CAR T: NOW AND NEXT GENERATION

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Disclosures

Carl H. June, MD, has affiliations with Novartis *(Royalty and Contracted Research)*; Tmunity Therapeutics *(Ownership Interest)*; Patents with CAR T cells *(Receipt of Intellectual Property Rights/Patent Holder)*.

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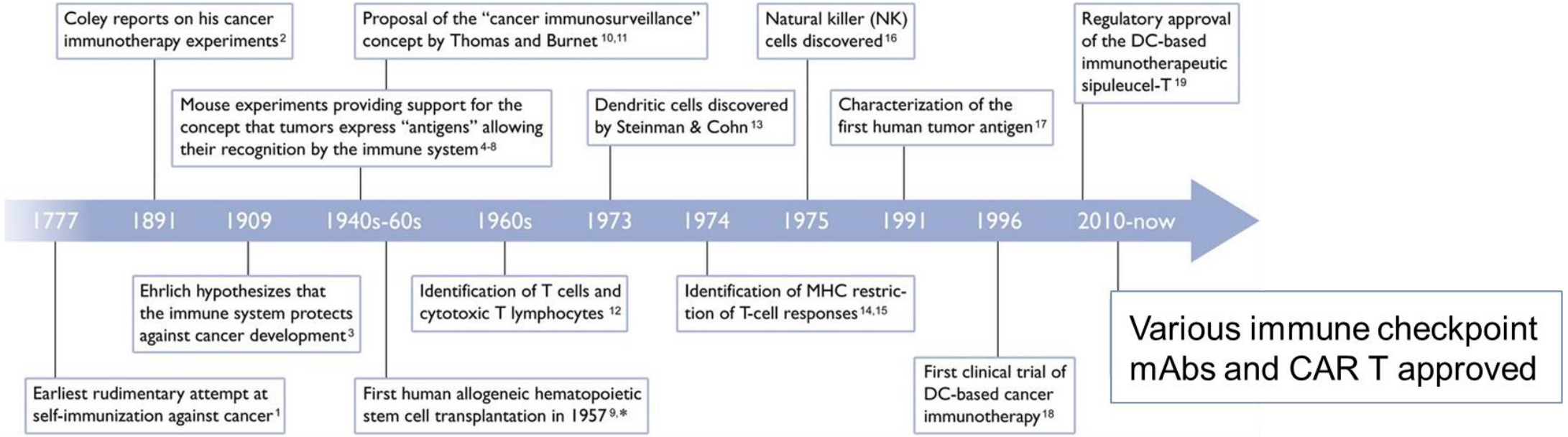
Cancer Immunotherapy: Overnight Sensation 100 years in the Making Checkpoints and CAR T



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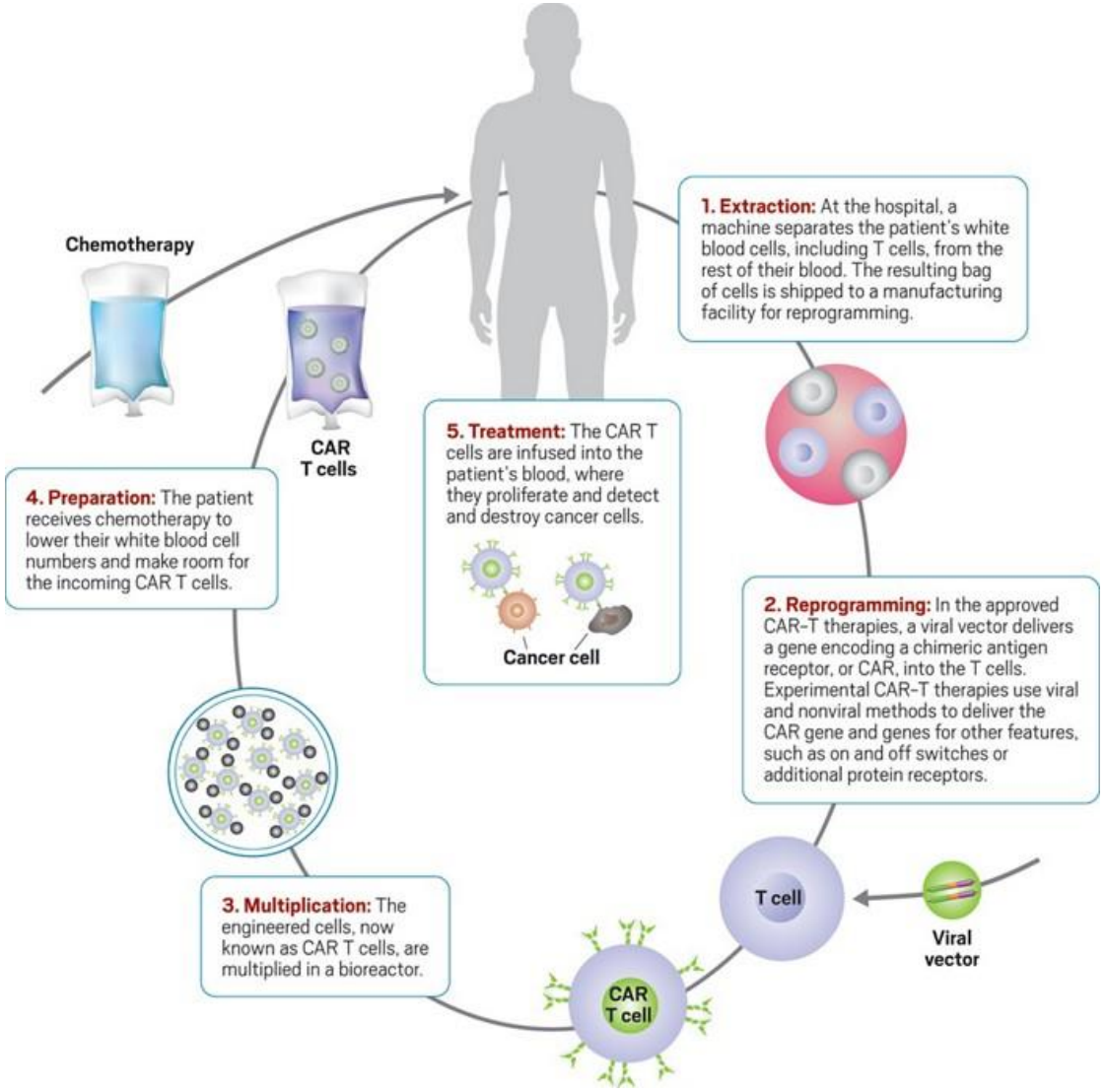
Anguille S, et al. *Pharmacol Rev*. 2015;67(4):731-753.

CAR T Cell Therapy: A Process Not a Drug



- Autologous T cells
- Allogeneic "3rd party" T cells
  - Cord blood
  - Healthy donor
  - iPSC

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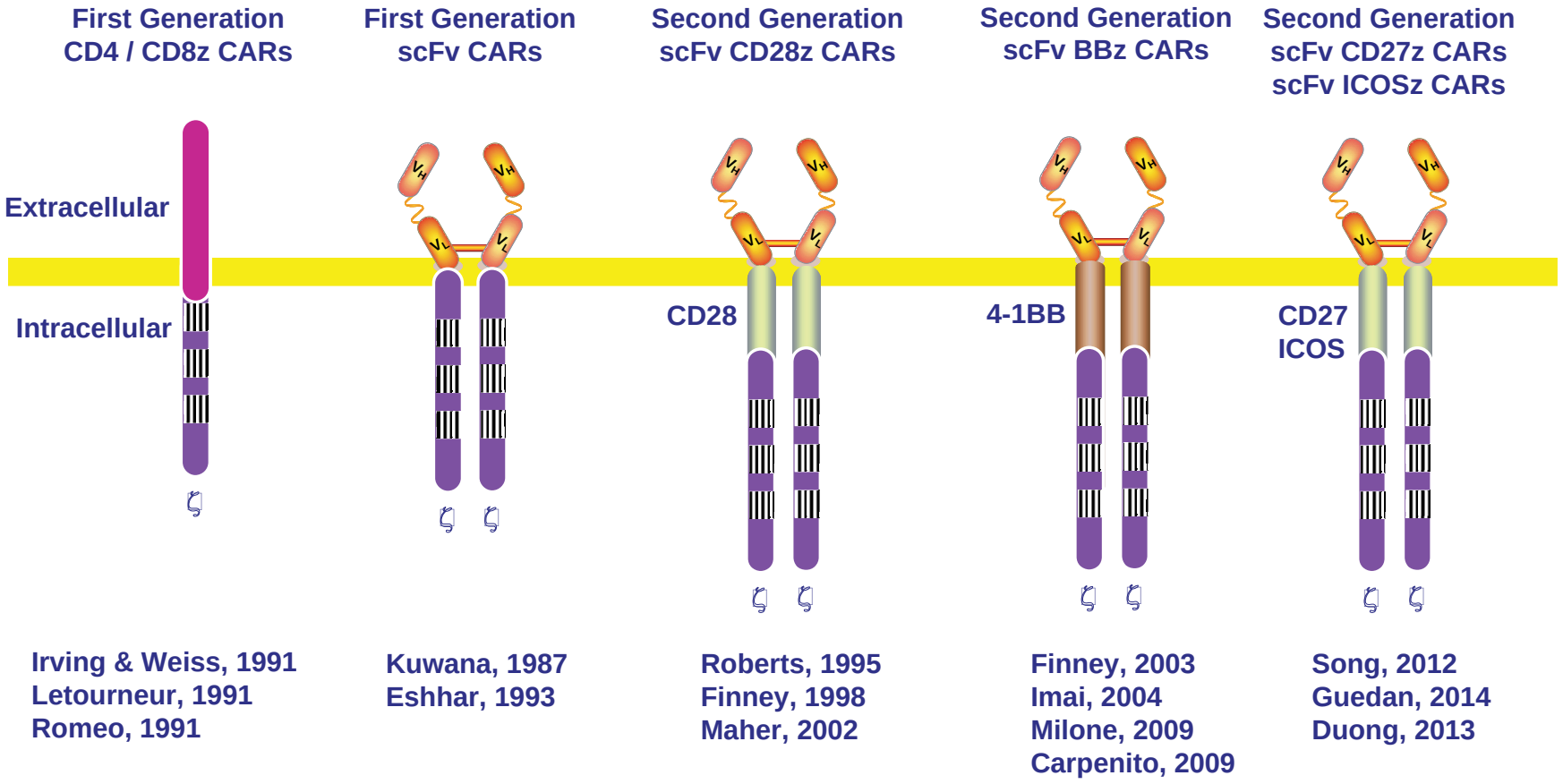
Chemical & Engineering News: https://cen.acs.org/pharmaceuticals/oncology/Controlling-CAR-T-scientists-plan/96/i19. Accessed October 26, 2018.

Using Synthetic Biology to Overcome Tolerance Creation of Bi-specific CAR T cells

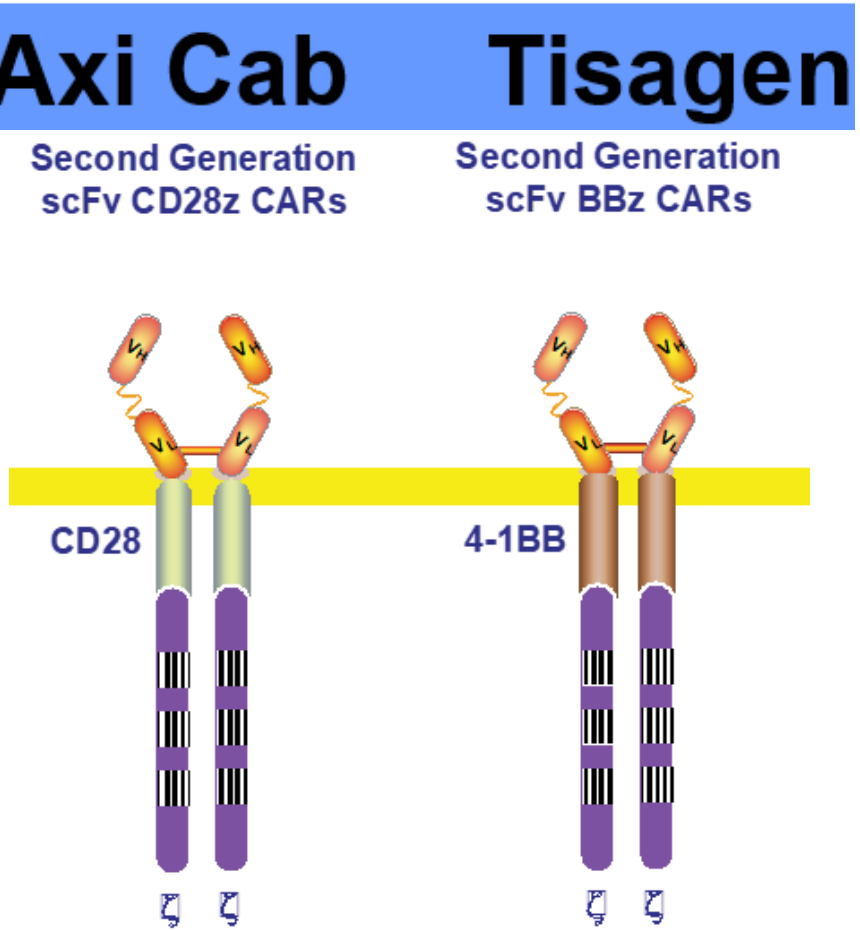
Axi Cab **Tisagenlecleucel**



Design of CAR T Cells

Design Features of Axi Cab and Tisagenlecleucel: Differential Persistence

Attribute	Tisagenlecleucel	Axi Cab
Vector	Lentiviral	Retroviral
Promoter	EF1a	MSCV
ScFv (CD19)	FMC63	FMC63
Signaling Domain	4-1BB zeta	CD28 zeta
Hinge and TM	CD8 alpha	CD28
Cell Culture	Frozen CD3/28 beads	Fresh PBMC/CD3
CAR T Persistence	Long term >1 to 7 years	Short term (<6 weeks)

