T cell therapy moving into new territory

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HARVARD MEDICAL SCHOOL

Dana-Farber/Harvard Cancer Center

NCI Comprehensive Cancer Center

A Cancer Center Designated by the National Cancer Institute

MASSACHUSETTS GENERAL HOSPITAL

CANCER CENTER

Disclosure Information

Updated November 6, 2019

Marcela V. Maus, MD, PhD

- I have the following relevant financial relationships to disclose:

  Consultant for: *Adaptimmune, Arcellx, Cellectis (SAB), CRISPR therapeutics, Incysus (SAB), GSK, Kite Pharma, Micromedicine, Novartis, TCR2 (SAB), and WindMIL (SAB)*

  Speaker's Bureau for: none

  Grant/Research support from: CRISPR therapeutics, Kite Pharma

  Stockholder in: Century Therapeutics, TCR2

  Honoraria from: listed under Consultant

  Employee of: Massachusetts General Hospital

  - AND –

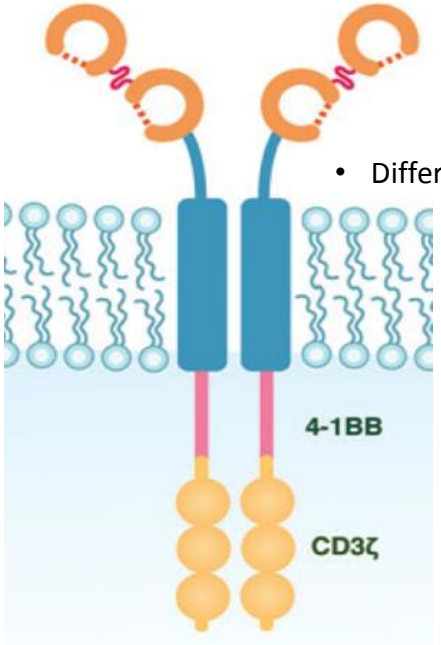
- I may discuss the following off label use and/or investigational use in my presentation: new CAR T cells

  - AND -

- My career is intricately tied to CAR T cells now

CAR T cell “anatomy”

- Different gene delivery technologies
- Gene editing enhancements (PD1 KO)
- Orthogonal cytokines
- Nanoparticle backpacks
- Regulatable expression



- Different affinities
- Different binders (mAb,humAb, nano)

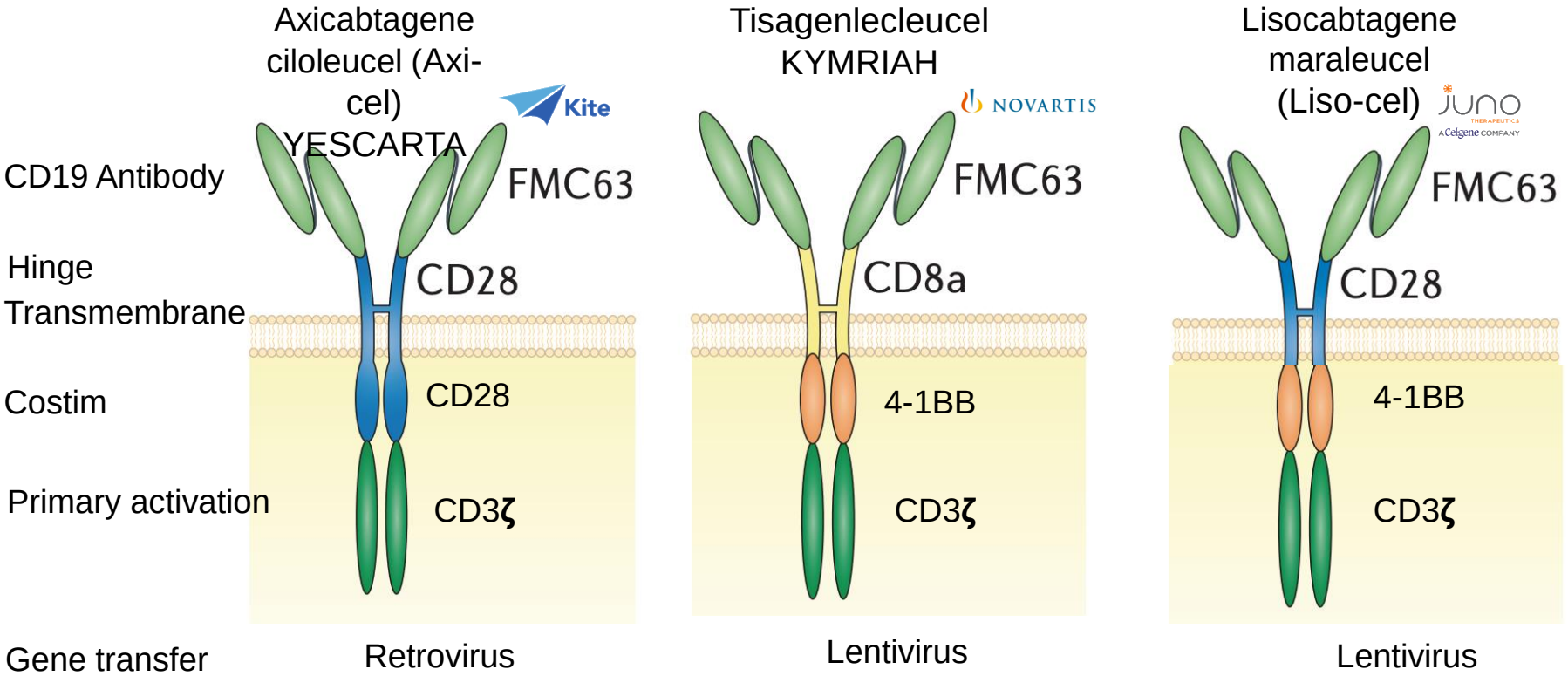
- Different “hinges”

- Different immune effector cells
- Gamma-delta, NK, ips-derived
- “off the shelf” based on gene editing

- Different costimulatory domains
- (4-1BB, CD28, GITR, CD27)
- Mutations in costim domains

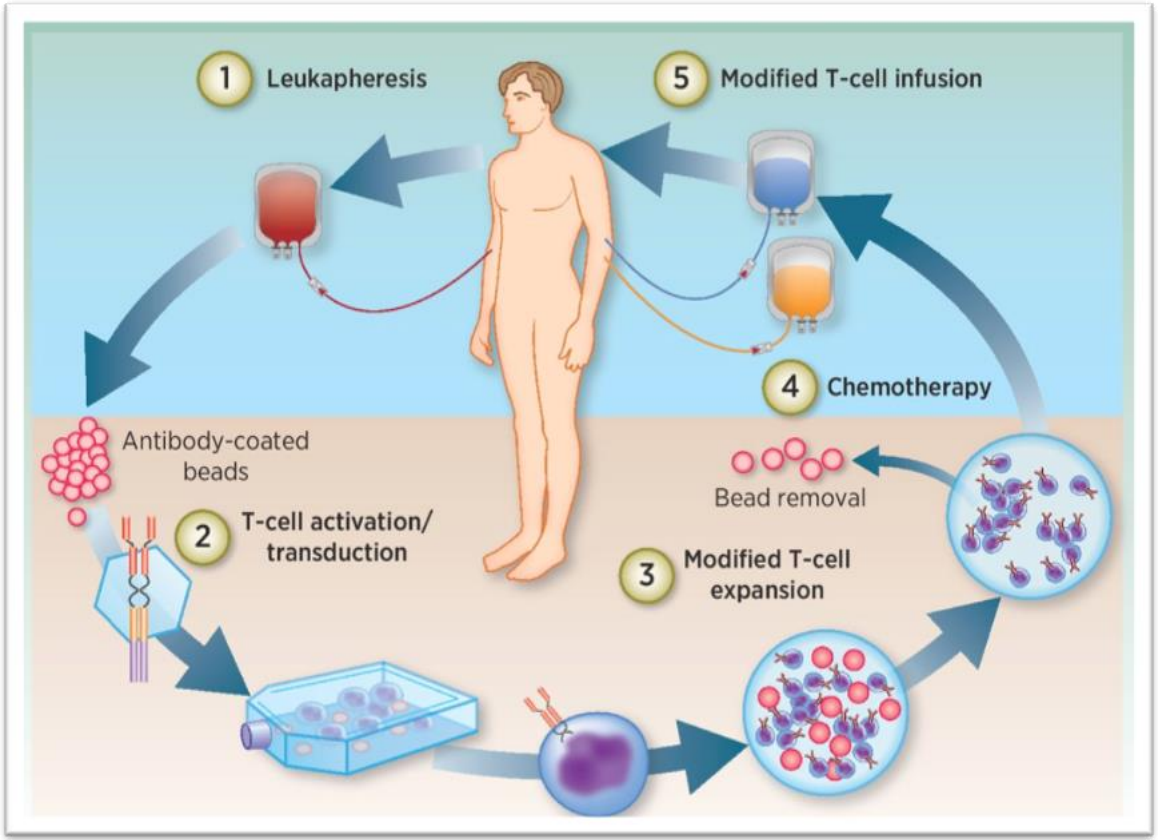
- Mutations in CD3z

CD19-directed chimeric antigen receptor-T cells in the clinic



Adapted from Nat Rev Drug Discov. 2015 Jul; 14(7): 499–509.

