D+2	0.01
D+3	0.00
D+21	9.7
D+23	10.7
D+28	11.9

Autologous CART-19 Study Status: Subject 3
Delayed onset tumor lysis syndrome



Porter et al. NEJM, 2011

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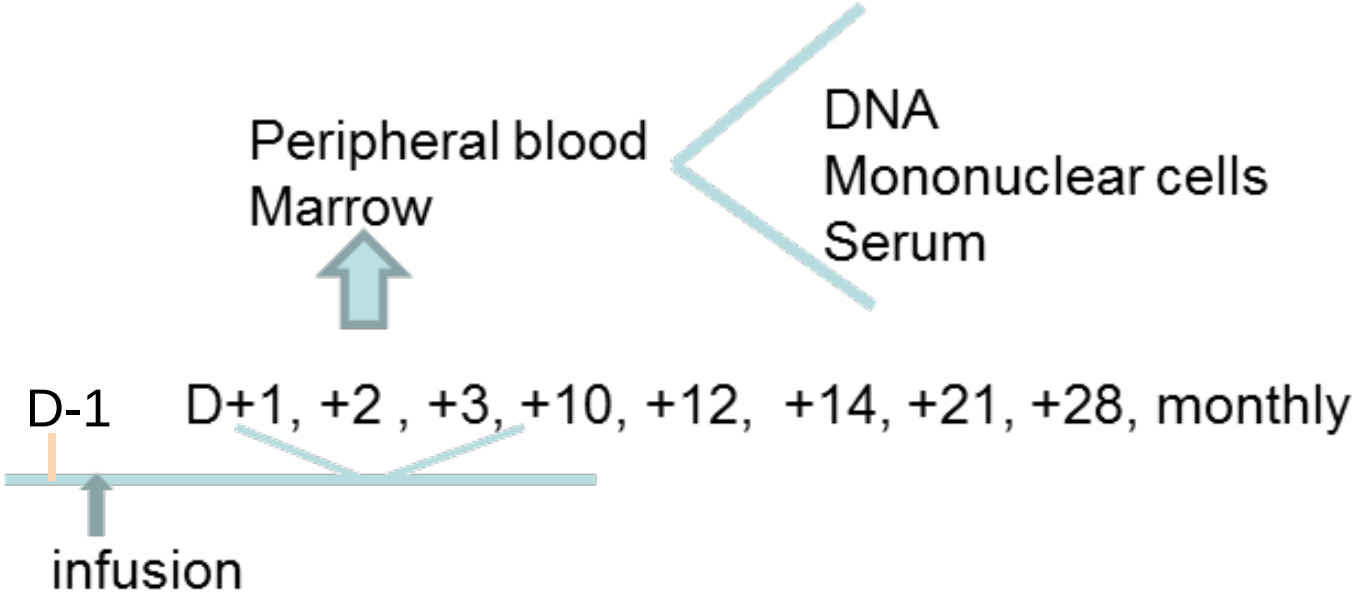
Secondary endpoints: Research Assessments



Aphereses:
Pre-infusion
Month 3

Secondary endpoints:

- Persistence: Q-PCR
- Cytokine modulation: Luminex
- Ex-vivo immunophenotyping *
- Ex vivo functional assays *