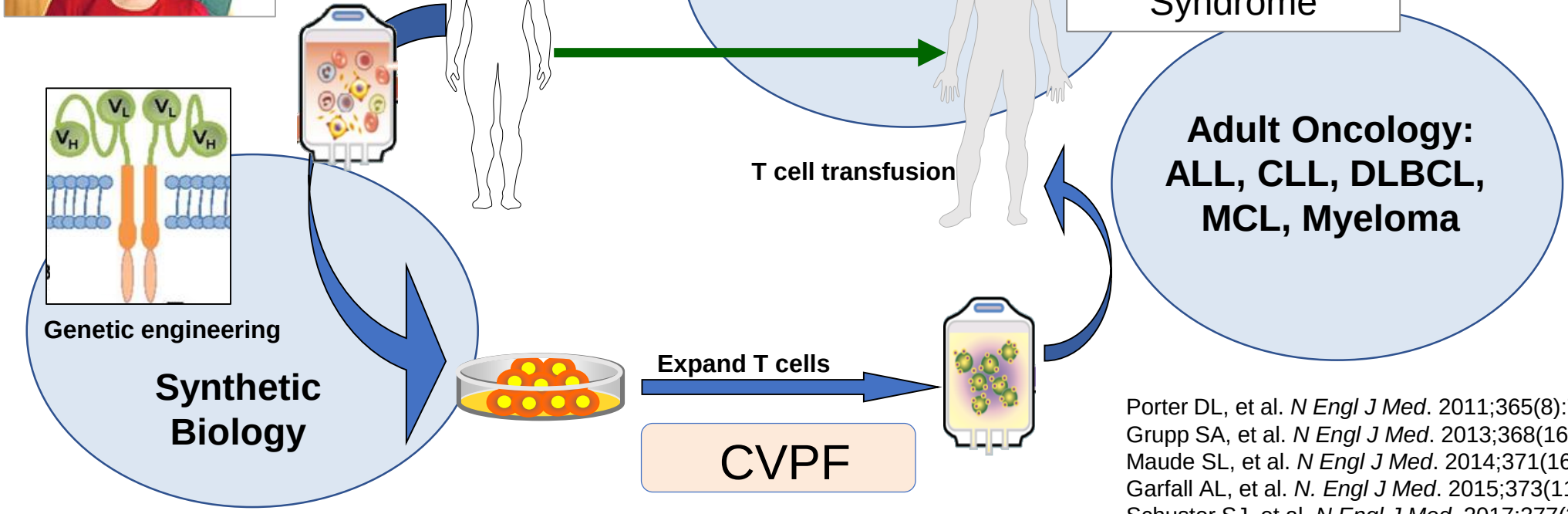


CT019 (tisagenlecleucel)

The NEW ENGLAND JOURNAL of MEDICINE

July 31, 2010
1st CART19 Infusion

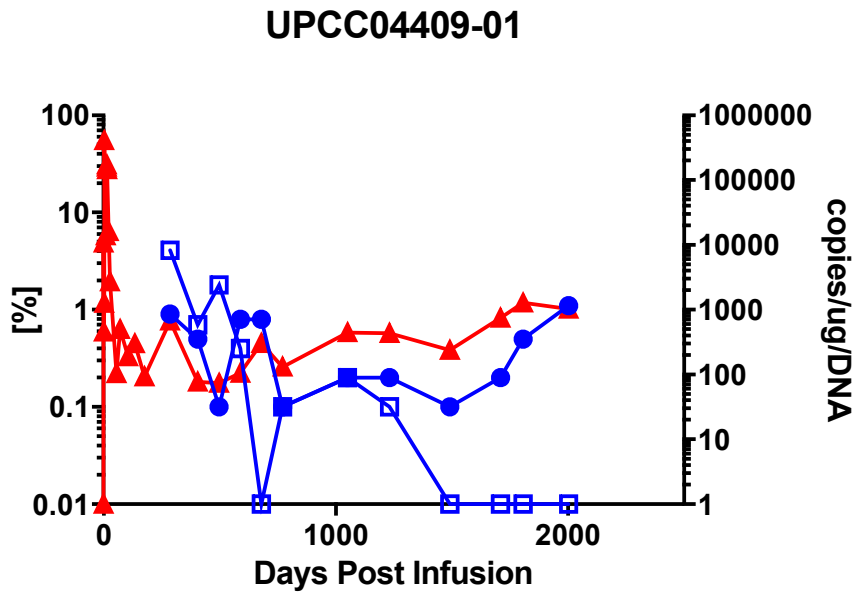
The New York Times

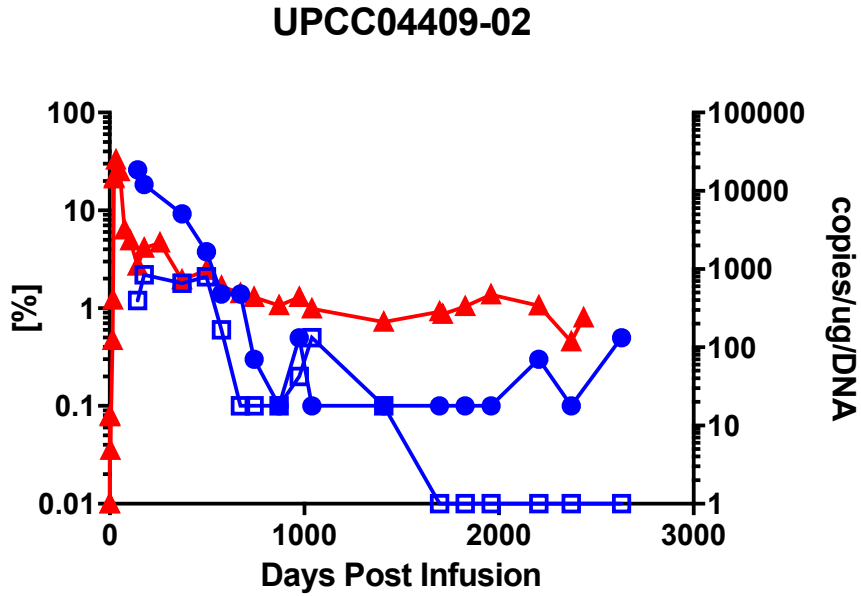


Porter DL, et al. *N Engl J Med*. 2011;365(8):725-733;
Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518;
Maude SL, et al. *N Engl J Med*. 2014;371(16):1507-1517;
Garfall AL, et al. *N. Engl J Med*. 2015;373(11):1040-1047;
Schuster SJ, et al. *N Engl J Med*. 2017;377(26):2545-2554;
Maude Sl, et al. *N Engl J Med*. 2018;378(5):439-448.

FDA approval August 30, 2017

Long-Term Persistence of CTL019 in CLL Patients





Note: For Flow 0 = 0.01; qPCR 0 = 1

CAR B cell qPCR

1. 7-year survival and function of CTL019 in patients 1 and 2
2. First “living drug”

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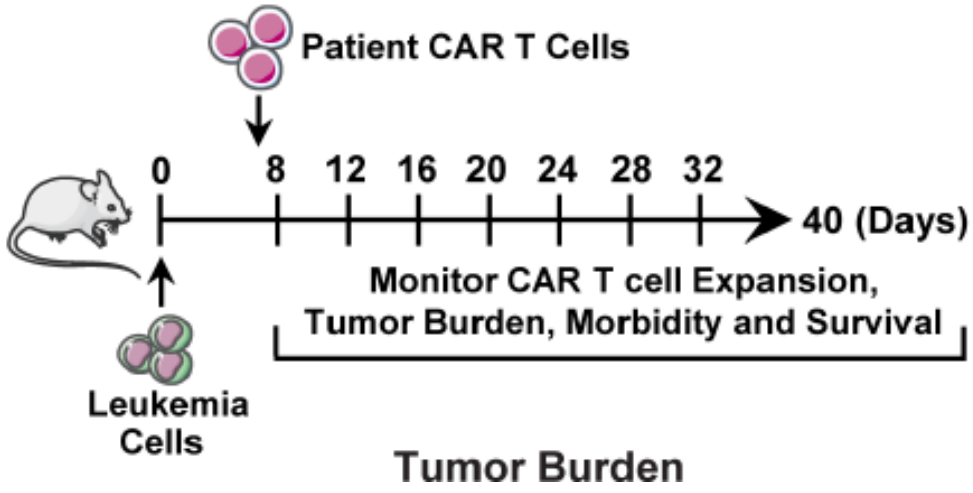
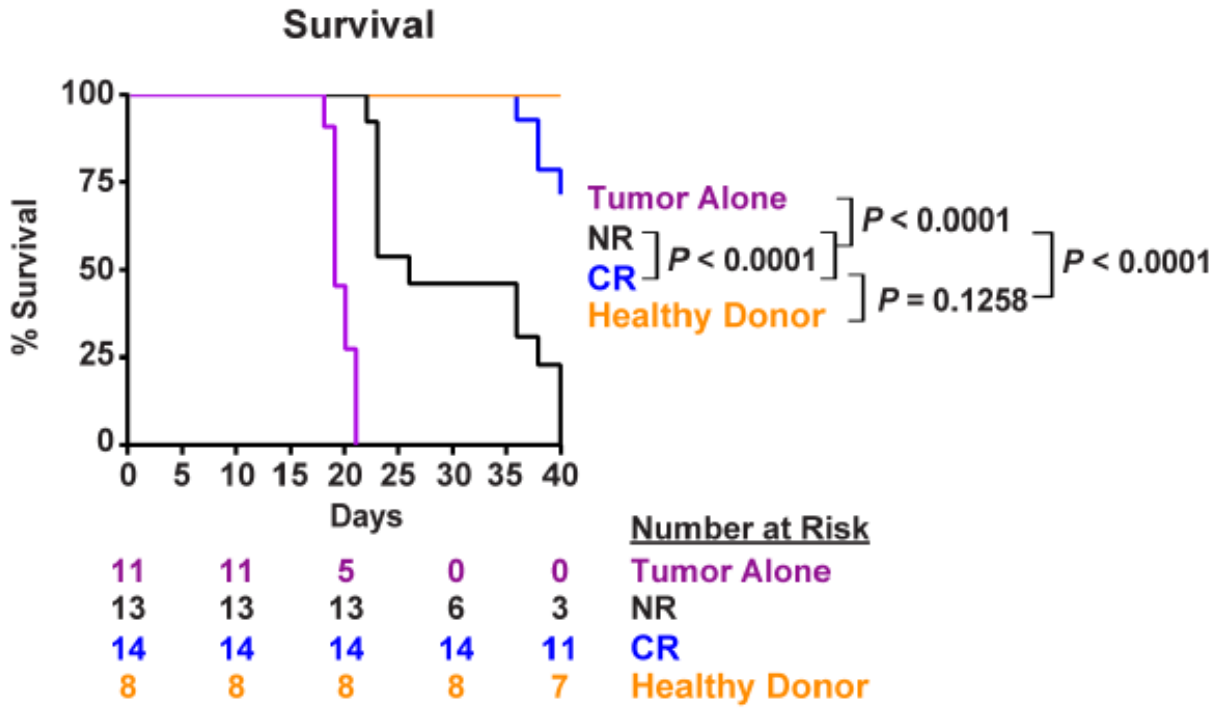
Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571; Imai C, et al. *Leukemia.* 2004;18(4):676-684; Milone MC, et al. *Mol Ther*. 2009;17(8):1453-1464; Carpenito, et al. *Proc Natl Acad Sci U S A.* 2009;106(9):3360-3365.

Characterization of CLL CAR T Cells in NSG CLL Model





Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571; Imai C, et al. *Leukemia*. 2004;18(4):676-684; Milone MC, et al. *Mol Ther*. 2009;17(8):1453-1464; Carpenito C, et al. *Proc Natl Acad Sci U S A*. 2009;106(9):3360-3365.

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Characterization of Long-Term CAR T Cells in CLL Patients

- Transcriptomic profiling revealed that CAR T-cells from complete responding CLL patients were enriched in memory-related genes, including IL-6/STAT3 signature
- CAR T-cells from non-responders upregulated programs involved in effector differentiation, glycolysis, exhaustion, and apoptosis.
- Sustained remission was associated with an elevated frequency of CD27+CD45RO- CD8+ T-cells in baseline blood samples
- CD27+PD-1- CD8+ CAR T-cells expressing high-levels of IL-6R predicts therapeutic response and is responsible for tumor control.