Cell Therapy: Collection, Manufacturing, Administration



Maus and June, CCR 2016

Anti-CD19 CAR T-cell Pharma Trials: B-ALL

Novartis – ELIANA

- N=75* (16 patients did not get their infusion)
- Pediatric/young adult B-ALL

  - 6 month – **90%**
  - 12 month – **76%**

Maude SL et al NEJM 2018;378:439

Juno - ROCKET

- N=51 (30 morphologic; 21 MRD+)
- Adult B-ALL
- JCAR015 (co-stimulatory domain CD28)
- CR rate
  - Morphologic: **77%**
  - MRD positive: **90%**
- 6 month OS:
  - Morphologic: **57%**
  - MRD positive: **73%**

Park JH, et al. ASCO 2016:a7003

Kite ZUMA-3

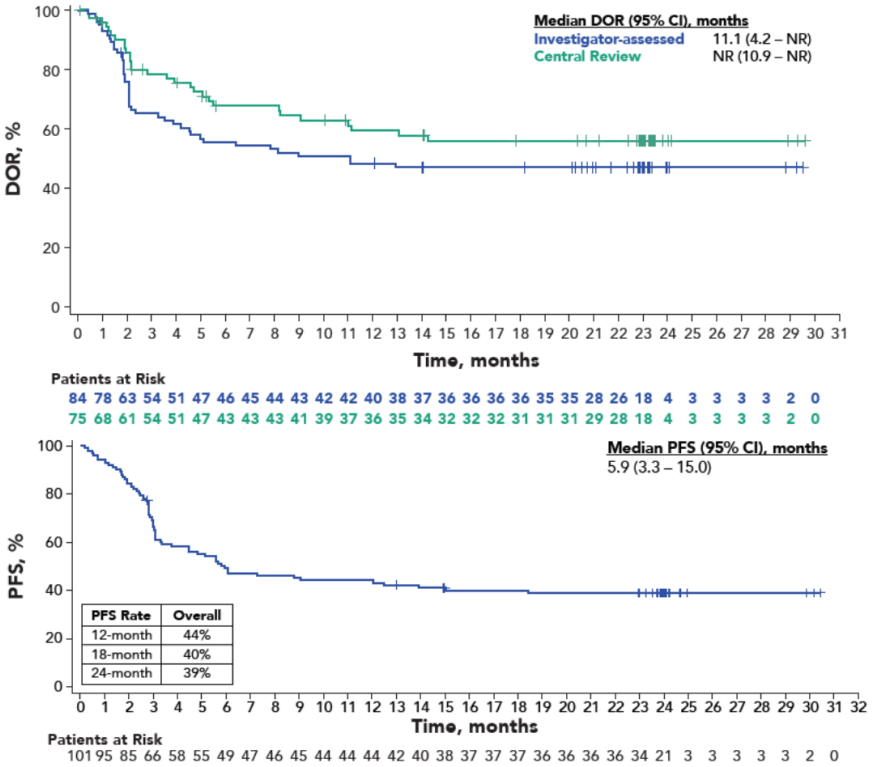
- Phase 1: N=45
- Adult B-ALL
- KTE-X19 (co-stimulatory domain CD28)
- CR rate
  - Overall: **68%**
  - RP2 dose: **84%**
- Median f/u 16 months:
  - Median DOR 12.9m
  - RP2 dose: **75%** in ongoing response

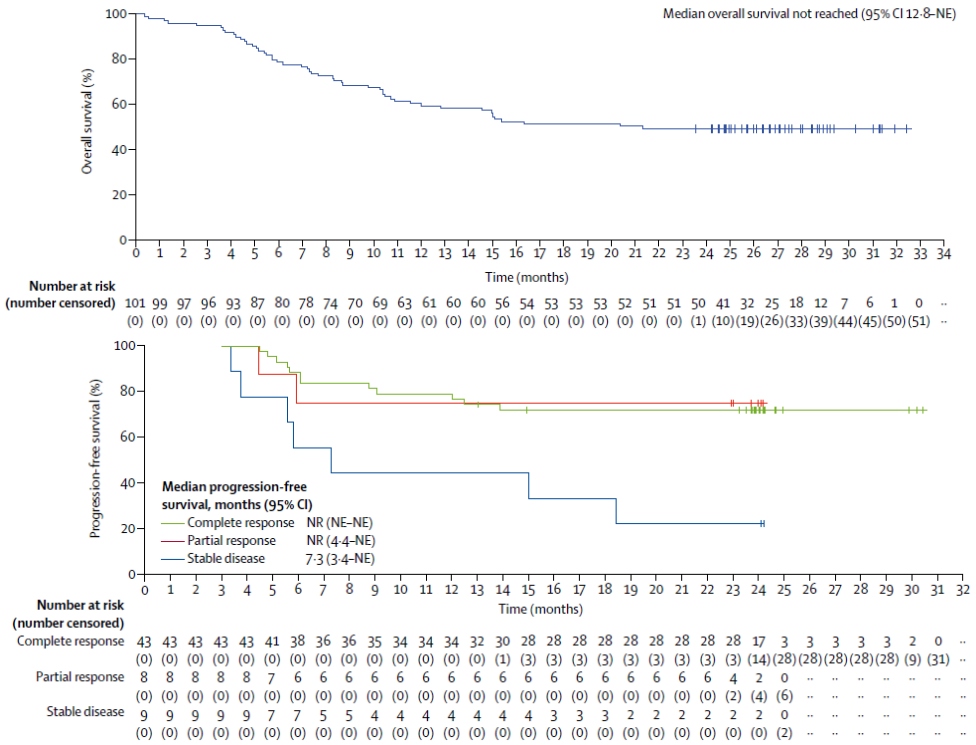
ASCO 2019:a7006

Anti-CD19 CAR T-cell Pharma Trials: DLBCL

	ZUMA-1	JULIET	TRANSCEND CORE
Product			Liso-cel
# pheresed			NR
# treated			NR
ORR (%)			80
CR (%)			59
6m ORR (%)	41	37*	47
6m CR (%)	36	30*	41

ZUMA-1: 2-year Results





Locke et al Lancet Oncology 2019;20:31

CAR T-cell Toxicity

- Cytokine Release Syndrome (CRS)
  - Caused by activation/expansion of CAR T-cells and increased levels cytokines like IL-6, IL-15, INF-γ, GM-CSF and others
    - Monocytes and macrophages are a source of some of these cytokines
  - Onset: 1-3 days
  - Duration: 3-5 days
  - Risk variable but gr3+ up to 20-30%

- Neurotoxicity
  - Mechanism less well understood
  - Onset: 5-7 days
  - Duration: 5-10 days
    - Fully reversible except in cases of fatal cerebral edema
  - Risk variable but gr3+ in up to 30-40%

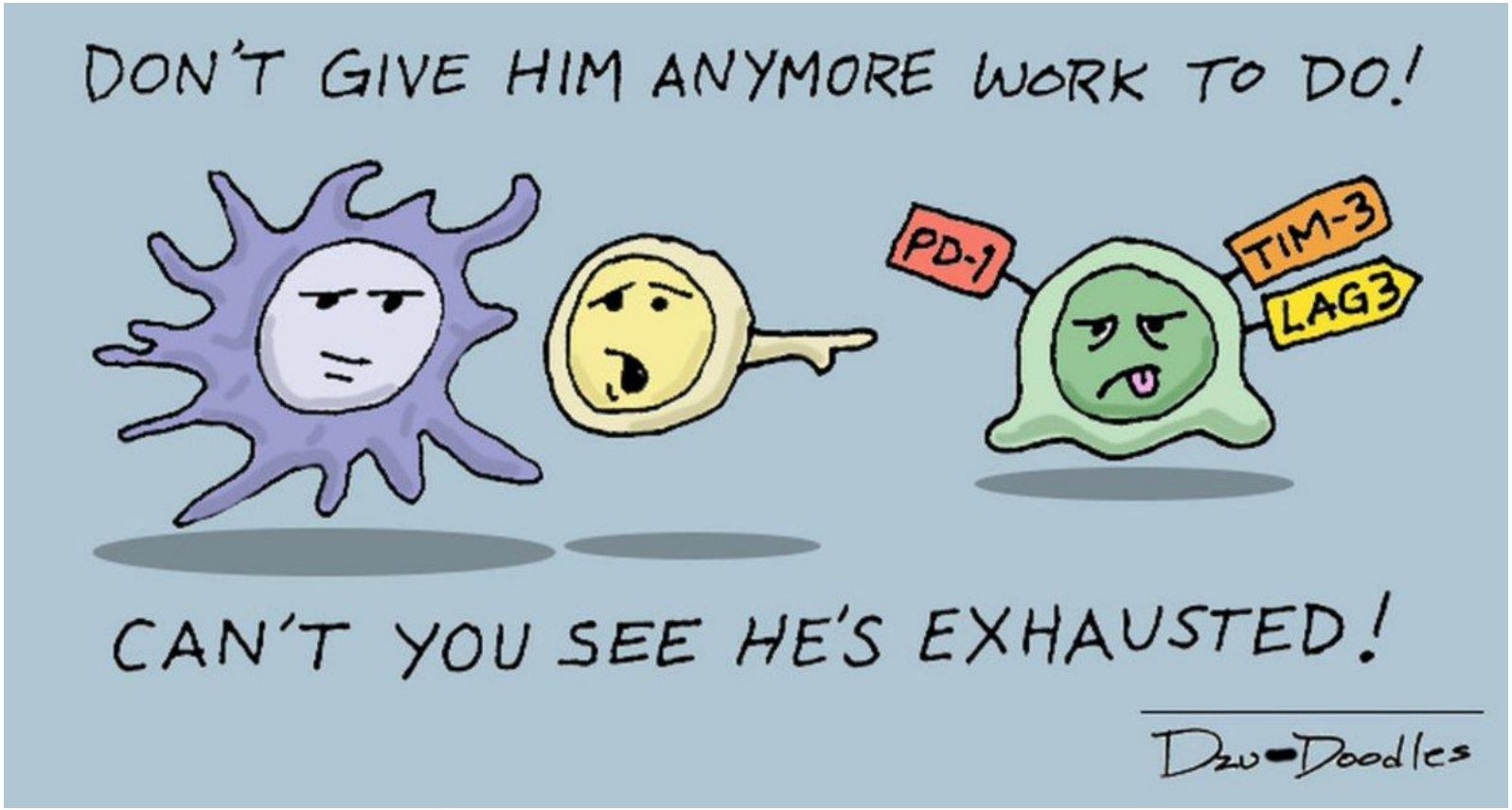
Novel approaches in clinical development center on cytokine blockade