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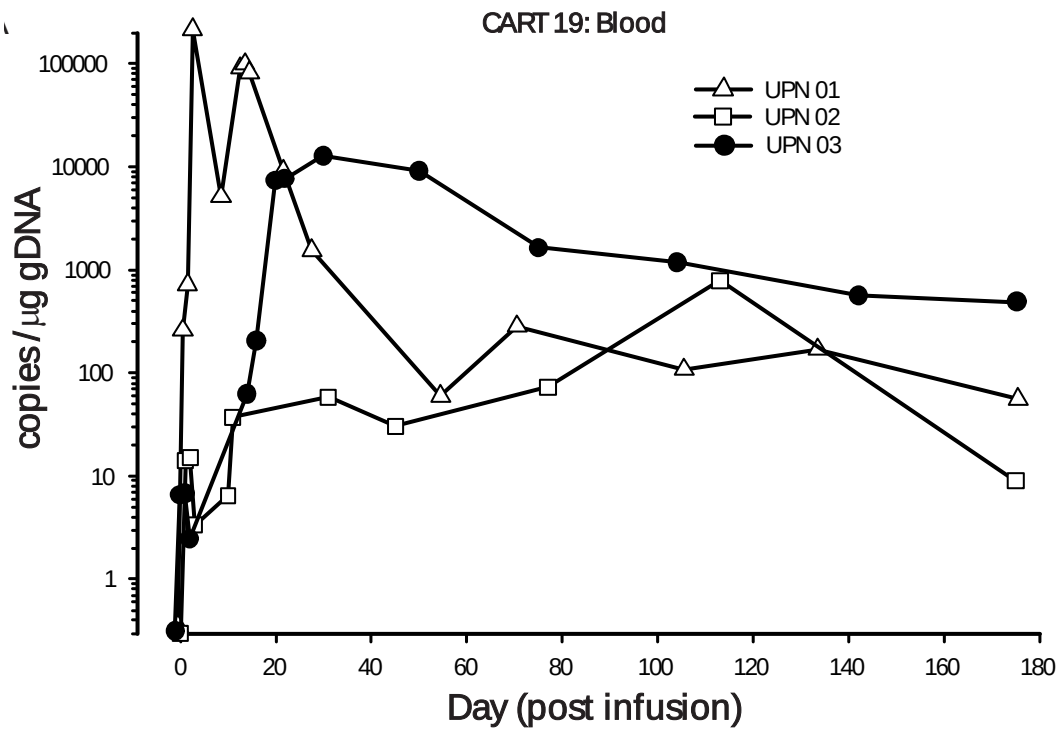
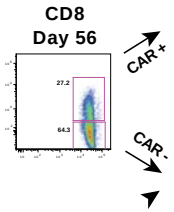
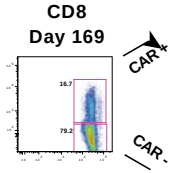
Pharmacology and Pharmacokinetics of CART-19







1. CAR moiety expressed for at least 6 months