Nature Medicine, 2018; Imai C, et al. *Leukemia*. 2004;18(4):676-684; Milone MC, et al. *Mol Ther*. 2009;17(8):1453-1464; Carpenito C, et al. *Proc Natl Acad Sci U S A*. 2009;106(9):3360-3365.

CAR T Cells for Relapsed/Refractory Acute Lymphoblastic Leukemia

Hematologic Malignancies:

- CD19 Phase I/II trials at the University of Pennsylvania

  CLL[1]
  ALL[2]
  ALL[3]
  Myeloma[4]
  DLBCL[5]
  ALL[6]

85% of children are cured with intensive chemotherapy and radiation. However, major long-term disability from chemoradiotherapy:

- The 2-year survival for twice relapsed pediatric ALL is <2 years
- Tisagenlecleucel (Kymriah™) induced complete remissions in <85% young adults and children with multiply relapsed ALL
- FDA approved August 2017

1. Porter DL, et al. *N Engl J Med*. 2011;365(8):725-733.
2. Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518.
3. Maude SL, et al. *N Engl J Med.* 2014;371(16):1507-1517.
4. Garfall AL, et al. *N. Engl J Med.* 2015;373(11):1040-1047.
5. Schuster SJ, et al. *N Engl J Med*. 2017;377(26):2545-2554.
6. Maude Sl, et al. *N Engl J Med.* 2018;378(5):439-448.

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6
years
cancer
free!

Pediatric CART19 ALL Study
PI: Stephan Grupp, MD, PhD

- Subject #1: 7yoF pre-B ALL. Karyotype: high risk
- Dx May 2010: Standard COG ALL induction
- Relapse #1: 10/2011
- Relapse #2: 2/2012
- 3/2012: High-dose cytoxan/clofaribine: persistent ALL
- Marrow 4/16/2012: 60% blasts w/kidney, liver, spleen lesions
- Autologous CART19: 4/17/2012
- Total dose CART19: 1.2 $x10^7$ CAR cells/Kg
- CAR T cells infused with no additional chemotherapy

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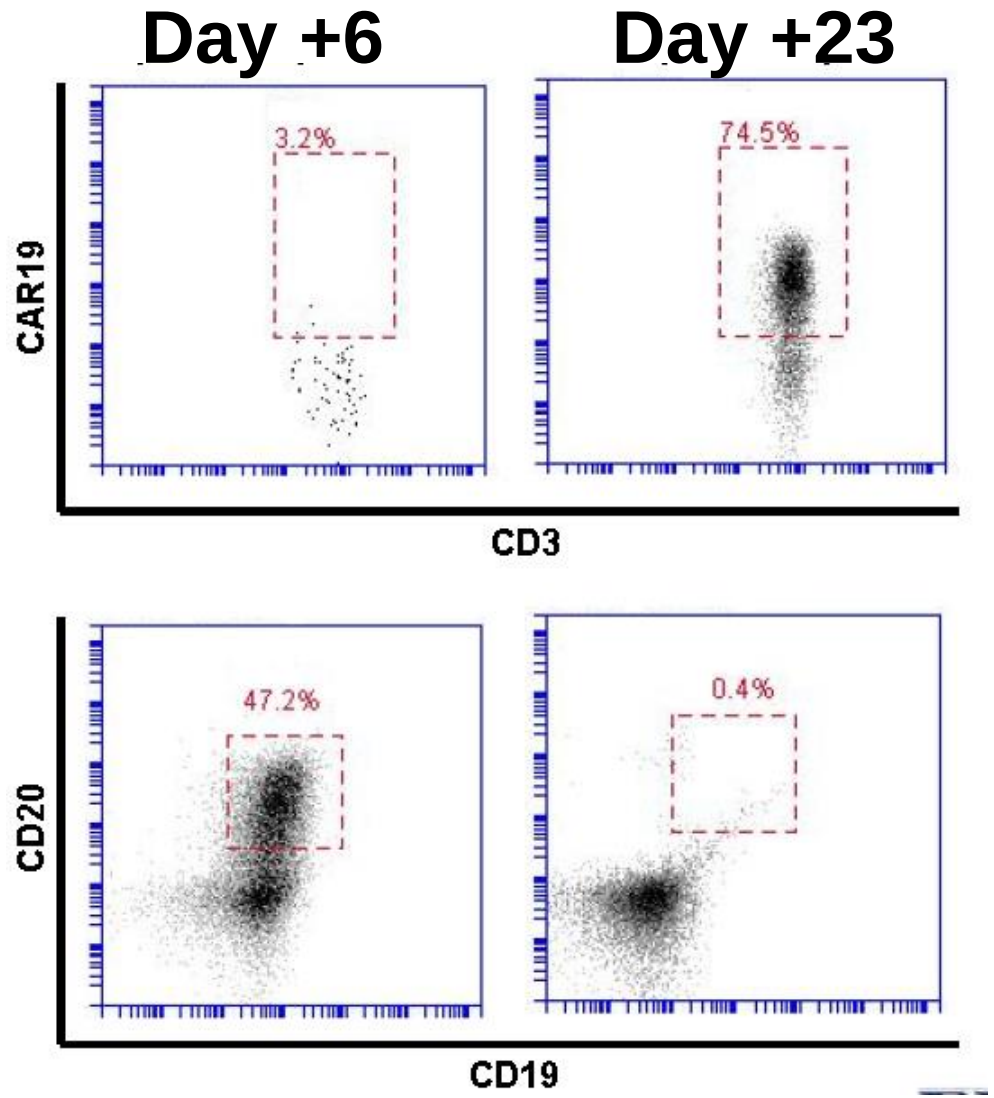
First Pediatric ALL Patient



- Deep remission induced in 23 days
- 0% blasts seen
- Flow MRD negative
- CR maintained >5 yrs

Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518.
Sotillo E, et al. *Cancer Discov.* 2015;5(12):1282-1295.

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Considerations for T Cell Therapy

- 1 kg of tumor = 10^{12} cells
- Our first 3 patients had 3 to 7 lbs of tumor!
- It is not realistic to expect tumor eradication unless the killing machinery (T, NK, macrophage) is equivalent to tumor burden, ie, "E:T" ratio ~= 1
- Failure to achieve critical mass of T cells likely explains previous trials with disappointing results
- Two potential solutions:
  - Infuse huge numbers of T cells (TILs)
  - Infuse small numbers of T cells programmed to divide

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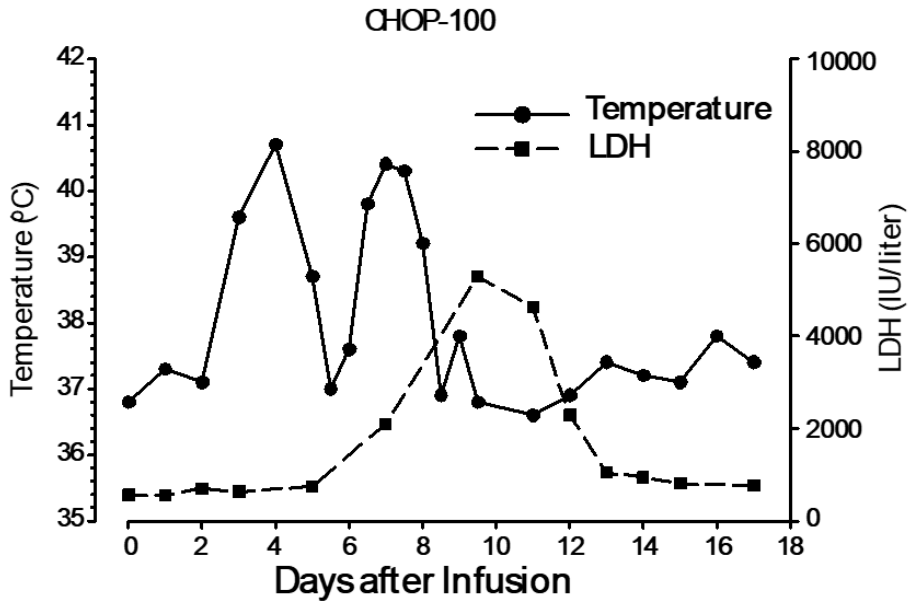
Cytokine Release Syndrome (CRS)

- High fever, flu-like syndrome
- Severe CRS – unstable hypotension, can proceed to need for mechanical ventilation
- Only occurs in responding patients:

  <u>On-target toxicity</u>



Steroids

Etanercept, Tocilizumab

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Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518.

Tocilizumab (Actemra)

- Interleukin 6 (IL-6) receptor antagonist
- Blocks IL-6 mediated effects
- Indicated in:
  - Juvenile idiopathic arthritis(JIA)
  - Rheumatoid arthritis (RA)
  - In Japan, indication for Castleman’s disease
- Typically given monthly
- Rare side effects of transaminitis and neutropenia
- Co-labeled with CD19 CAR T

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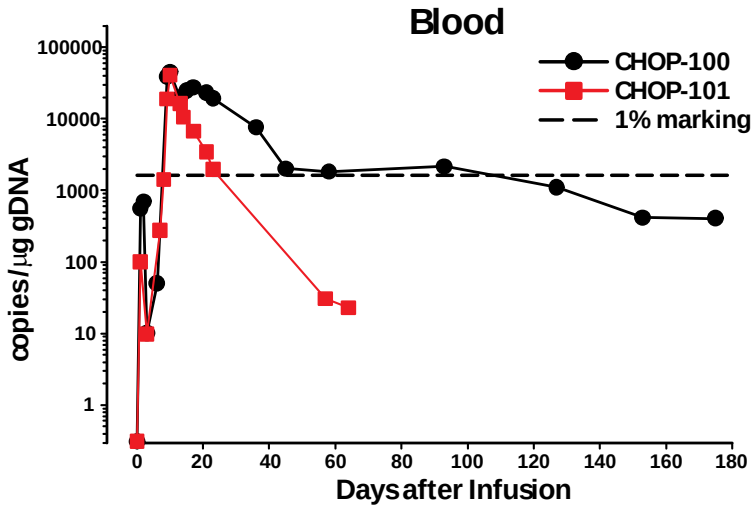
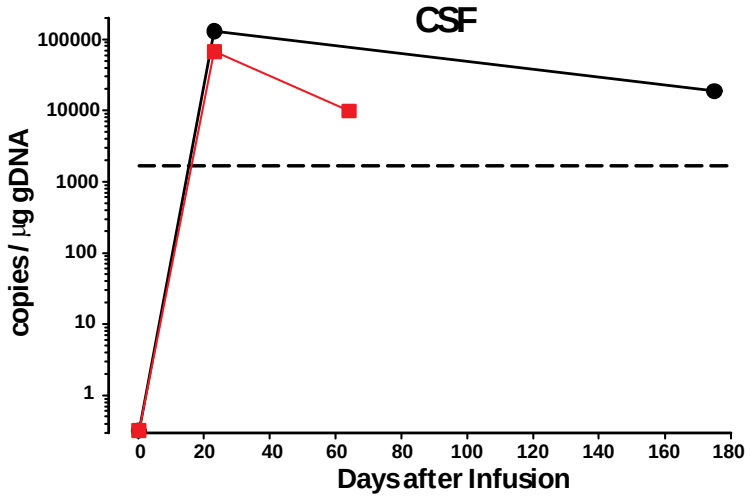
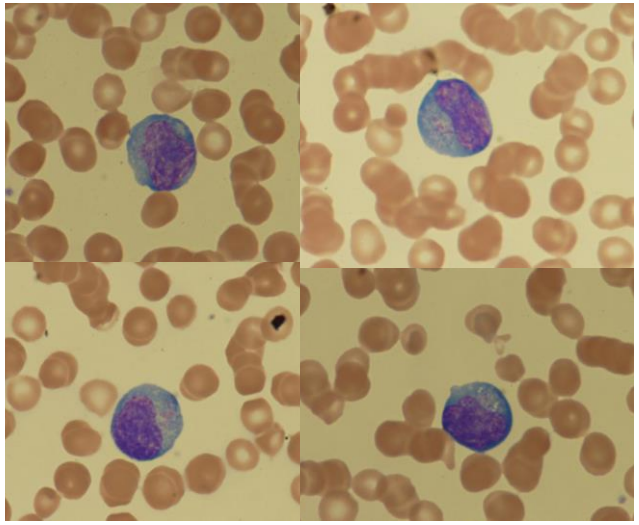
Efficient Trafficking of CTL019 T Cells to CNS in ALL



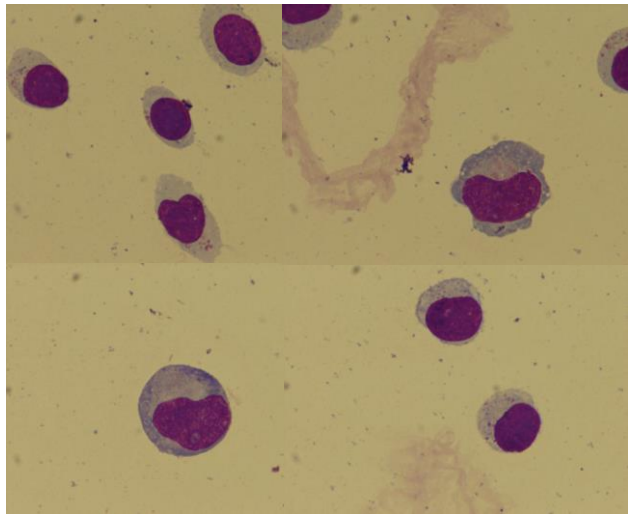


Morphology of CARs *in vivo*

Blood
Day 10



CSF
Day 23



Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518.