Opening soon at MGH: Phase 2 study of anakinra prophylaxis with axi-cel

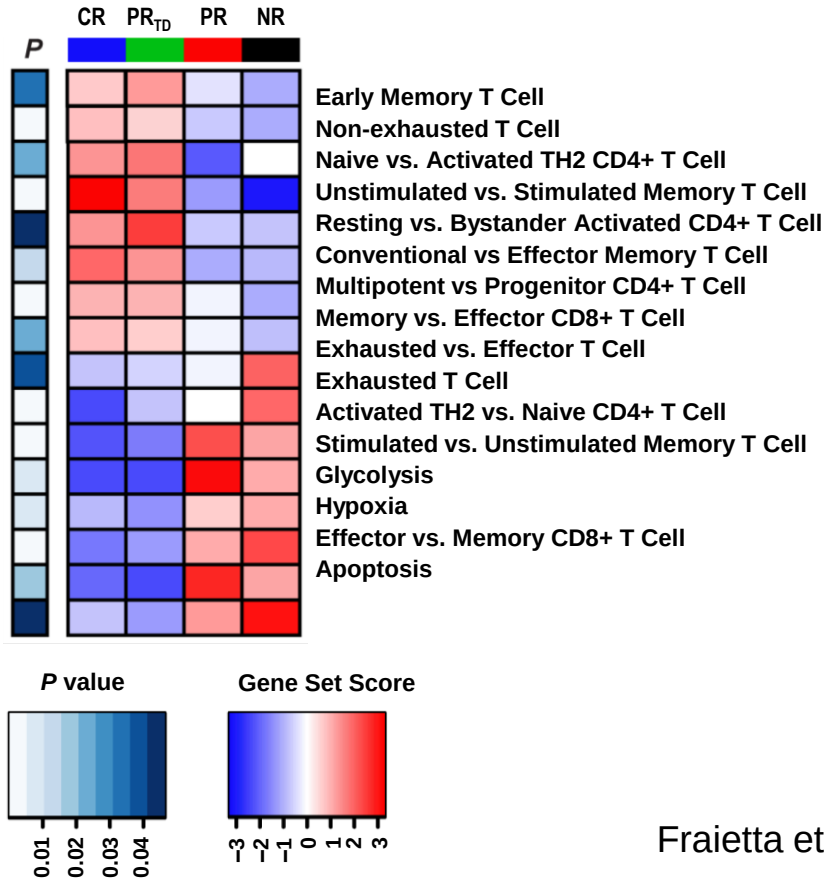
Mechanisms of Resistance

- Poor T cell fitness (lack of proliferation and/or persistence)
  - PD1/PDL1 blockade
  - Ibrutinib pre-treatment
  - Allogeneic source?
- Target antigen loss
  - Splice variants
  - Reversion to myeloid phenotype
  - Mutations
  - B cells transduced with CARs and masking
  - Trogocytosis of CD19

T cell Exhaustion



T cell attributes determine responses in CLL: it's the product, not the disease!

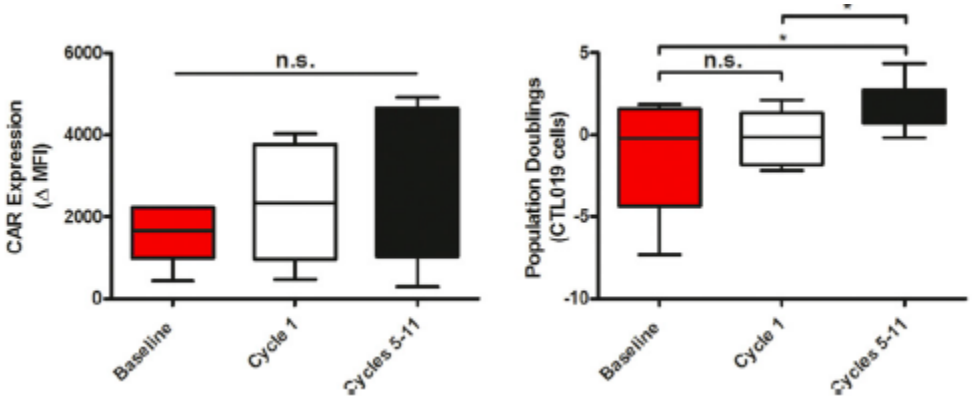


Fraietta et al., Nat Med, 2018

Improving T cell Health: Ibrutinib Pre-treatment

- CLL patients s/p >6 months of ibrutinib had a healthier and more potent CAR T-cell product
  - Improved T cell expansion and engraftment
  - Decreased expression of inhibitory molecules like PD1
  - Improved antitumor activity in mouse models

- Pilot trial of CTL-019 in CLL patients with less than a CR after at least 6m of ibrutinib (n=18)
  - Morphologic CR: 94%
  - MRD negative CR: 78%
  - 10/11 patients remain in response at 12m
  - Suggestion of an improved safety profile



Fraietta et al. Blood 2016:127:1117
Gill ASH 2018

Mechanisms of Resistance

- Poor T cell fitness (lack of proliferation and/or persistence)
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  - Ibrutinib pre-treatment
  - Allogeneic source?
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  - Splice variants
  - Reversion to myeloid phenotype
  - Mutations
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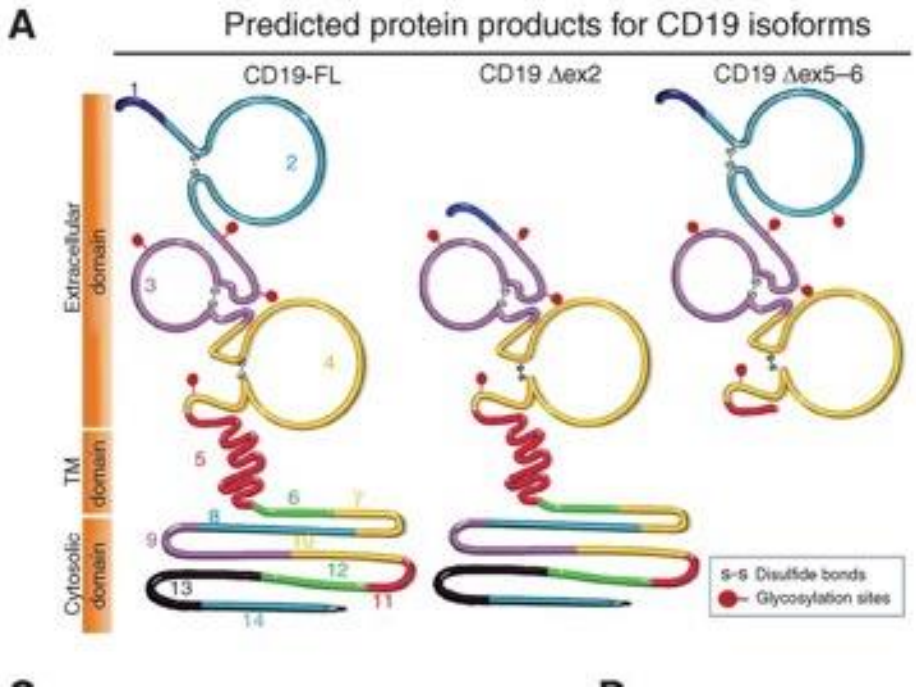
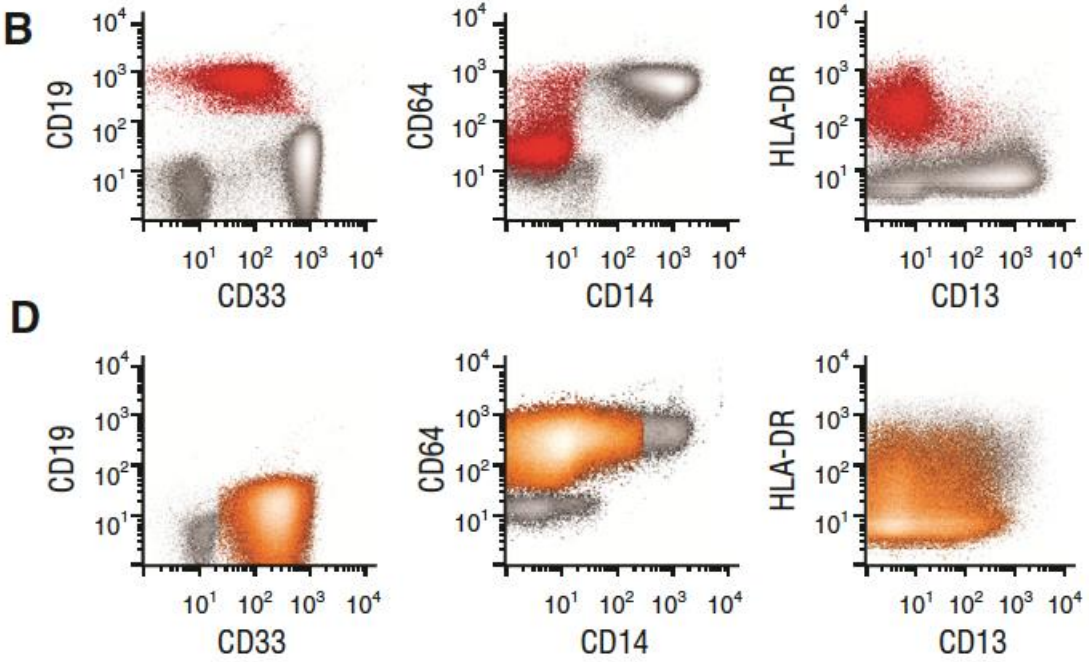
CD19 splice variants that lose epitope bound by CAR