2. Sustained antibody delivery with a single infusion of engineered T cells

CART19 cells proliferate
2 to 4 log10 in all patients in vivo

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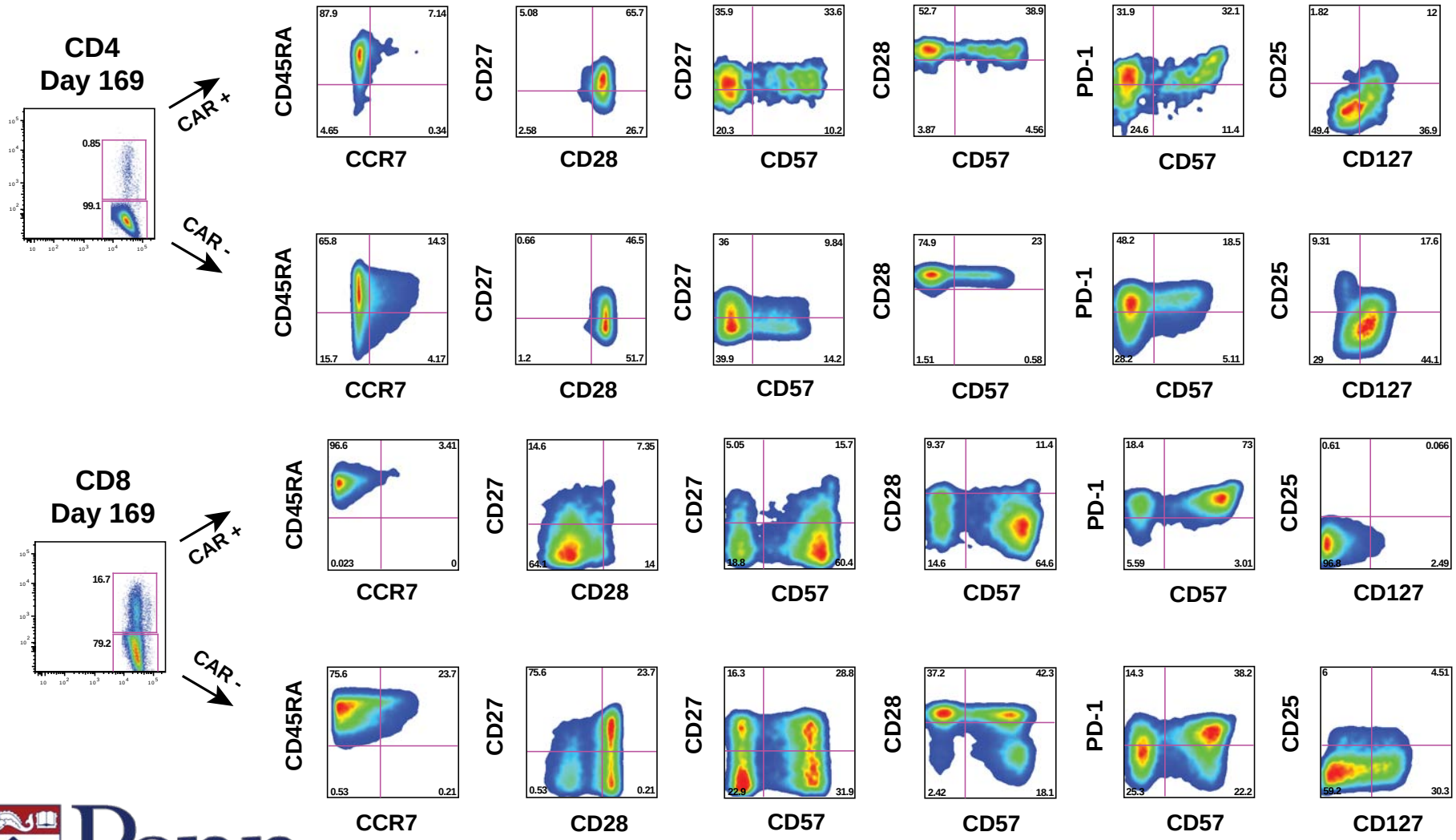
Evolution of CAR+ T Cells In Vivo: UPN 03 at 6 months