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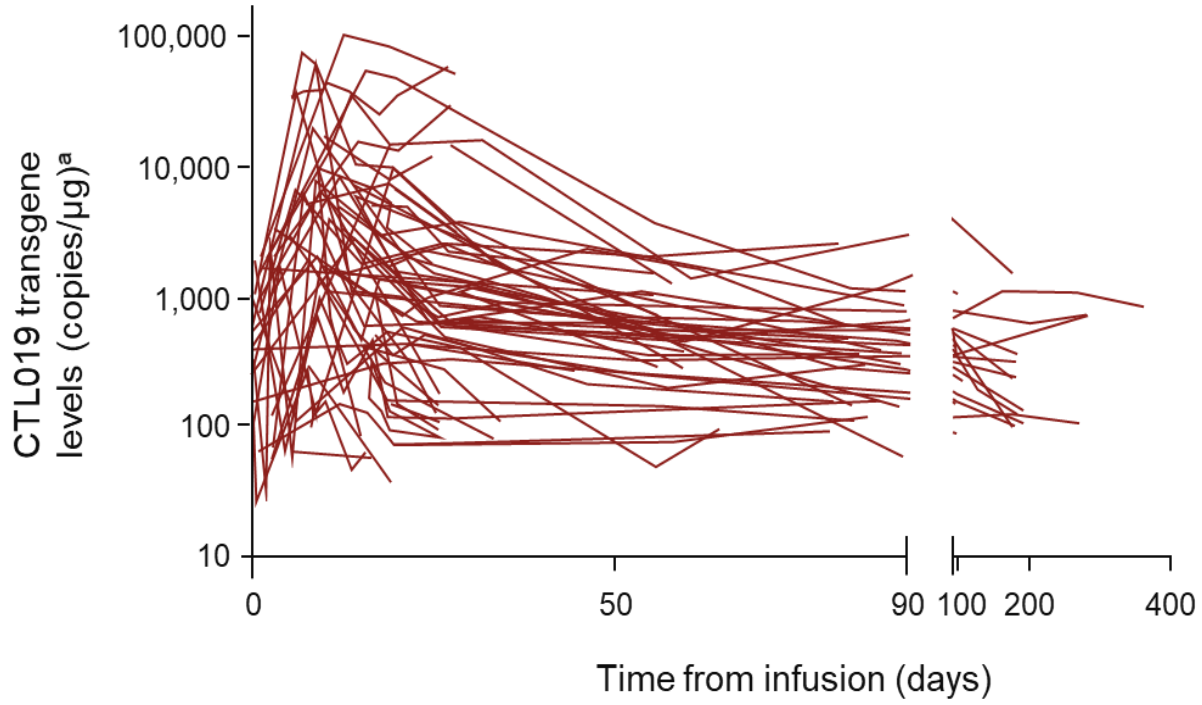
Cellular Kinetics of Tisagenlecleucel

- CTL019 transgene levels were observed to undergo significant expansion and demonstrated measurable persistence *in vivo* >1 year
- Persistence and expansion similar to ALL and CLL patients



[a] Includes all patients with ≥1 quantifiable transgene level post infusion (N = 83).

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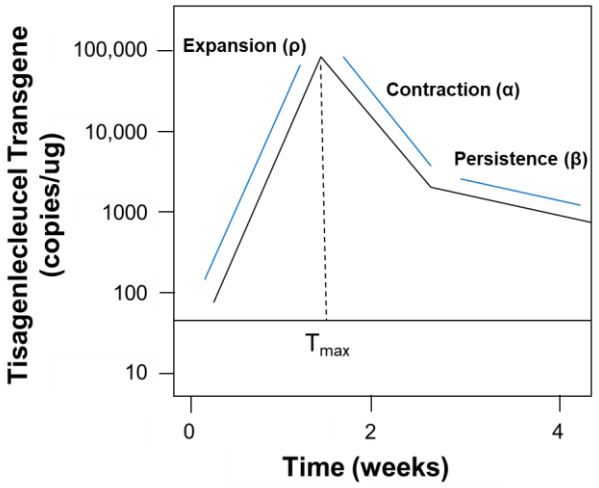
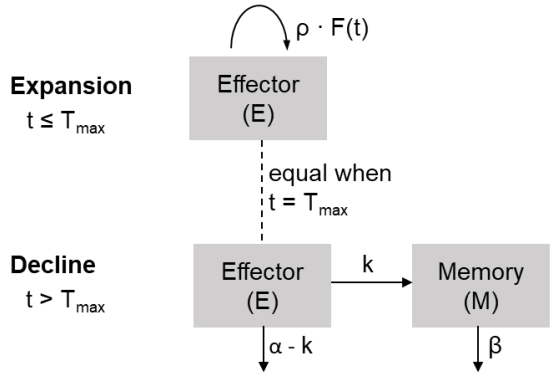
Mueller KT, et al. *Clin Cancer Res.* 2018 Sep 6. [Epub ahead of print]
Andrew Stein, Karen Thudium et al, unpublished.

Modeling the Cellular Kinetics of Tisagenlecleucel



Compartmental model



- Blood samples from 90 patients with r/r ALL analyzed
- For tisagenlecleucel, the initial doubling time (ln 2/ρ) was 0.78 days, the half-life for the initial rate of decline (ln 2/α) was 4.3 days, and the terminal half-life (ln 2/β) was 220 days
- α slope corresponds to a rapid contraction due to AICD of CAR T effector and the β slope corresponds to a gradual decrease of memory CAR T
- Unlike a traditional drug, no relationship was detected between the dose of tisagenlecleucel and C_{max} or any other model parameter
- C_{max} was associated with more severe CRS

Mueller KT, et al. *Clin Cancer Res*. 2018 Sep 6. [Epub ahead of print]
Andrew Stein, Karen Thudium et al, unpublished

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Patient Safety Years of Genetically Modified T cells University of Pennsylvania

Trial	Engineered T Cell	# Patients Infused	Safety (Patient-Years)	# Patients Alive (as of last date enrolled in study/LTFU)
Sangamo ZFN (HIV)	Ad5/35 zinc finger nuclease	12	74.4	12
CD4z CAR (HIV) includes CG trials	Retroviral CAR	44	783.6	44
SB-728mR CCR5 ZFN	CCR5 ZFN	11	10.9	11
MAZ-Takara (HIV)	Retroviral MazF	10	22.7	10
VirxSys VRX496 (HIV)	Lentiviral antisense HIVenv	20	204.1	20
Adaptimmune (HIV)	Lentiviral gag TCR	2	10.8	2
Adaptimmune Myeloma and Sarcoma	Lentiviral NY-ESO1 TCR	21	100.8	21
Penn/Novartis CART19/CTL019	Lentiviral 19:BBz CAR	311	467.3	214
EGFR	Lentiviral CART-EGFR	10	8.5	3
UPCC19214 CART-MESO-19	CART-MESO-19	3	3.2	2
UPCC31213	CART-MESO	15	10.2	2
UPCC31415	CART22	3	1.5	1
UPCC14415	CART-BCMA	14	10.9	10
Total		**476**	**1709**	**352**

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CD19-DIRECTED CAR T CELLS IN RELAPSED/REFRACTORY AGGRESSIVE B-CELL LYMPHOMAS

Stephen J. Schuster, et al.

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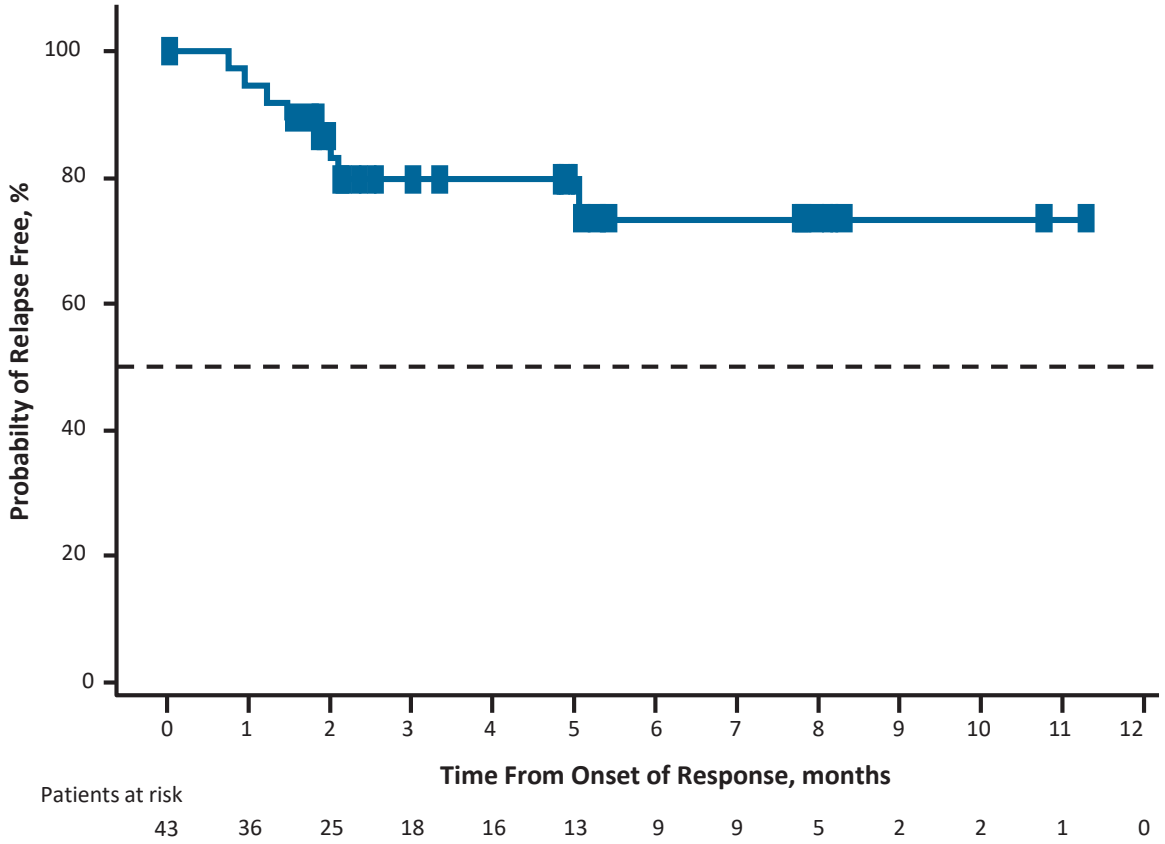
JULIET: Duration of Response 74% Relapse Free at 6 Months



- **Median DOR and OS not reached**
- Almost all patients in CR at month 3 remained in CR
- No patients proceeded to transplant while in response

Efficacy analysis set = all patients who received a tisagenlecleucel infusion ≥ 3 months prior to data-cut date.

CR, complete response; DOR, duration of response; OS, overall response.

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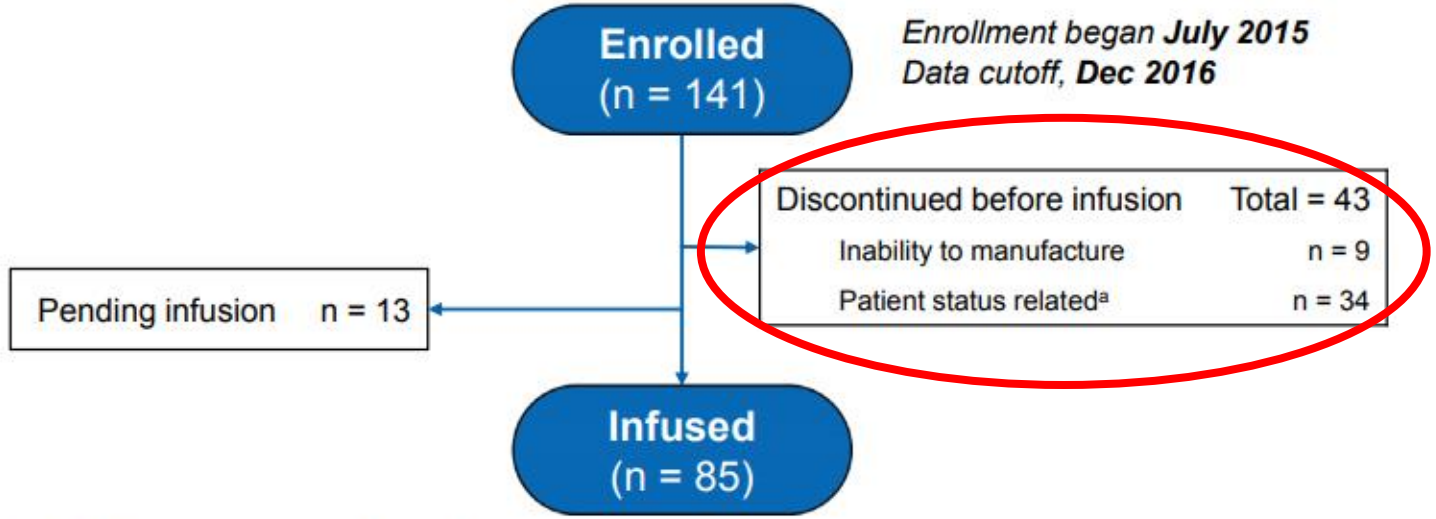
"Vein-to-Vein": Current Limitations

JULIET Patient Disposition



- 85 patients evaluated for safety
- 51 patients evaluated for response (completed ≥3 months follow-up or discontinued earlier)
  - Median time of 3.7 months from infusion to data cutoff (20 Dec 2016)
- CTL019 cell dose[b]:
  - Median (range), 3.1 x 10^8 (0.1-6.0 x 10^8) cells

[a] Progressive disease (n = 28; including 16 deaths); adverse event (n = 2), investigator decision (n = 2), withdrawal (n = 1), protocol deviation (n = 1).
[b] 1 patient received < and 3 patients received > the target dose range.