Sotillo, Cancer Discovery, 2015

ALL with rearranged mixed lineage leukemia (MLL) gene can revert to myeloid phenotype



Gardner, Blood, 2016

Frameshift mutations in CD19 with loss of TM domain

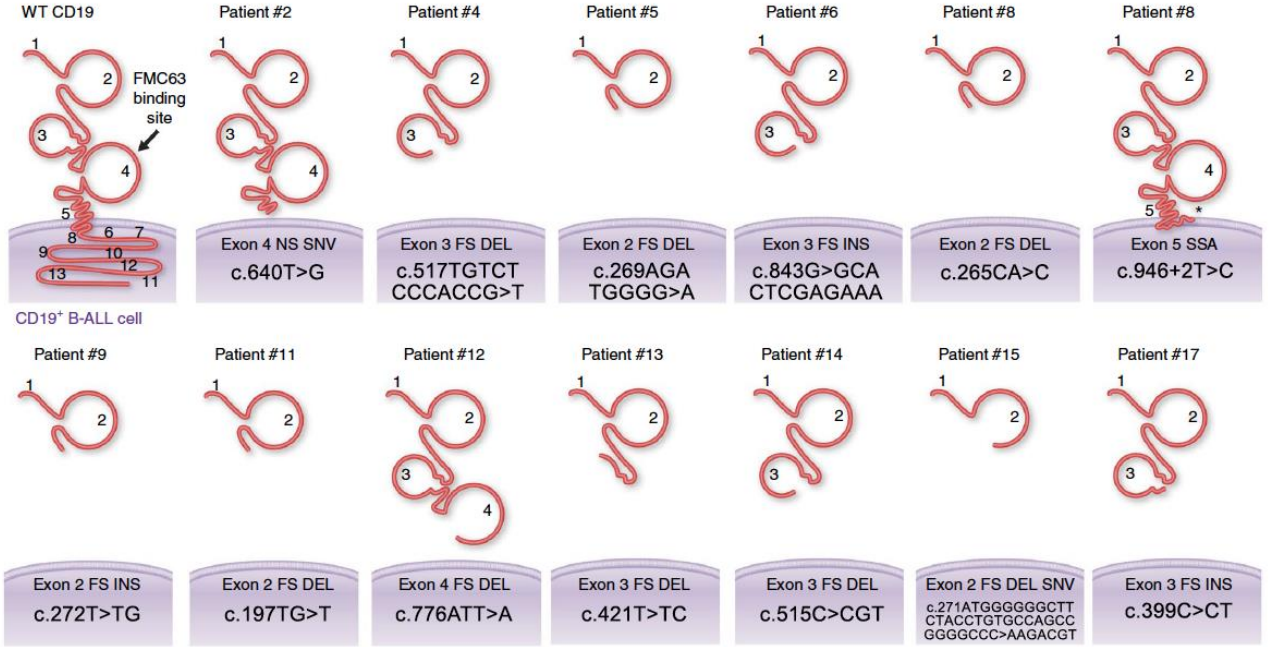
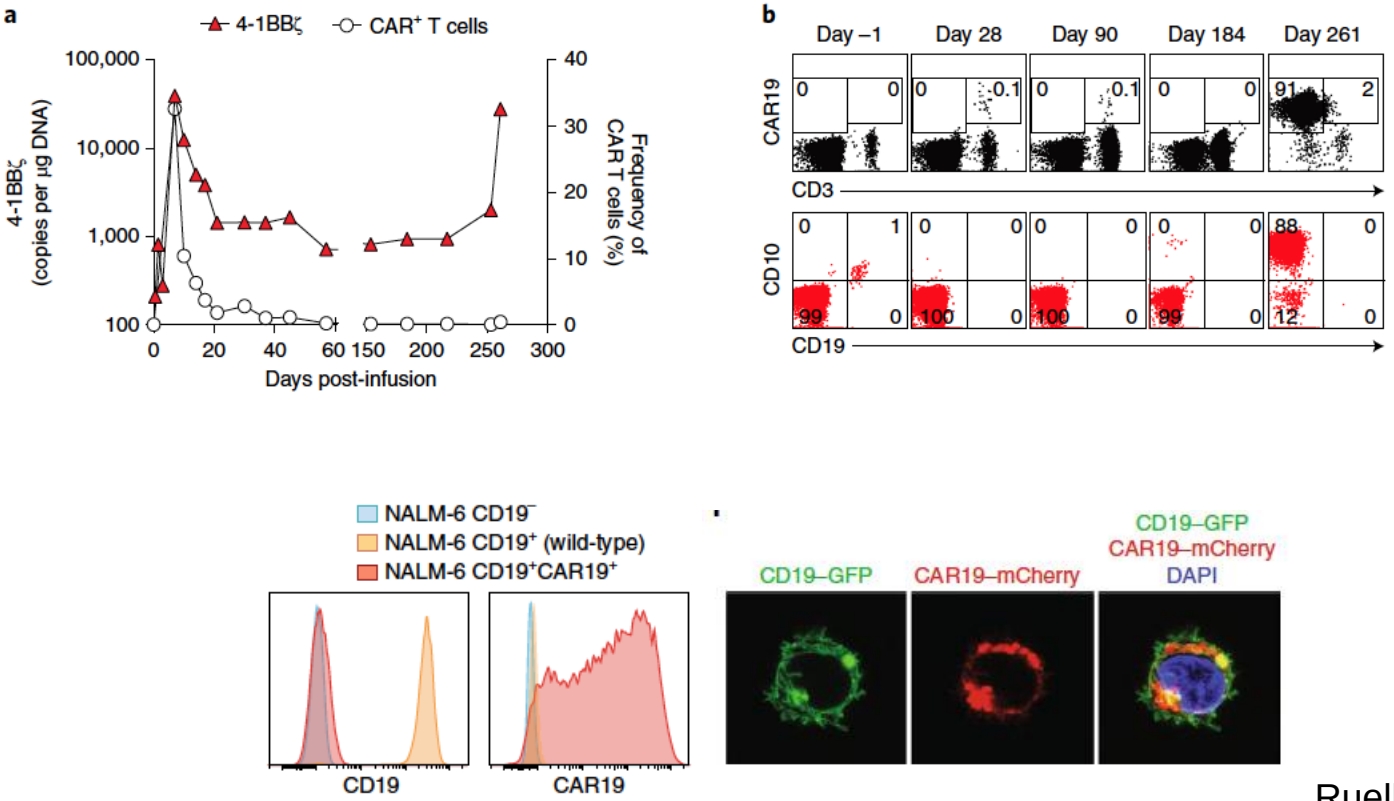


Fig. 1 | Wild-type (WT) CD19 and the predicted mutated CD19 protein structures for the CD19^{-}/r patients. Mutations in *CD19* were found in relapse samples from all 12 patients and are predicted to lead to a truncated protein lacking the transmembrane domain. Exons before the mutation are numbered. For each patient, the mutation with the largest AF is displayed, except for patient #8, who has two mutations with nearly equal AFs (both shown). The anti-CD19 antibody (FMC63)-binding site is found on exon 4. * indicates a mutation in the splice-site acceptor (SSA) leading to intron retention and destabilization of the transmembrane domain. NS, nonsynonymous; SNV, single-nucleotide variant; FS, frameshift; DEL, deletion; INS, insertion.