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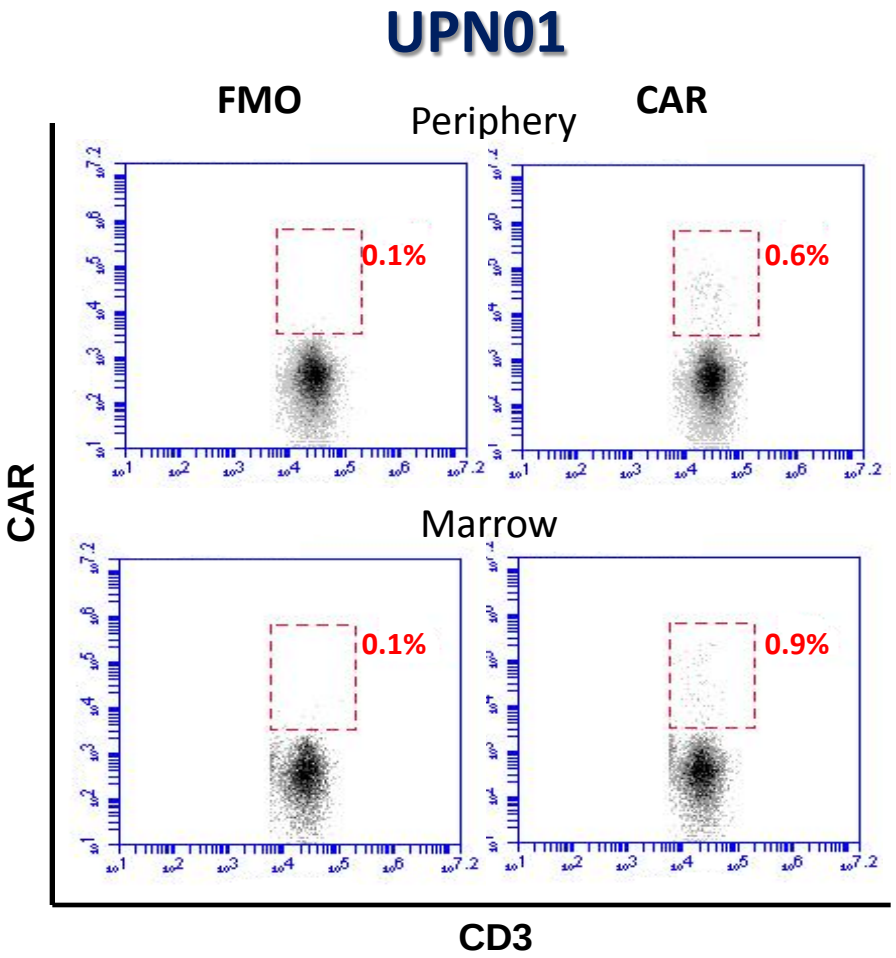
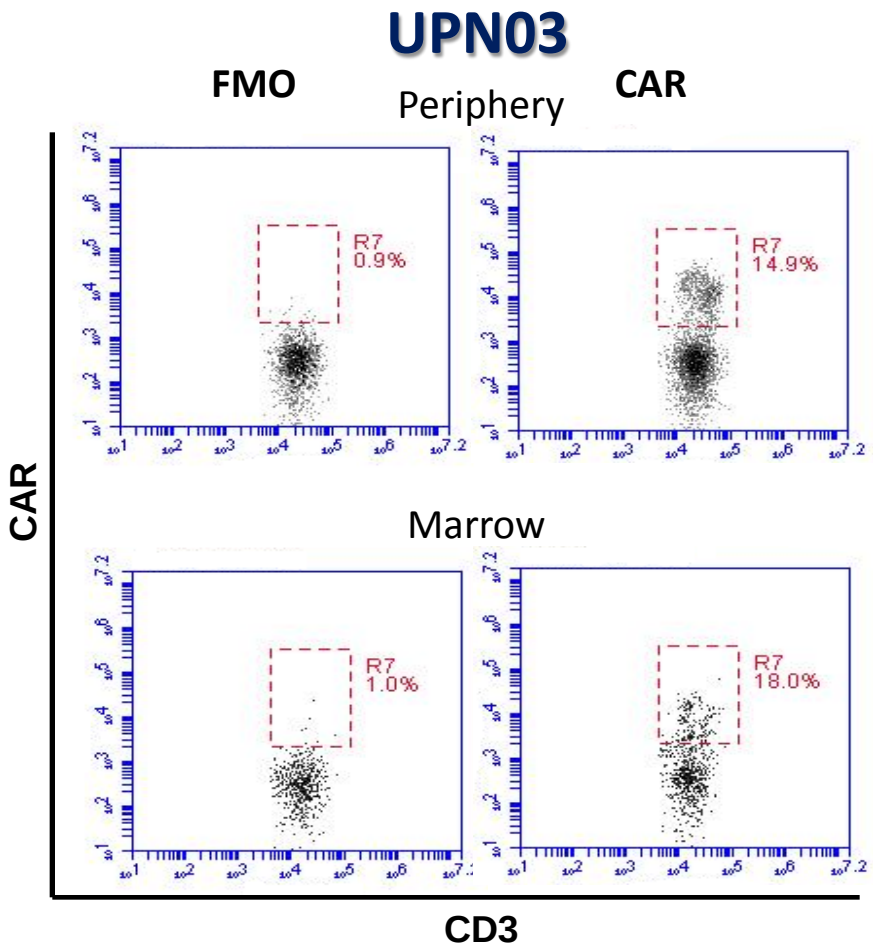
Kalos et al, Science Translational Medicine, 2011