NOVARTIS

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CAR T Cell Manufacturing

Other limitations:

- Apheresis availability → **Financial toxicity**
- GMP suites availability → **Financial toxicity**
- Testing of each product → **Not enough blood bank donors**
- “Peak serum” → **Not enough blood bank donors**

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Mechanisms of Resistance to CD19 CAR T

- CD19 escape: A combination of genomic mutations and shifts in splicing that favor retaining some CD19 protein. Incidence is 28% in ALL[1-3]
- T cell exhaustion and/or AICD
- “CARB”: Inadvertent transduction of tumor blast cells with CAR19

1. Grupp SA, et al. *N Engl J Med*. 2013;368(16):1509-1518.
2. Sotillo, et al. *Cancer Discov.* 2015;5(12):1282-1295.
3. Orlando EJ, et al. *Nat Med.* 2018;24(10):1504-1506.

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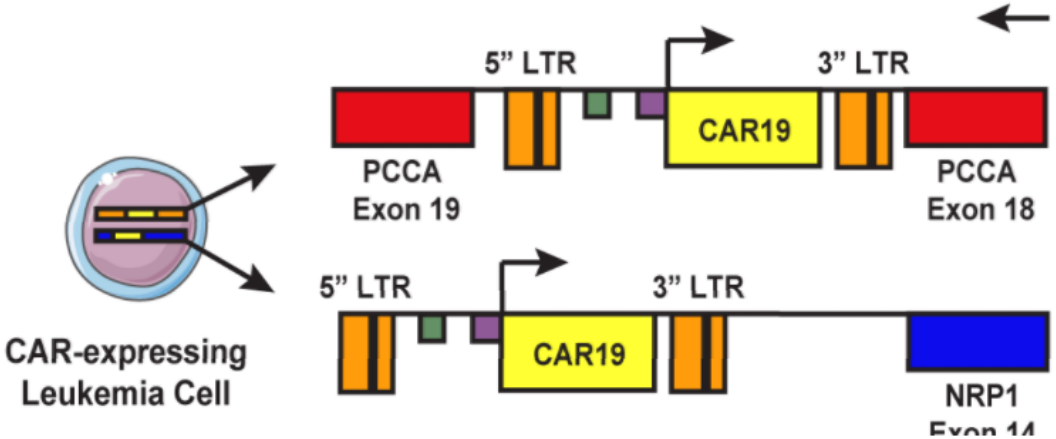
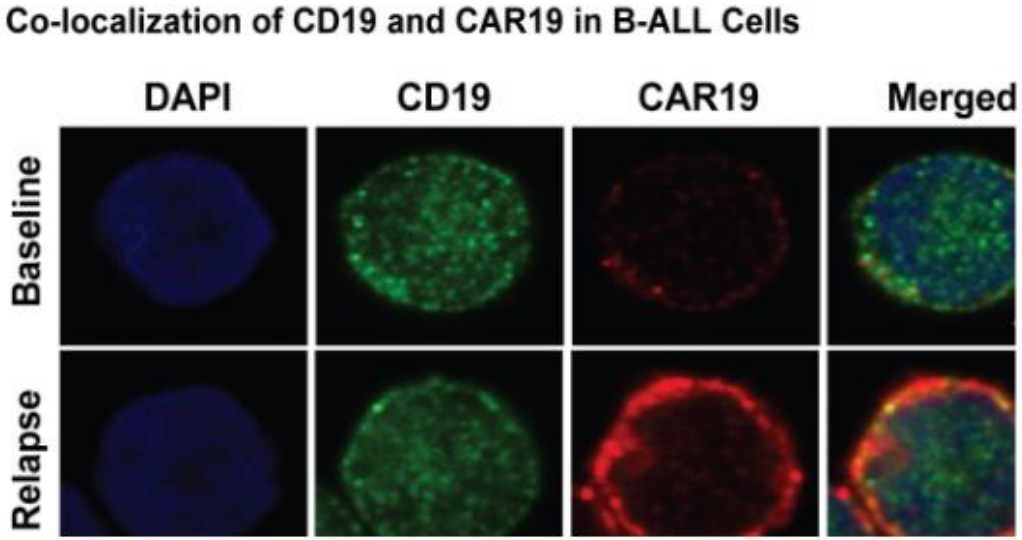
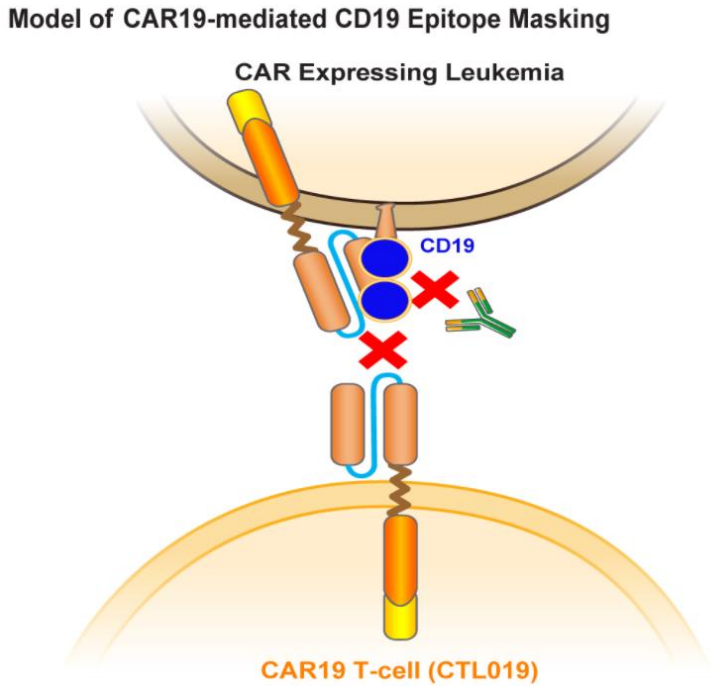
CARB Cells “Lethality of a Single Rogue CAR Cell” Mechanism



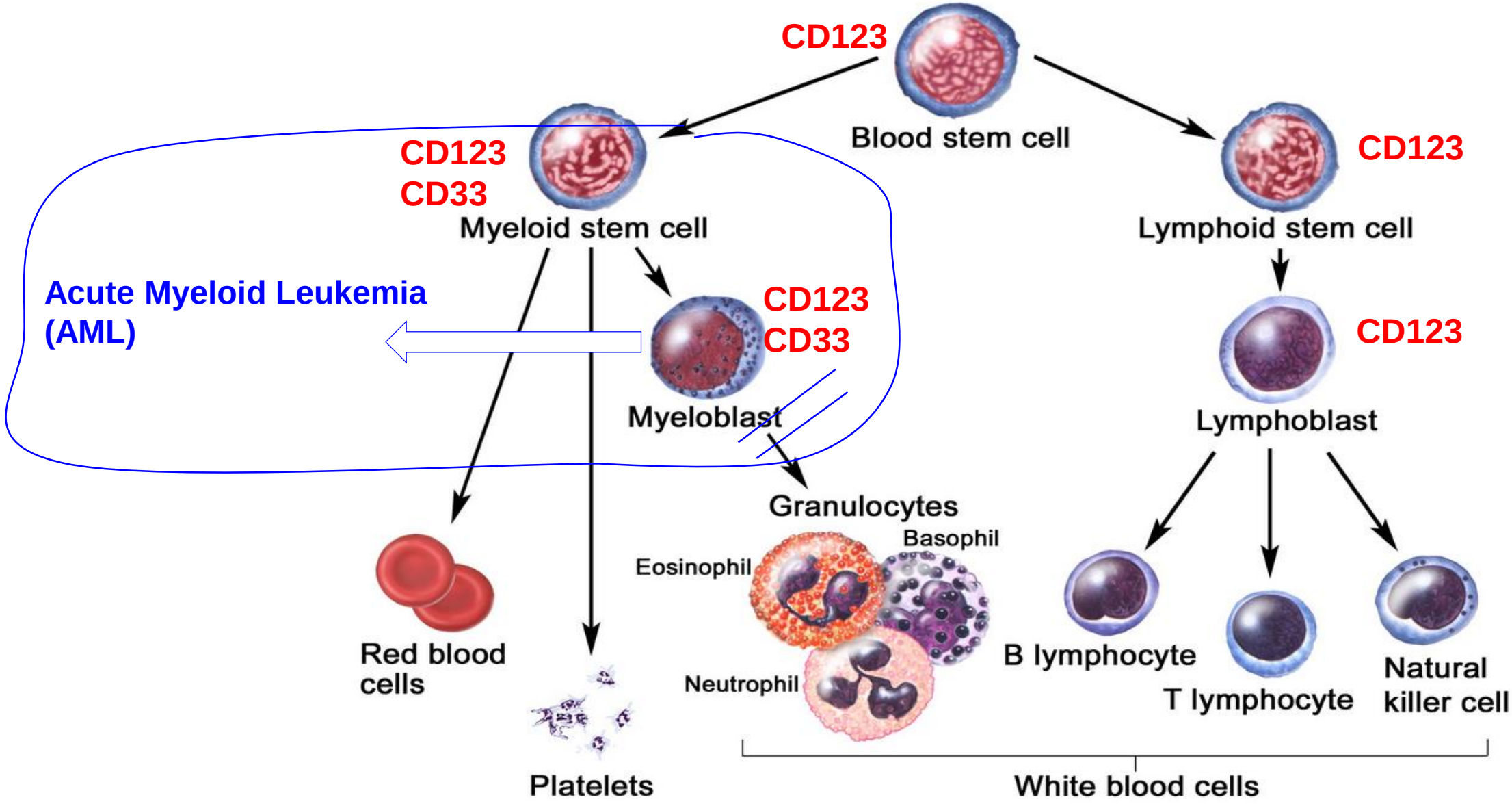




- 2 patients with clinical CARB
- 80% blasts at apheresis
- Infused product was 99.7% T cells
- 7/18 patients with low-level CARB in product

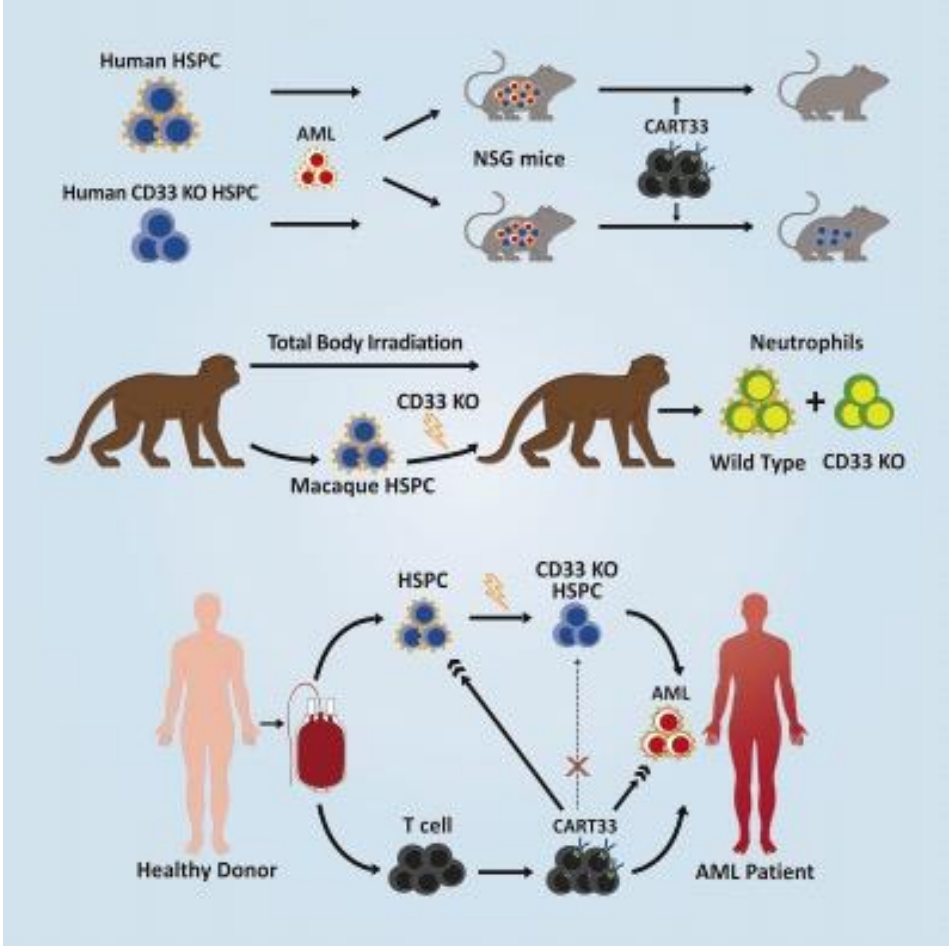
Ruella M, et al. *Nat Med*. 2018;24(10):1499-1503.