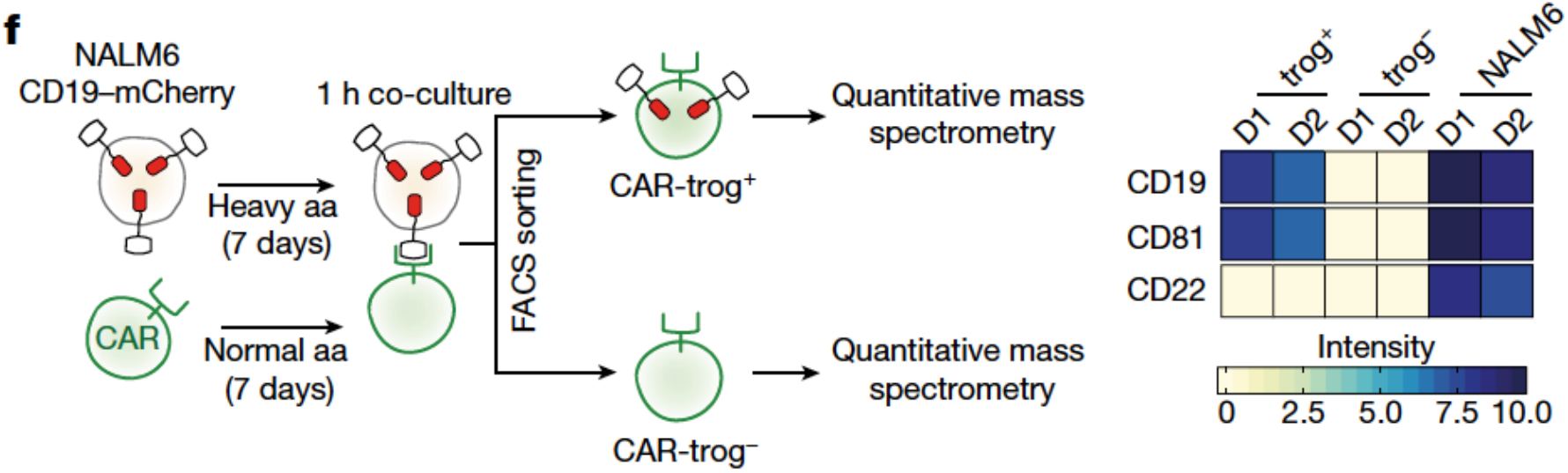
Orlando et al, Nature Med, 2018

CAR-transduced B-ALL cells ”mask” the CD19 antigen



Ruella, Nat Med, 2018

Trogocytosis of target antigen by CAR T cells



Hamieh, Nature, 2019

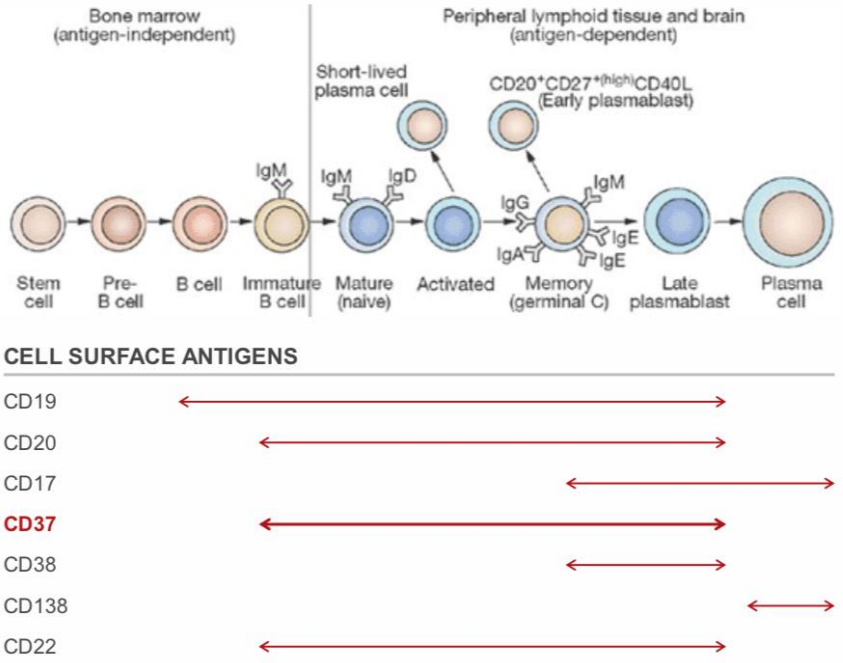
CD37 antigen as a potential therapeutic target in B & T cell lymphomas

CD37 antigen

- Tetraspanin family protein (TSPAN26)
- Cell membrane organization and co-signaling
- Modulate cellular adhesion, motility and proliferation
- Both pro-survival and pro-apoptotic capacities

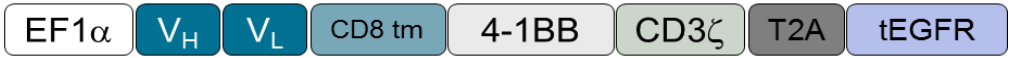
Expression on multiple tumor subtypes

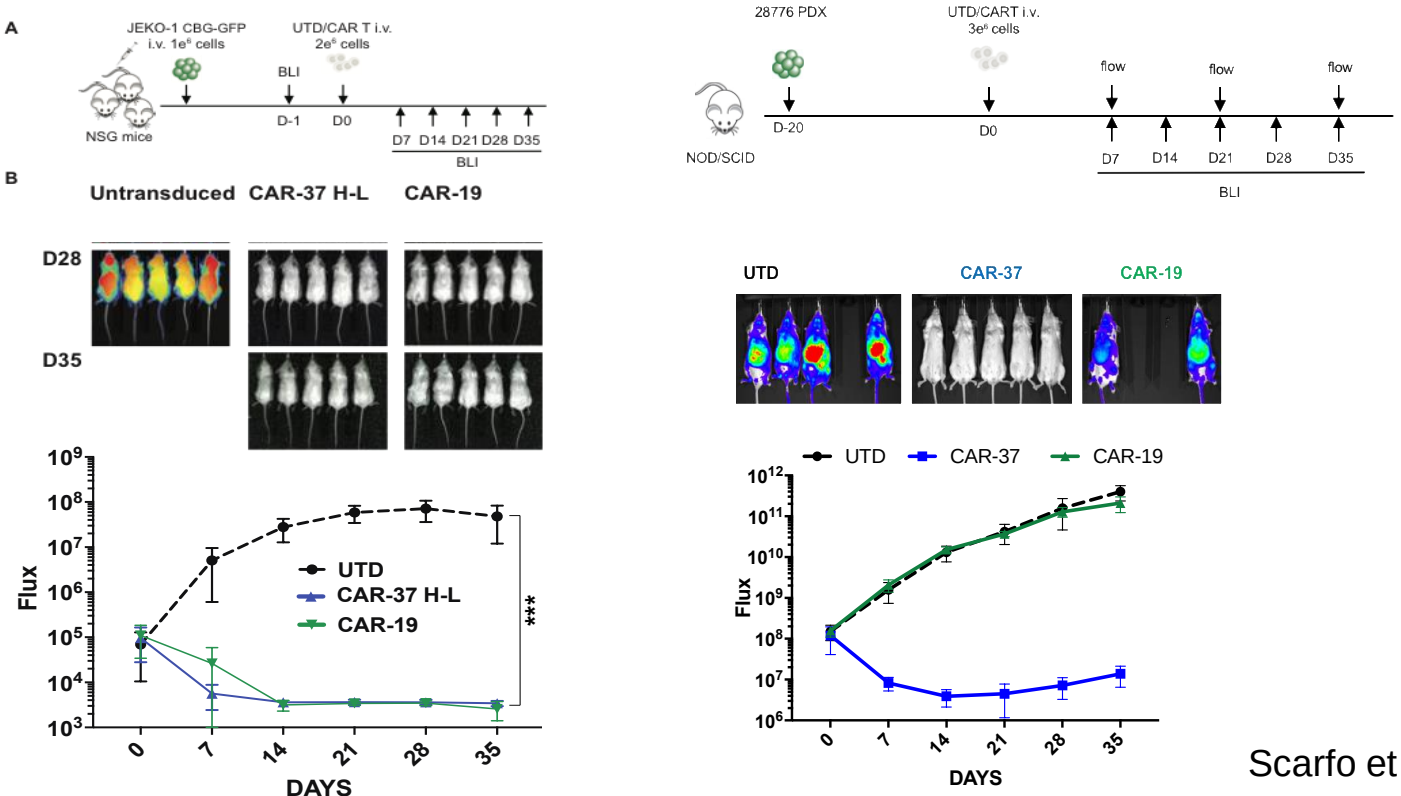
- B-cell NHL
- CLL
- Waldensrom's
- T-ALL/PTCL
- AML