Persistence- flow cytometry

CART-19 cells can be detected in periphery and marrow at 12 months-post infusion





Anti-CAR19 idiotype reagent courtesy of B. Jena and L. Cooper, MDACC

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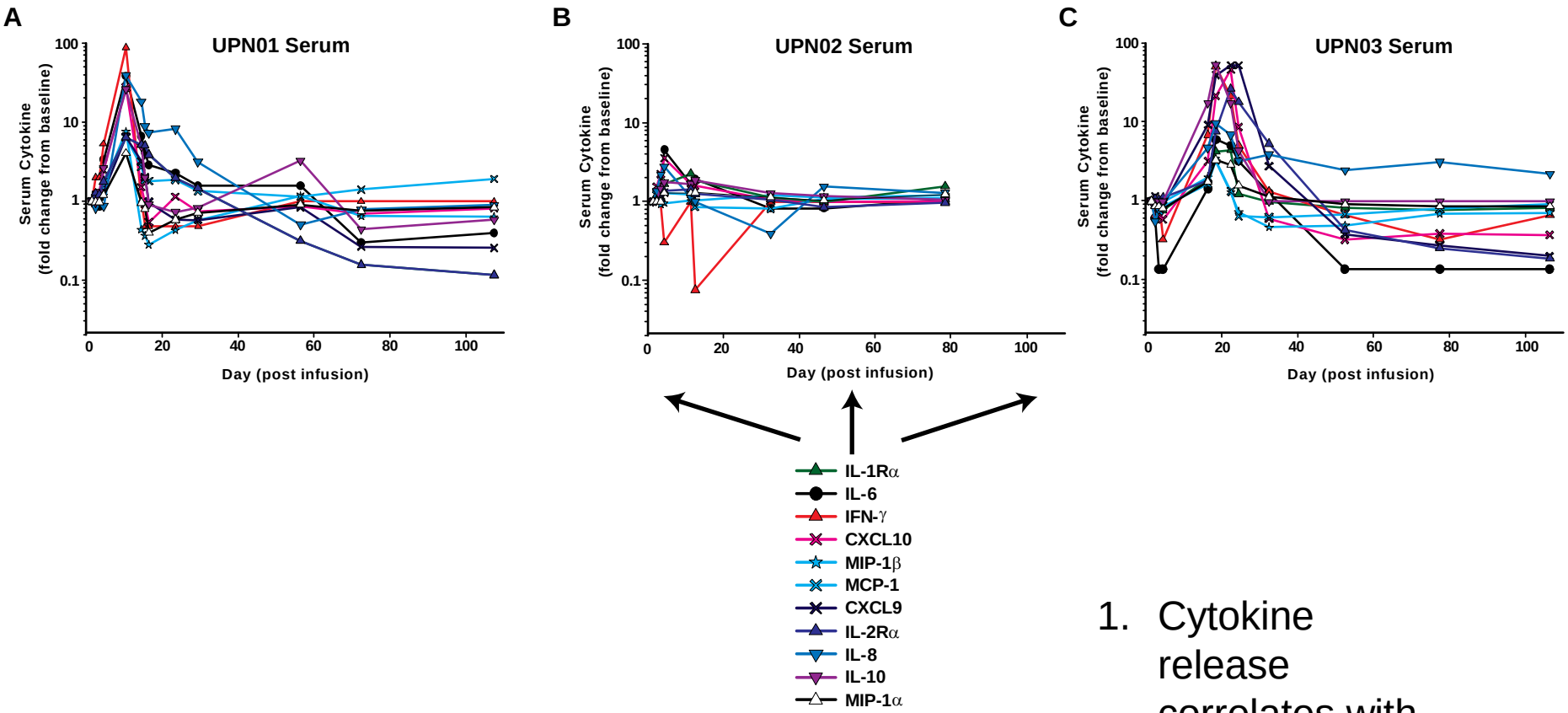
Pharmacology and Pharmacokinetics of CART-19