CAR T Cell Therapy of AML



Genetic Inactivation of CD33 in Hematopoietic Stem Cells to Enable CAR T Cell Immunotherapy for Acute Myeloid Leukemia

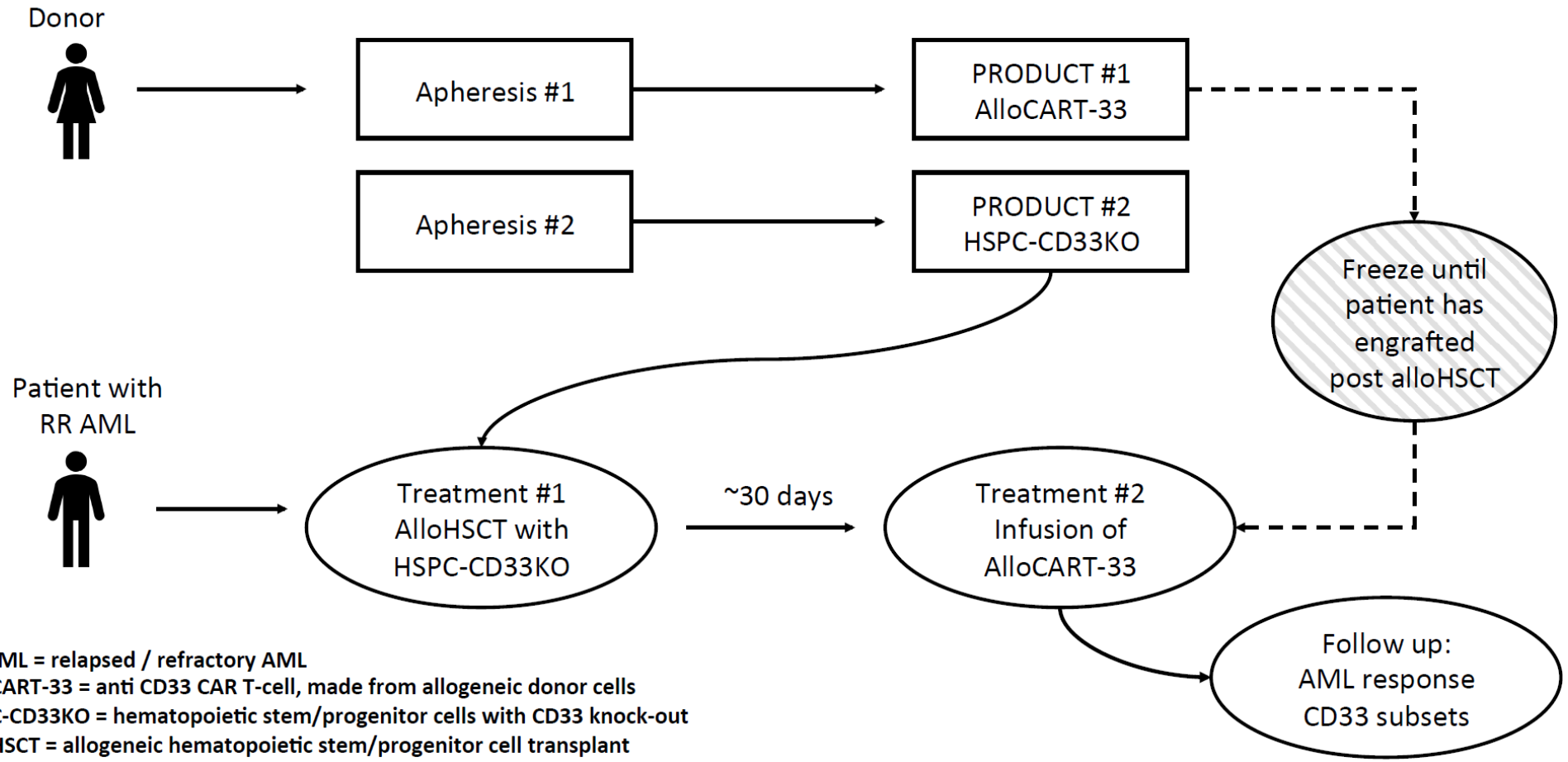
- CD33 is not required for human myeloid development and function
- CD33-deficient non-human primate myeloid cells are fully functional
- Anti-CD33 CAR T cells can eradicate AML while sparing CD33-deficient hematopoiesis
- This is a synthetic biology approach to generating a leukemia-specific antigen



Kim MY, et al. *Cell.* 2018;173(16):1439-1453

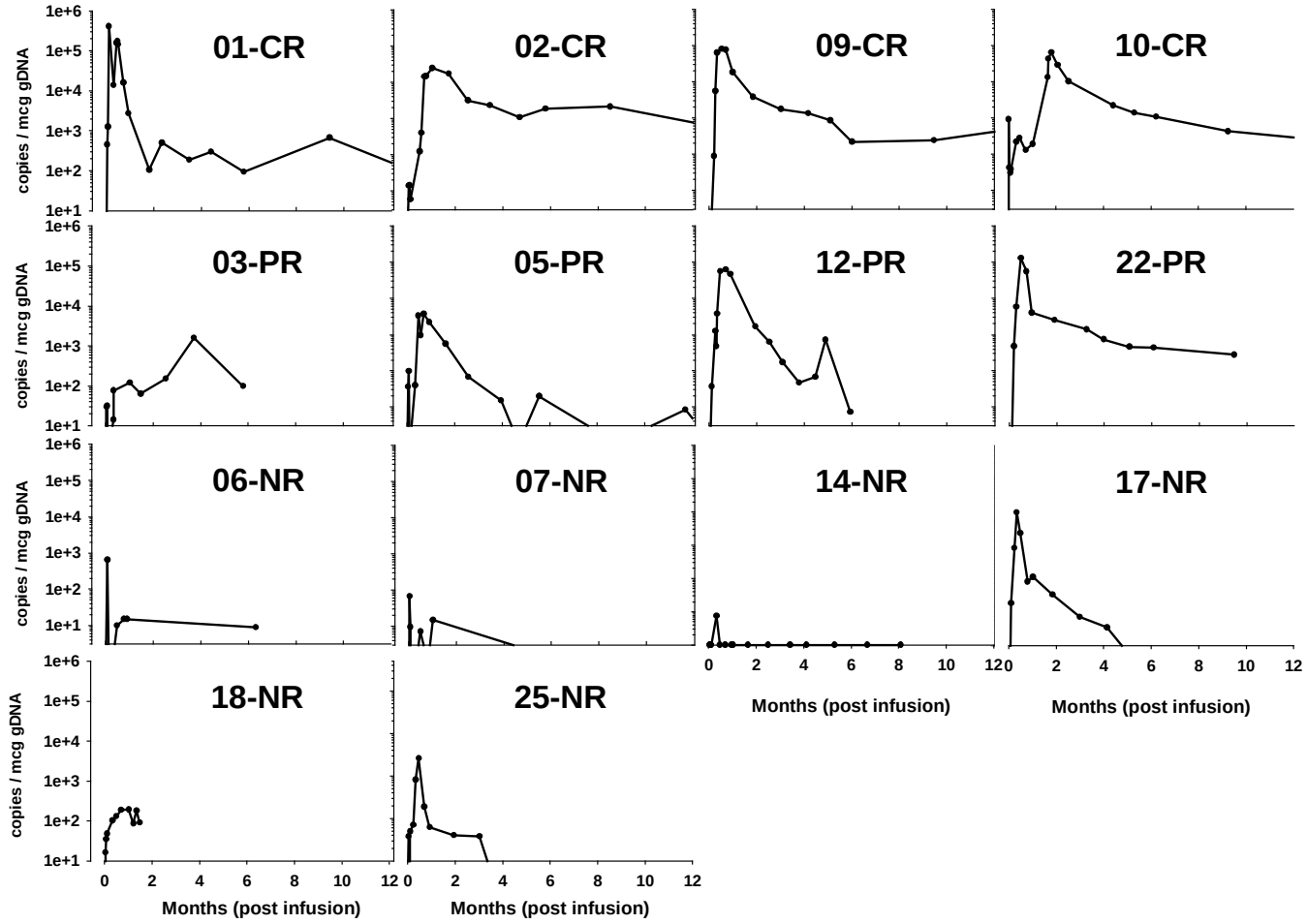
Dual Engineered CD33 CAR T and CD33 Deleted HSC for r/r AML: Pilot Trial

Saar Gill MD, PhD



RR AML = relapsed / refractory AML
AlloCART-33 = anti CD33 CAR T-cell, made from allogeneic donor cells
HSPC-CD33KO = hematopoietic stem/progenitor cells with CD33 knock-out
AlloHSCT = allogeneic hematopoietic stem/progenitor cell transplant

Long-Term Persistence and Expression of CTL019 is Associated With Durable Remission in Leukemia: Predictive Biomarker



CAR T Persistence for first year after infusion

Porter DL, et al. *Sci Transl Med.* 2015;7(303):303ra139.

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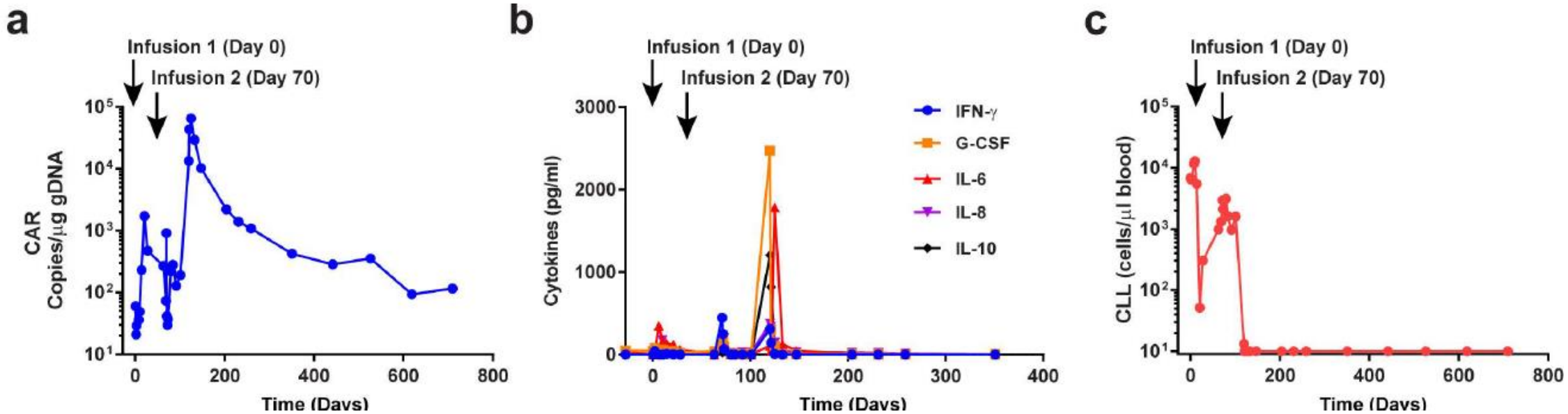
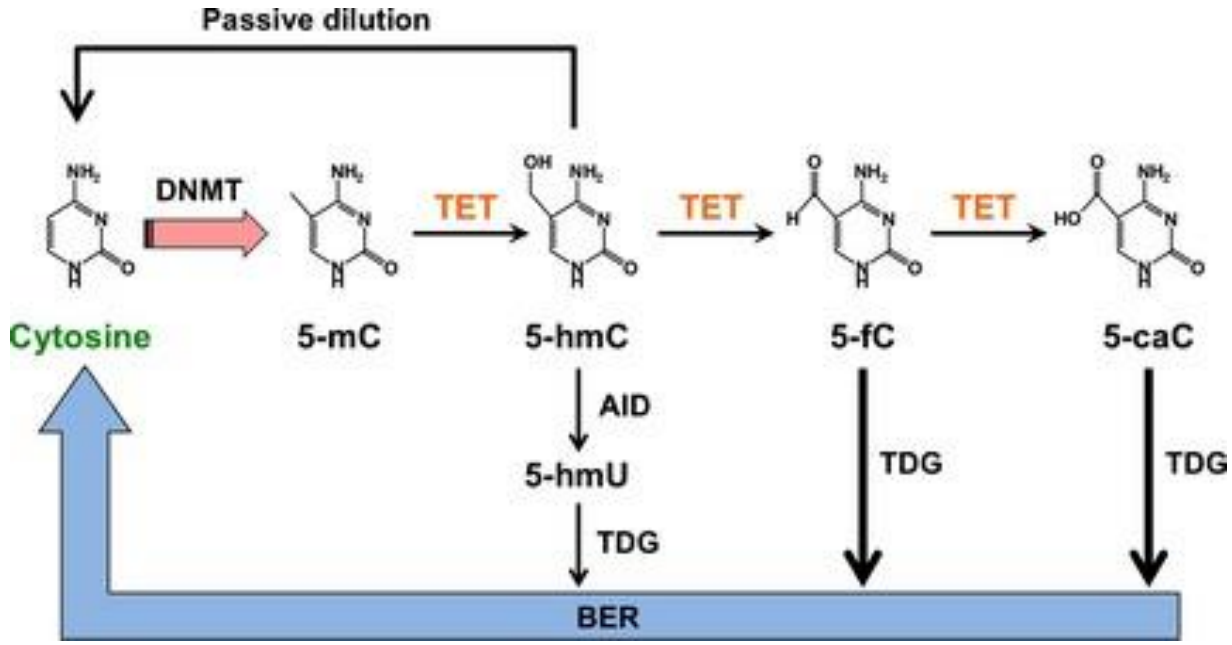
Delayed Tumor Lysis Syndrome in Pt #10