Dahle et al. Anticancer Res. 2013
Deckert et al, *Blood* 2013

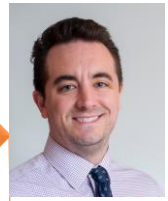
CAR 37 in B and T cell lymphoma





Scarfo et al, Blood, 2018

MGH Team and parallel streams enable rapid translation



Clinical Protocol Design

Preclinical Pharm/Tox Studies

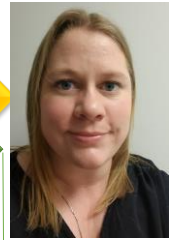
In vitro | In vivo | confirmatory

Establish correlative PK/PD assays

T cell CMC manufacturing process validations

Initial run | Eng runs | Validation

Phase I Clinical Trial Setup



Lentiviral vector manufacturing

Lenti manufacturing waitlist and contract

Release

SRC/IRB/IBC review | Site activation

IND Preparation and Submission to FDA

Jan 2018

Jan 2019

CAR selection completed

Pharm/tox studies completed

GMP vector Ready but not fully released

Protocol submission

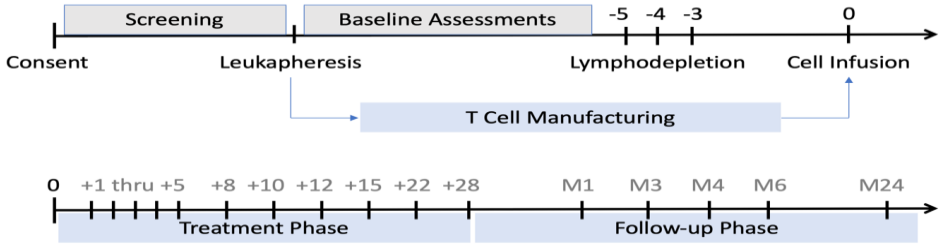
IND submission

FDA IND approval

FPFV

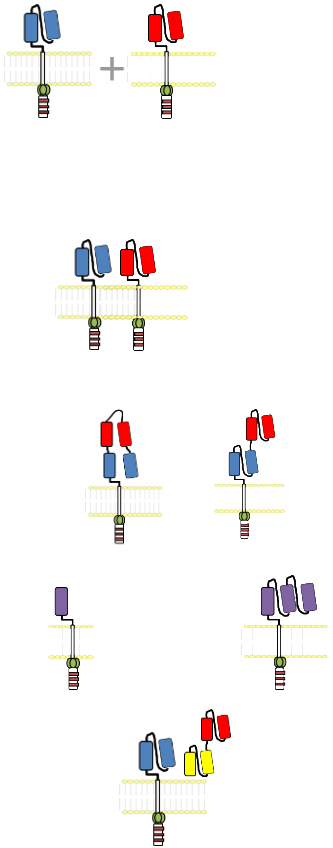
A Phase I Clinical Trial with CAR-37 T Cells for the Treatment of Patients with Relapsed or Refractory CD37+ Hematologic Malignancies

- **Primary:**
  - Evaluate safety and tolerability of CAR-37 T cells.
  - Determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of CAR-37 T cells in subjects with CD37+ hematologic malignances.
- **Secondary:**
  - Provide preliminary efficacy data on the anti-tumor effects of treatment with CAR-37 T cells in subjects with CD37+ hematologic malignancies
- **Exploratory:**
  - Evaluate the expansion, persistence, phenotype, and functional activity of CAR-37 T cells following infusion.
  - Evaluate cytokine profiles in the peripheral blood of subjects after infusion of CAR-37 T cells.
  - Explore potential mechanisms of resistance including, but not limited to, CD37 expression on tumor and host immune responses to CAR-37 T cells.

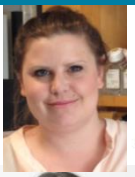


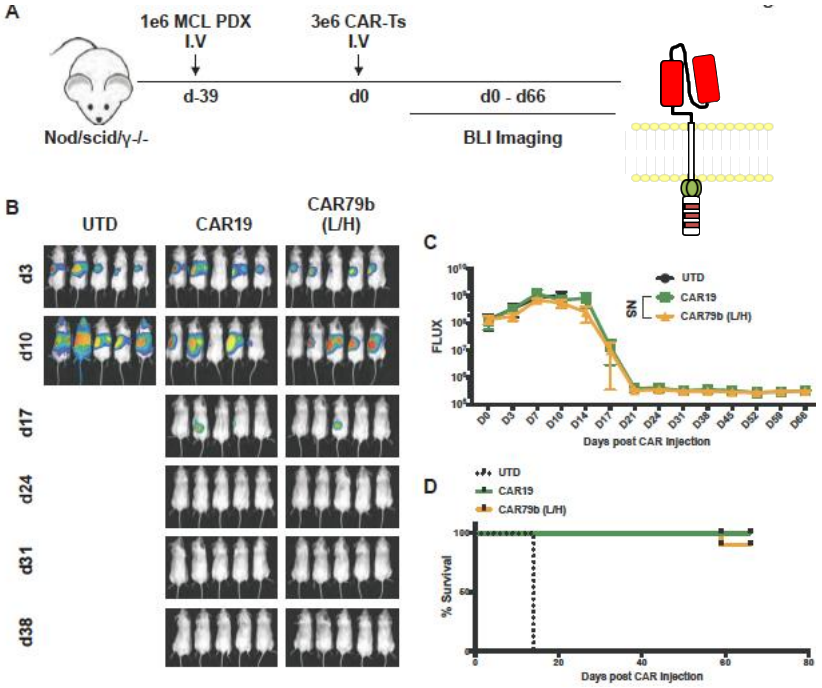
Approaches to multi-targeted CAR T cells

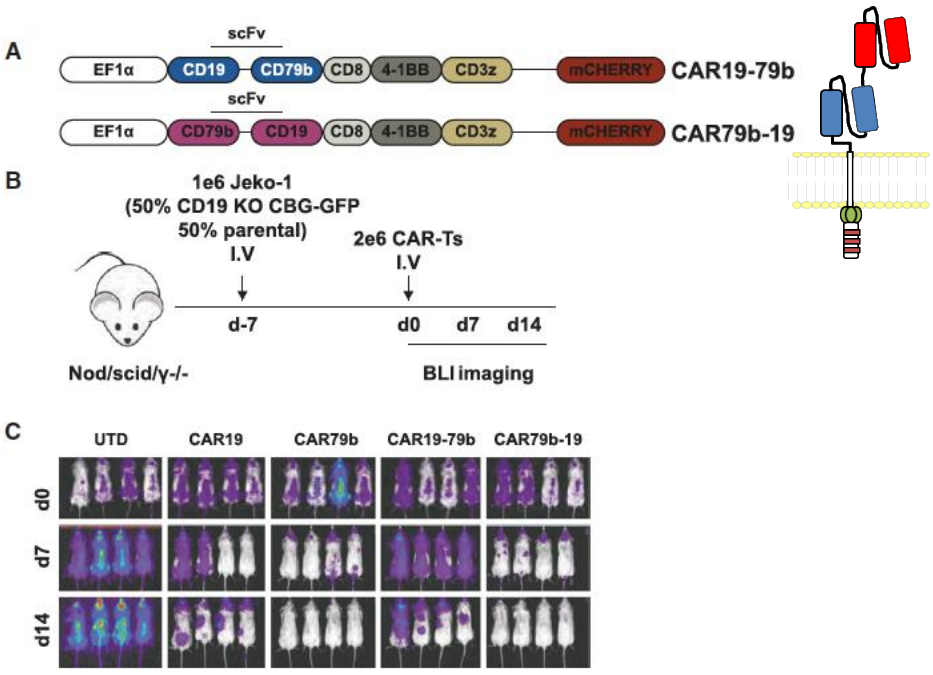
- Transduce 2 different cell populations with 2 different vectors
  - Savoldo JCI 2011 used this method to demonstrate 28z > z
- Transduce 1 cell population with 2 vectors
  - Ruella JCI 2016 used this method to show that 1 cell population > pooled cell populations
- Transduce 1 cell population with 1 “tandem” CAR
  - (Zah/Chen CIR 2016 with CD19/20; Qin/Fry Mol Ther Onc CD19/22)
- Binder that binds 2 related antigens (Schmidts Blood Adv, 2019)
- CAR T that secretes a BiTE (Choi Nat Biotech 2019)



A new CAR for the CD79b B cell antigen works in CD19+ and CD19- lymphoma as single and tandem CAR with CD19