1. Cytokine release correlates with tumor lysis
2. No cytokine storm

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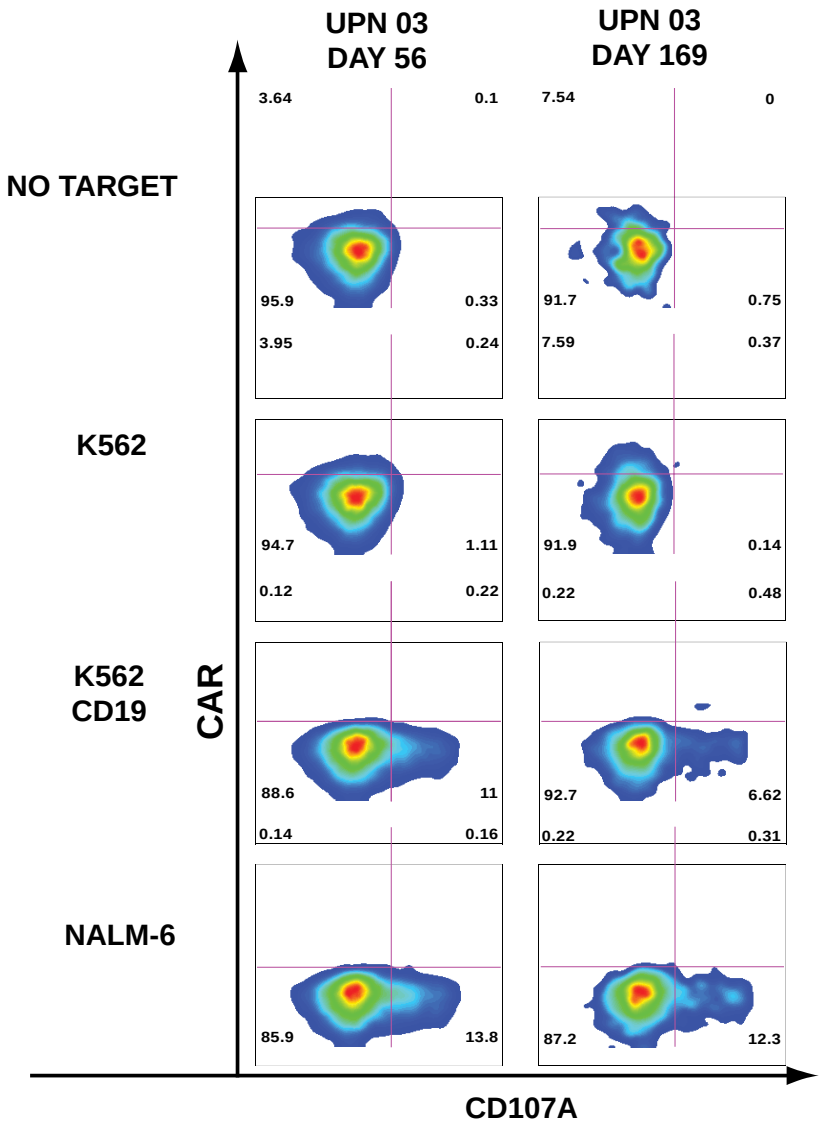
Long-term persisting UPN03 CART-19-cells retain anti-CD19 functionality directly ex-vivo



CAR antibody receptor functional for at least 6 months

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Deep sequencing to detect residual CLL and B cells

Patient ID	Time Point	Amount of DNA in PCR (ng)	Cell equivalents	Total Productive Sequence Reads	Total Productive Unique Clonotypes	Dominant Clone Reads	Clone Frequency (% Productives)
UPN03	Pre-Infusion	2,000	317,460	22,074,912	58,234	19,948,508	90.4
03	Day -1	386	61,270	1,385,340	4,544	1,231,018	88.9
03	D+31	1,000	158,730	0	0	0	0.000
03	D+176	2,000	317,460	0	0	0	0.000
UPN01	Pre-Infusion	1,000	158,730	184,786	24	184,256	99.7
01	Day -1	1,000	158,730	408,579	48	407,592	99.8
01	D+28	1,000	158,730	0	0	0	0.000
01	D+176	500	79,365	285,305	7,362	0	0.000

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Harlan Robins, Michael Kalos

CART19 Induced B Cell and Plasma Cell Aplasia

- B cell aplasia occurs in sygeneic mouse models treated with CD19 CARs (Brentjens)
- B cell aplasia first reported in humans after CD19 CARs (Kochenderfer, et al, Blood 116, 4099, 2010)
- In humans, CD19 is also expressed on plasma cells but not myeloma cells (Harada et al. Blood 81: 2658, 1993).
- Will CD19 CARs induce plasma cell aplasia?

Patient	Days after T cell infusion	% plasma cell ($CD138^+$)
UPN 01	-23	1%
	+39	0%
	+116	0%
	+174	0%
UPN 02	-45	2-3%
	-3	2-3%
	+188	1%
UPN 03	-3	3-5%
	+21	1-2%
	+174	1-2%