- Presented with fevers 45 days after infusion
- Day 50: Definitive evidence of TLS and MAS
  - Fevers, hypoxia (intubated), hypotensive (pressors)
  - Treated with tocilizumab
  - Expanding CART19 cells became detectable



TET (Ten-eleven translocation) Proteins



- All TET enzymes contain a C-terminal catalytic domain (CD) that belongs to the dioxygenase superfamily and oxidizes 5mC in a 2-oxoglutarate- (2-OG) and Fe(II)-dependent manner
- TET2 mutations frequently occur in hematological malignancies, including myeloid malignancies, T cell lymphomas, and adult T cell leukemia
- TET2 mutation not sufficient for transformation
- TET2 LOF mutations frequent in clonal hematopoiesis

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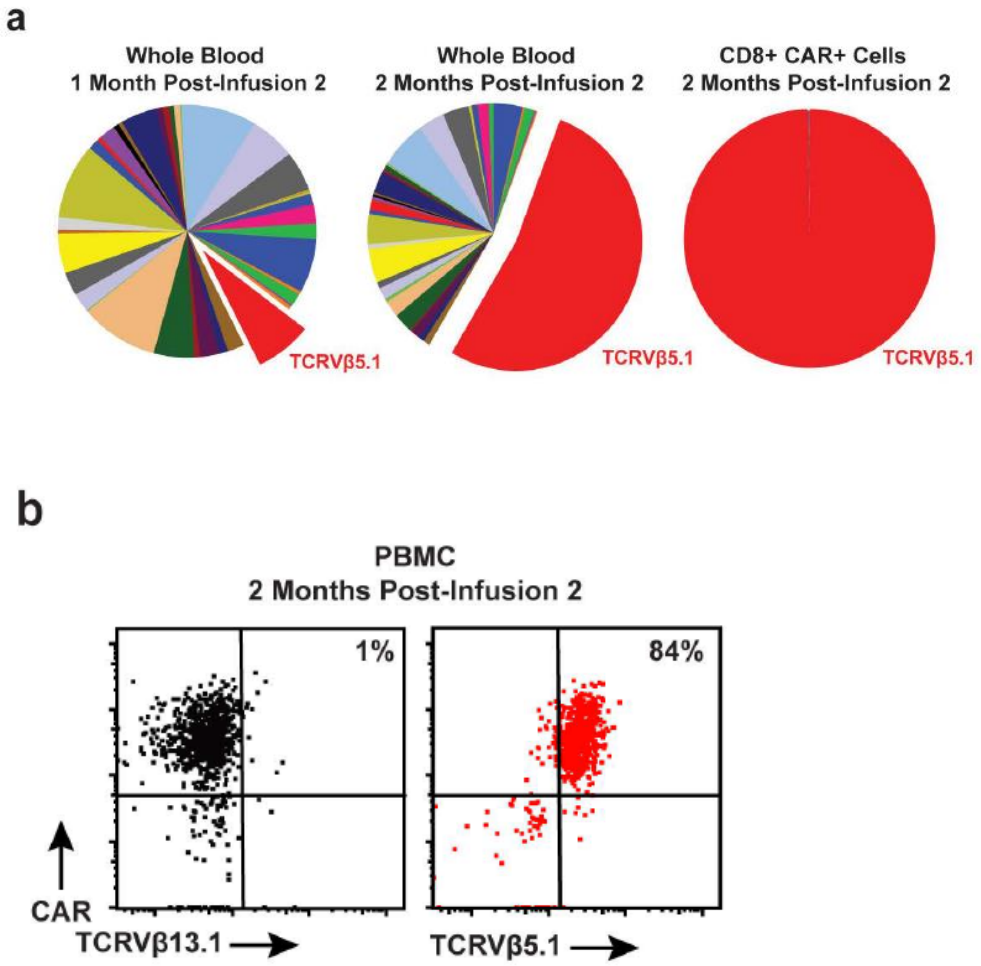
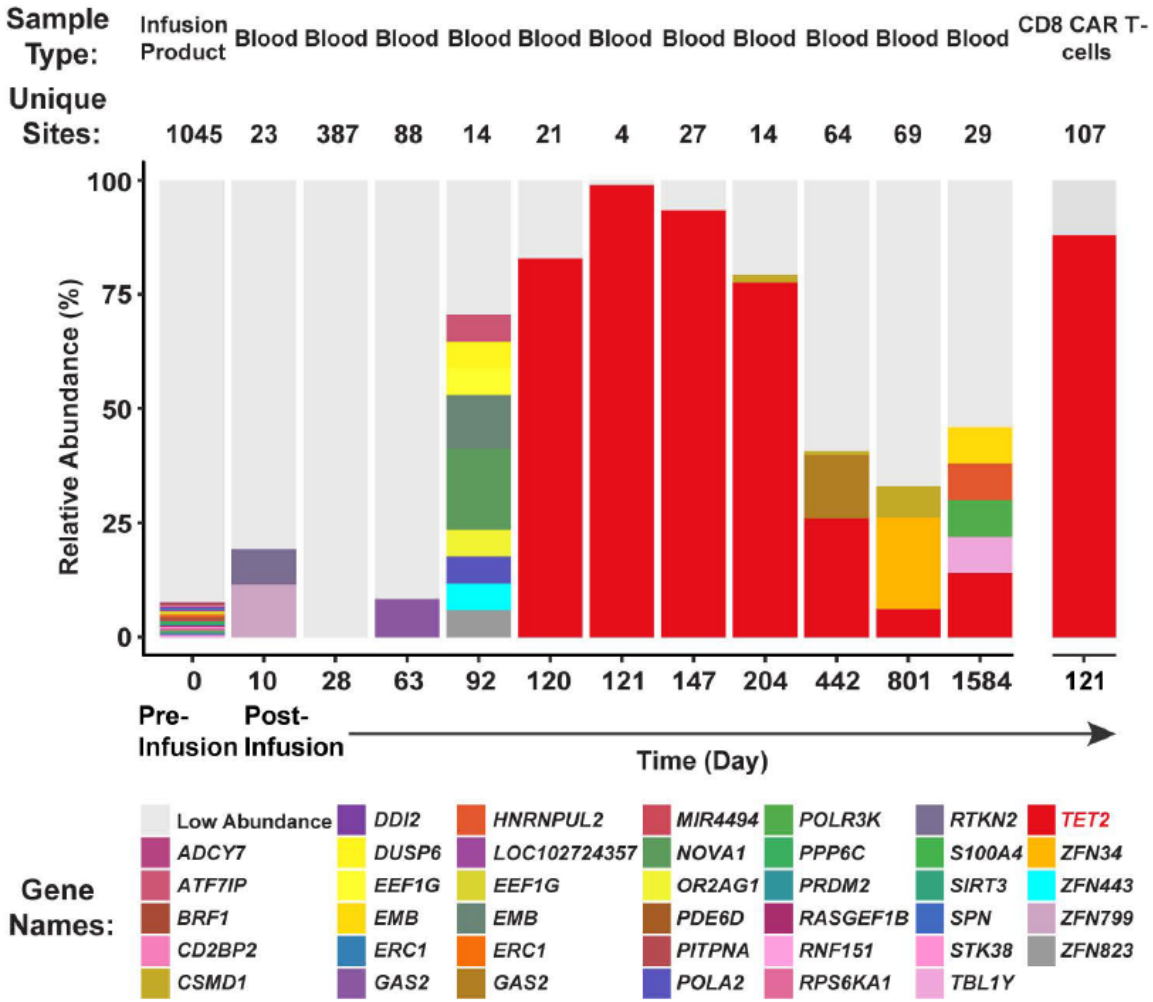
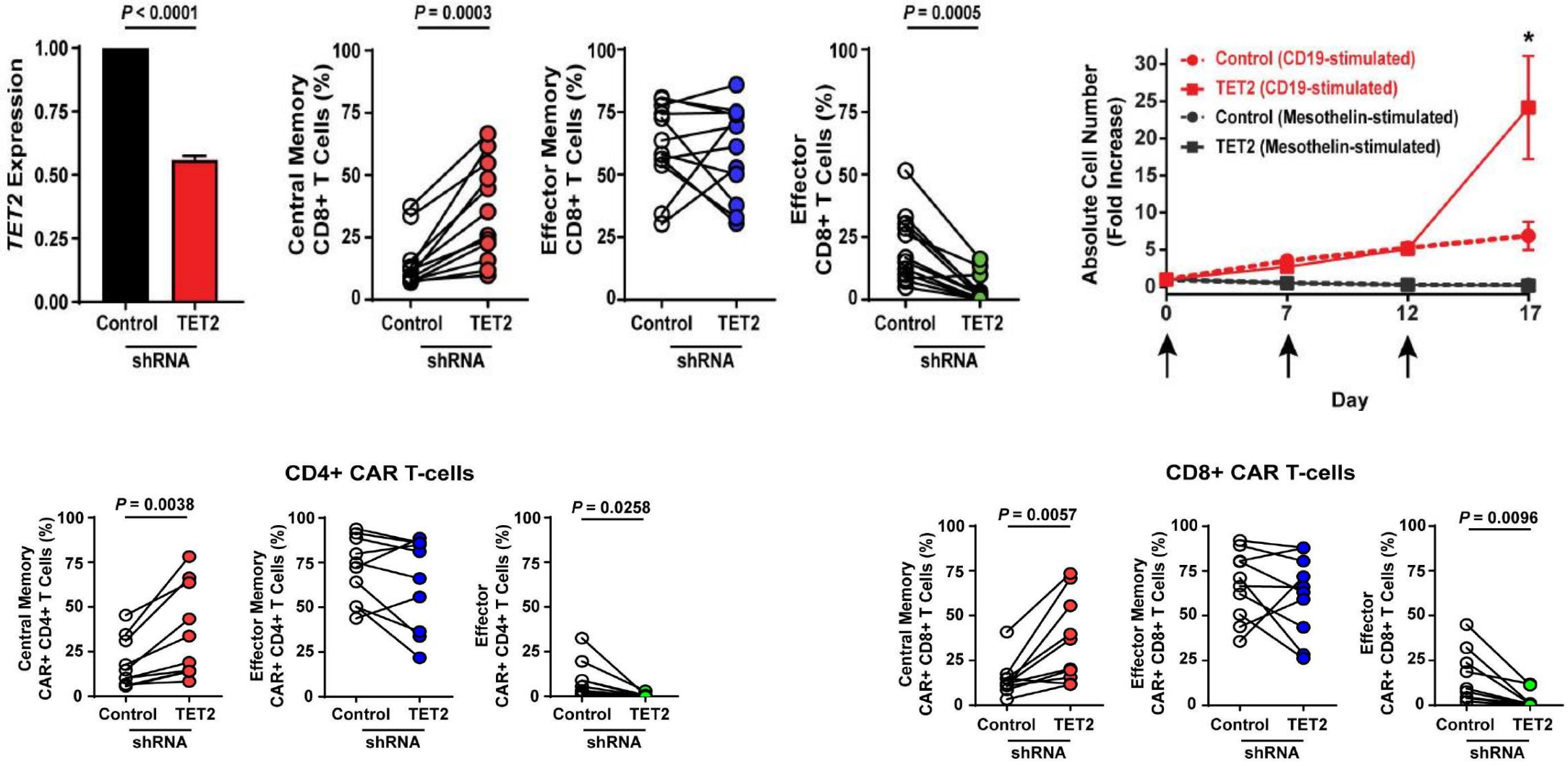
Rapid Massive Expansion of Clonal CART Cell Population in Patient #10





Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571.

Tet2 Knock Down in Healthy Donor T Cells



Fraietta JA, et al. *Nat Med.* 2018;24(5):563-571.

Lessons Learned from Tet2 Disruption in CLL Patient #10

- Progeny derived from a single CTL019 TCRVβ5.1+ CD8+ T cell were responsible for the eradication of massive tumor burden in patient #10.
- Can the lowest effective dose of CAR T be a single cell?
- Unintentional knock out of Tet2 was responsible for enhanced CAR T function
- Since Tet2 can increase HSC stem cell renewal, would inhibition or intentional disruption of Tet2 increase CAR T cell proliferation and/or function?