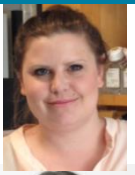


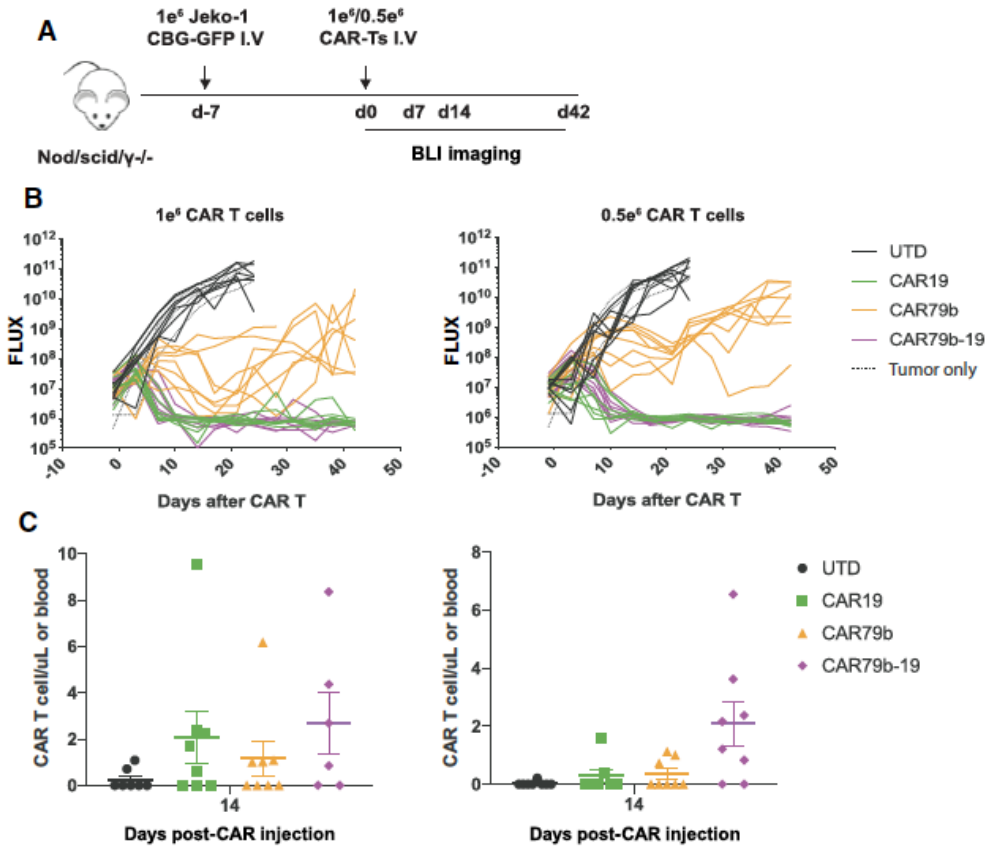




Ormhoj et al, Clin Canc Res, 2019

Tandem 1979 CARs are preferable in a stress test of “upfront” CD19+ CD79b lymphoma models

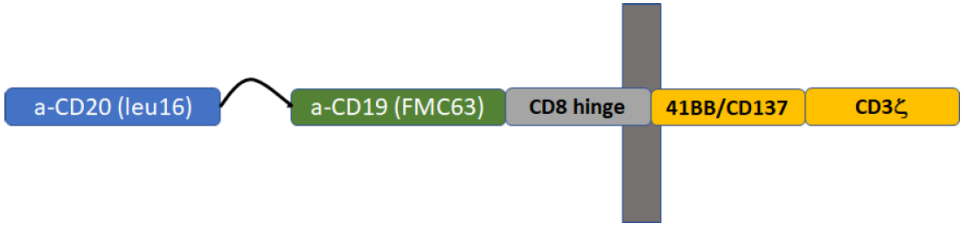


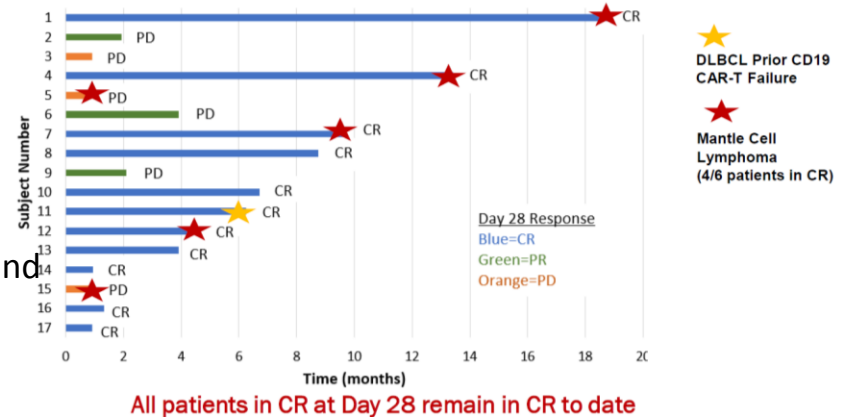


Ormhoj et al, Clin Canc Res, 2019

Dual Antigen Targeting

- Ph1 study of a bispecific, tandem anti-CD19, anti-CD20 4-1BBz CAR T-cell with point-of-care manufacturing
- Dose escalation:
  - $2.5x10^5$ cells/kg, n= 3
  - $7.5x10^5$ cells/kg, n= 3
  - *$2.5x10^6$ cells/kg, n= 3
- Dose expansion
  - Split dose (30/70), n=6
  - Single dose, n=2
- Results
  - 100% manufacturing success rate
  - Safety: No DLTs; no gr 3+ CRS; 2/17 grade 3 NT
  - Efficacy: ORR 82%, CR 65% overall; ORR 91%, CR 82% at RP2D; no relapses to date
  - Biomarkers: CR patients had better CAR T-cell expansion and persistence; patients with PD all retained CD19 and CD20

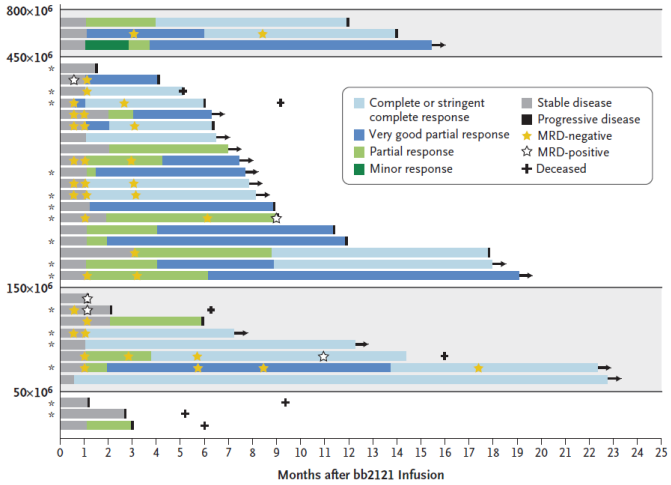


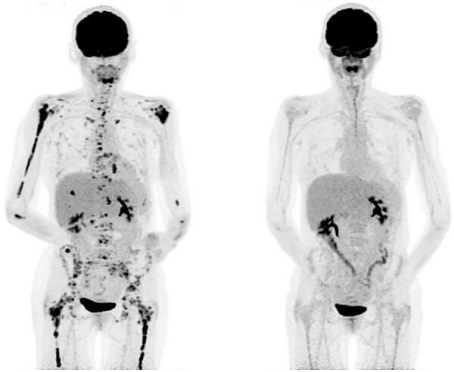


Shah N ASCO 2019

BCMA CAR T-cells in MM

- BB2121
  - 4-1BB CAR
  - N=33 at RP2D
  - ORR 85%; CR 45%; MRD neg in all responders evaluated
    - No correlation with BCMA level
  - mDOR 10.9m; mPFS 11.8m
  - CRS any grade 76%; gr3 6%
  - NT any grade 42%; gr4 2%
- UPenn
  - 4-1BB CAR
  - N=25 in dose escalation
  - ORR 64% in cohort 3 (n=11); 41% overall
  - mDOR 124.5d (3 ongoing, 1 CR at 32m)
  - CRS any grade 88%; gr3+ 32%
  - NT any grade 32%; gr3+ 12%
- NCI
  - CD28 CAR
  - N=16 at RP2D; median prior therapies 9.5
  - ORR 81%, VGPR/CR 63%
  - mEFS 31 weeks
  - Gr3+ CRS 38%