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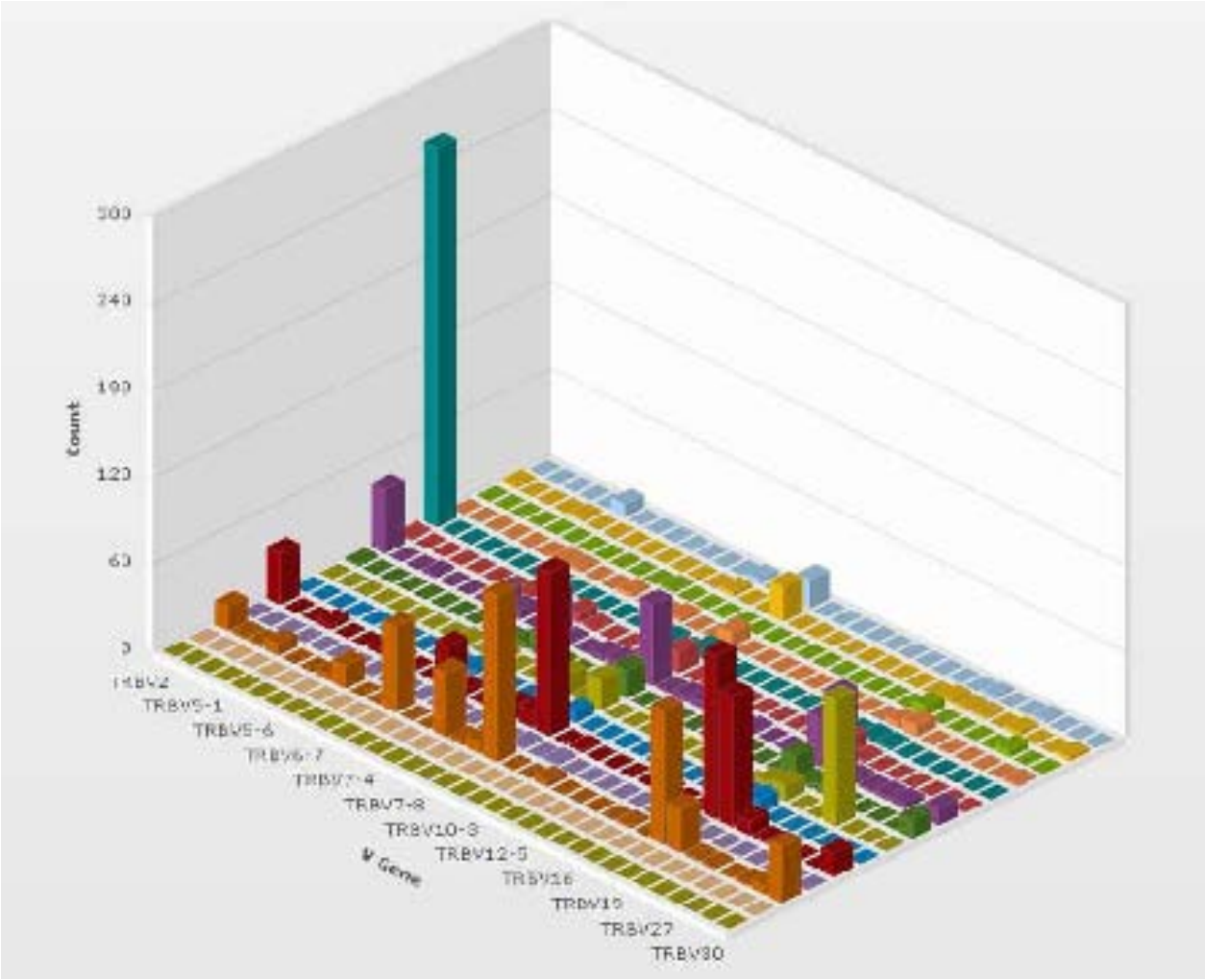
Deep sequencing reveals a polyclonal repertoire of CAR-modified CD8+ T cells



9 clonotypes >1%
20 clonotypes >0.1%

TCSL

PENN UNIVERSITY of PENNSYLVANIA

ABRAMSON family CANCER RESEARCH INSTITUTE

Summary: CD19 CAR T Cells

- CAR T cells have promise in B cell malignancies. Example of synthetic biology: “performance enhancing” drug
- On target toxicities (expected):
  - Short term: inflammatory syndrome
  - Long term: B cell / plasma cell aplasia
  - Long term safety is unknown
- Persisting CART19 cells (both CD8+ and CD4+) maintain surface expression of CAR antibody, have functionally evolved to include a memory subset, and maintain robust effector function in vivo for at least 1 year
- Questions
  - Will CD19 CARs have efficacy in other hematologic malignancies? Adenocarcinoma?
  - Can CARs trigger spreading immunity?

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