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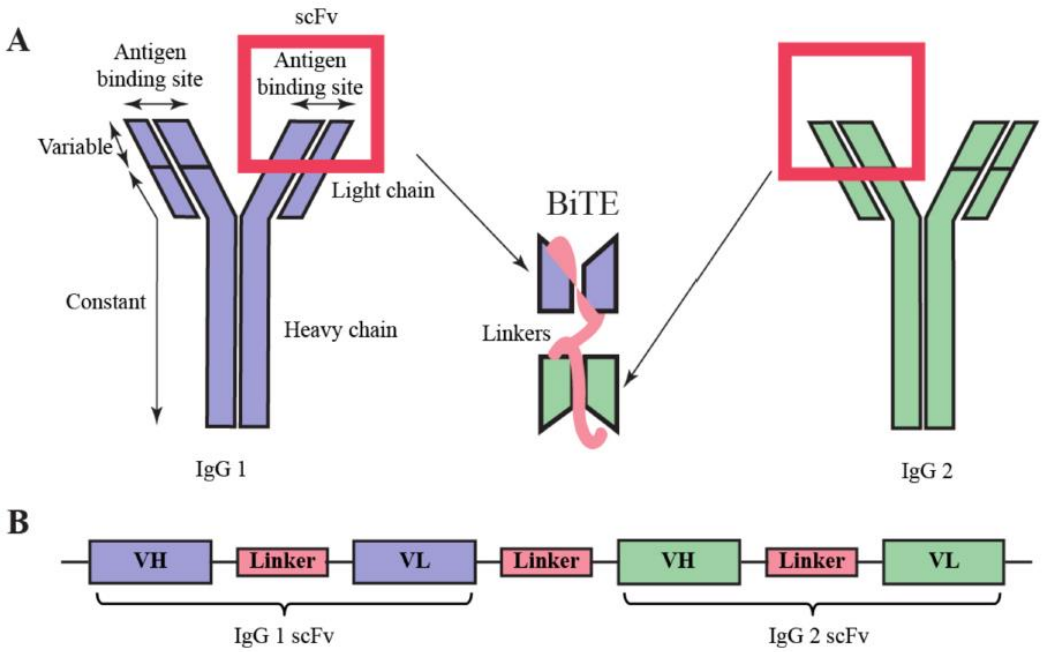
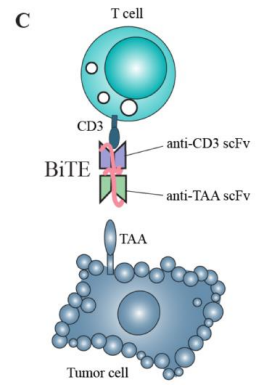
Fraietta JA, et al. *Nat Med.* 2018;558(7709):307-312.

CARs versus BiTEs: Quo Vadis?

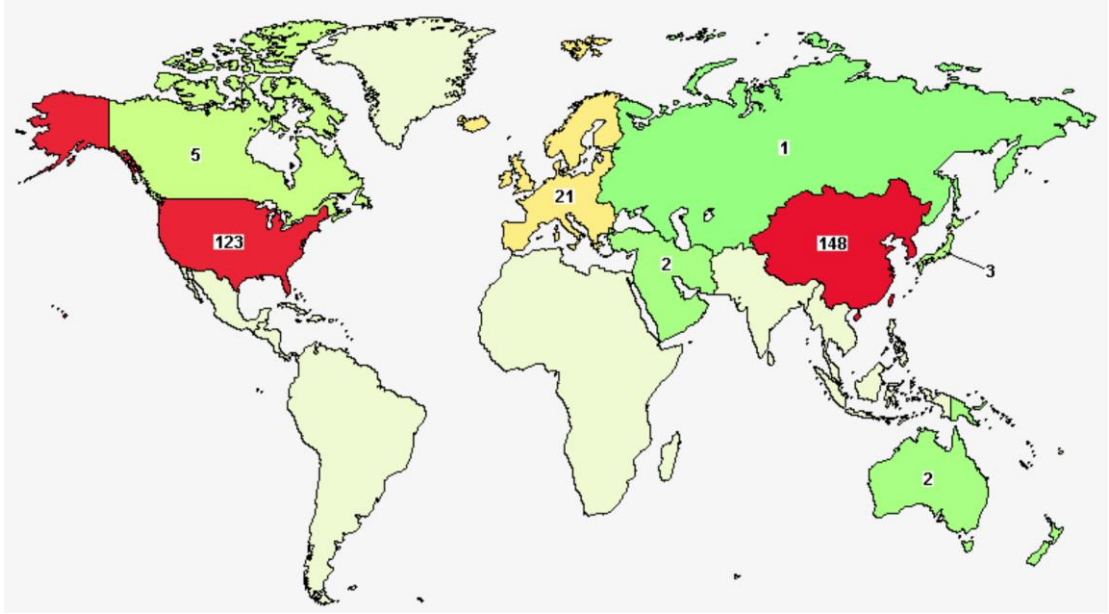
- Both BiTEs and CARs have similar adverse events: CRS and neurotoxicity
- Both strategies are MHC independent
- BiTEs are given without conditioning chemotherapy: host T cells are required
- CAR T have active trafficking to sanctuary sites; BiTEs have passive diffusion
- CAR T: Individual manufacturing
- BiTEs: Off the shelf





BiTE Design
Slaney et al

Social Challenges: Geographic Disparities and High Cost of Ultra-personalized Genetically Engineered Cells



Colors indicate the number of studies with locations in that region.

Least — Most

Labels give the exact number of studies.

Clinical trials.gov search term “chimeric antigen receptor”
305 trials listed as of August 2018

Rajkumar, ASCO 2018

THE WALL STREET JOURNAL.

U.S. Edition ▾ | September 4, 2018 | Today’s Paper | Video

BUSINESS

The Million-Dollar Cancer Treatment: Who Will Pay?

So far, few patients have received the new drugs, as commercial health plans and Medicare wrestle with how to cover the treatment

- The number of new myeloma patients in the US in 2017: ~30,000
- At current standard of care: total lifetime costs to treat all patients diagnosed in 2017: $22.4B
- The average cost of cancer drugs approved in the US in 2017: 150,000

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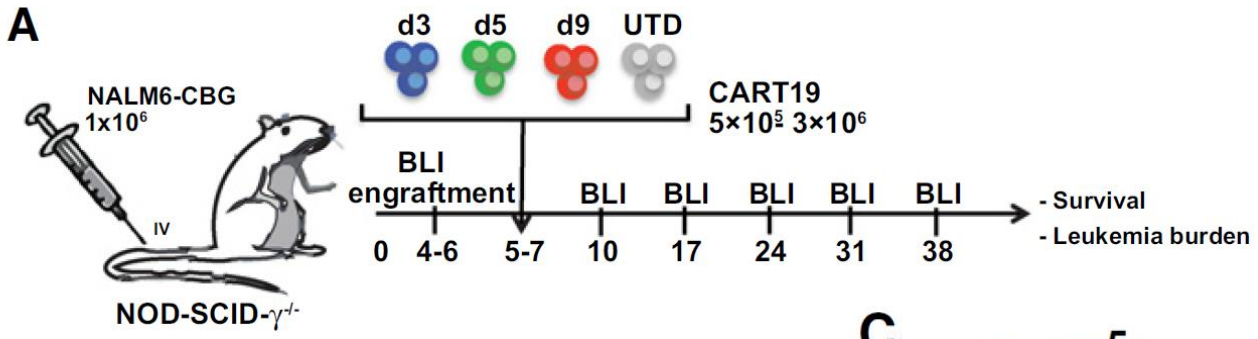
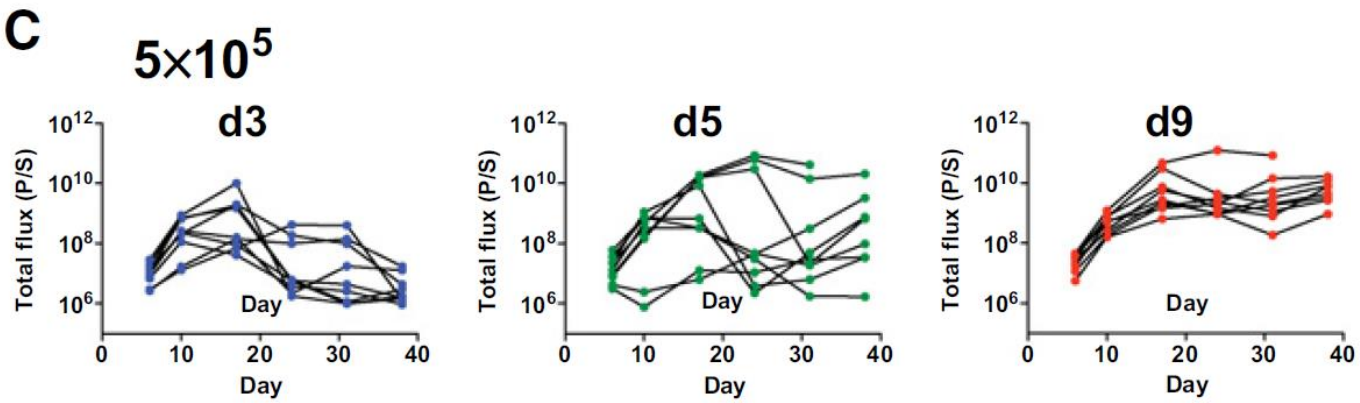
Making Better and Cheaper CAR T

Research Article

Cancer Immunology Research

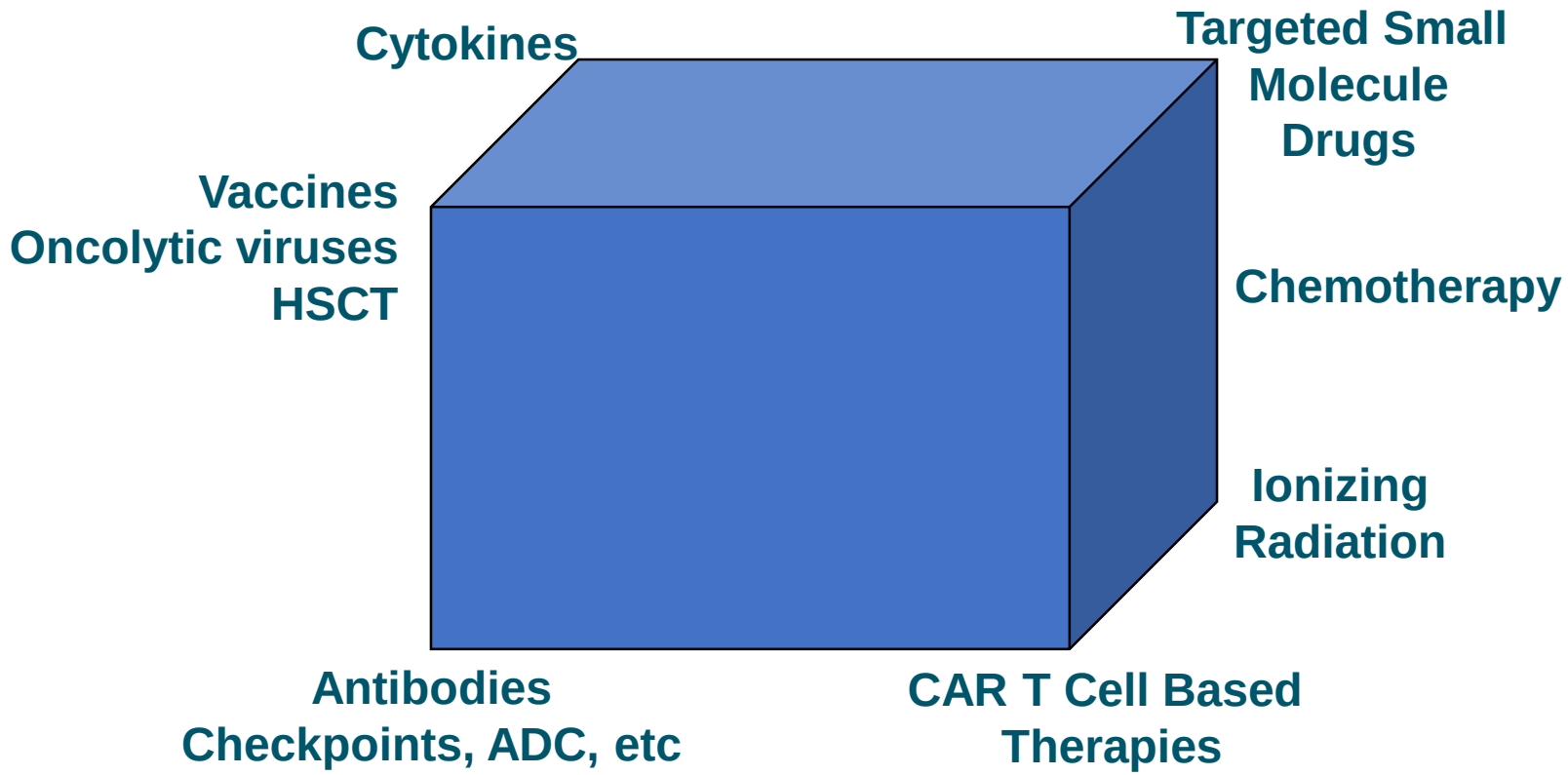
Reducing *Ex Vivo* Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T Cells





Ghassemi S, et al. *Cancer Immunol Res*. 2018;6(9):1100-1109.

Combinatorial Cancer Immunotherapies: Many Possibilities



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Colleagues and Patients: Thank you!

Center for Cellular Immunotherapies
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Institute for Immunology
John Wherry

Prostate Cancer
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Vivek Narayan
Whitney Gladney
Gabriela Plesa
Shannon Maude
James Gulley

Prostate Cancer Foundation
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Patients and Families

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