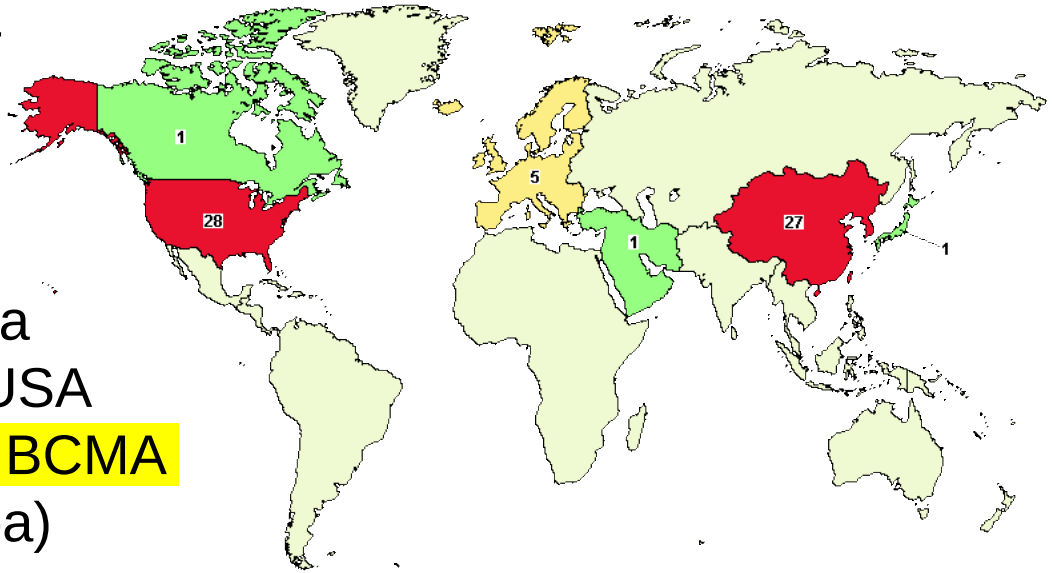


Raje et al NEJM 2019
Cohen et al JCO 2019
Brudno et al ASCO 2018

Clinical trials of CAR T cells in multiple myeloma

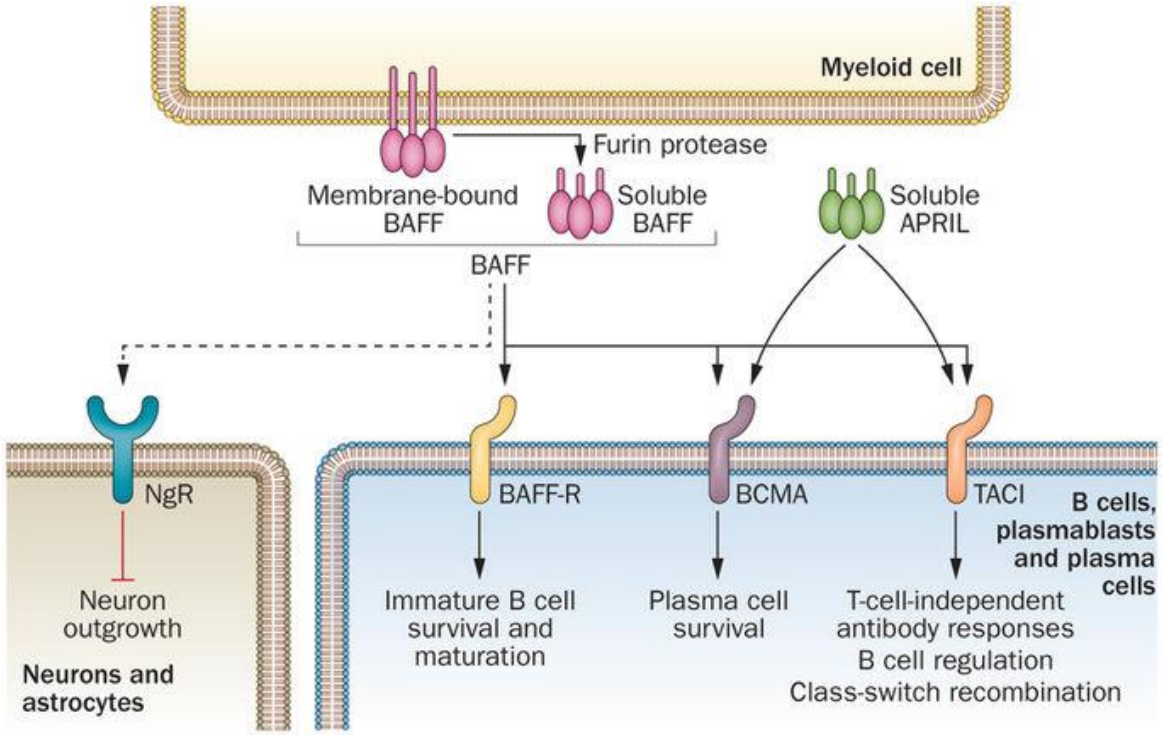
Multiple myeloma and CAR-T

59 trials worldwide
38 trials recruiting worldwide
- 27 trials recruiting in China
- 18 trials recruiting in the USA
  - all but 3 are targeting BCMA (NKG2D, CD38, kappa)



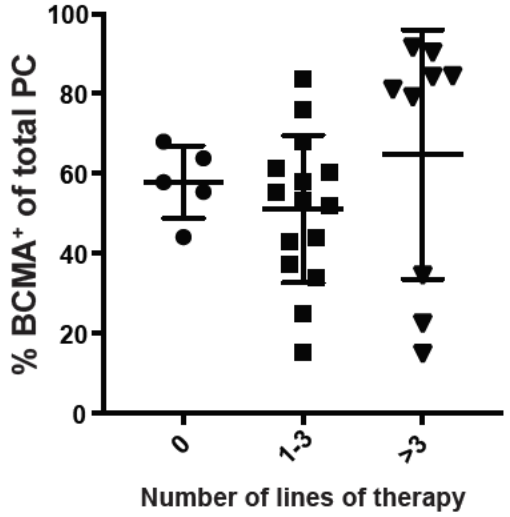
Targeting BCMA and TACI on multiple myeloma

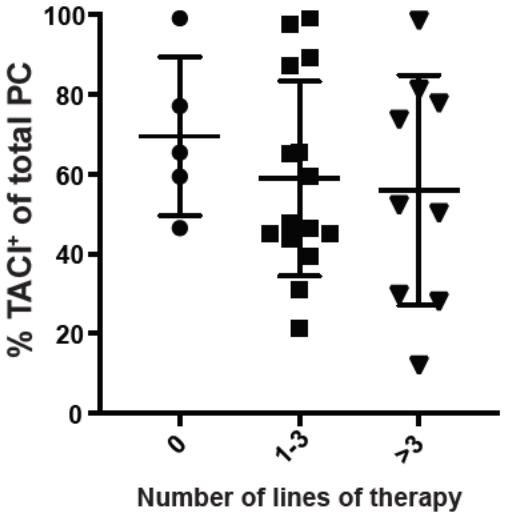
- APRIL (a proliferation-inducing ligand) is produced by myeloid cells in the BM microenvironment
- soluble APRIL binds to BCMA and TACI and promotes proliferation and survival of MM

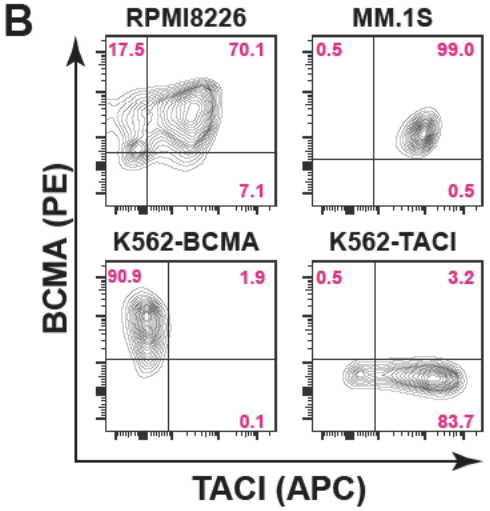


Vincent et al. Nature Reviews Rheumatology 2014

Patients with MM retain BCMA and TACI expression on their plasma cells

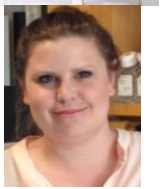


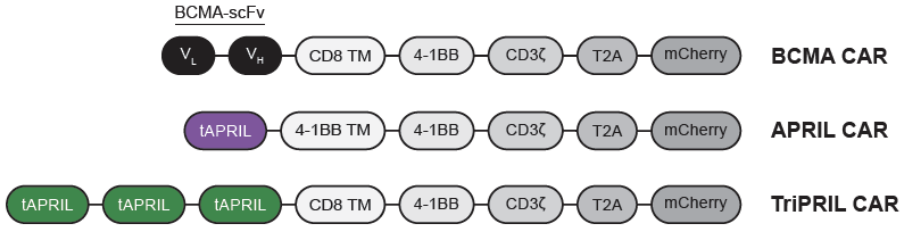


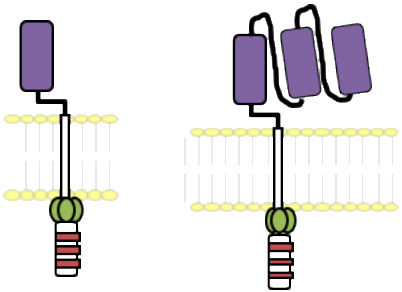


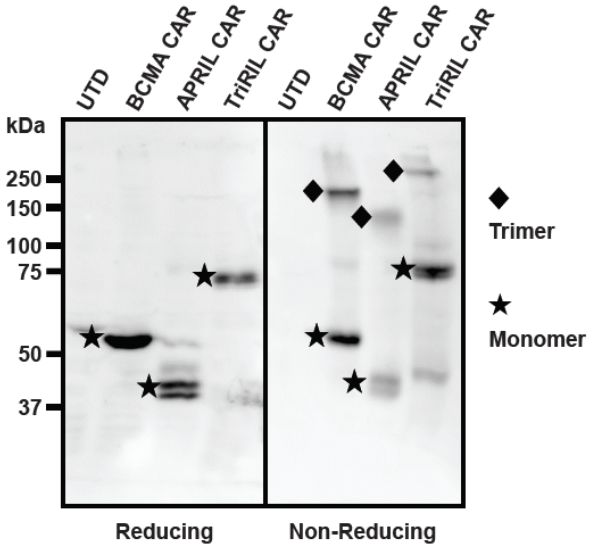
Schmidts et al, Blood Advances, in press

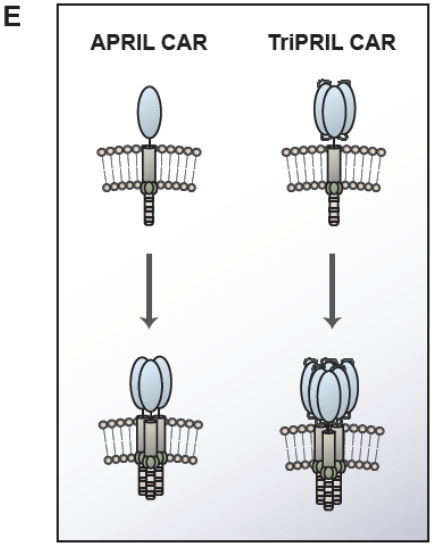
Design and trimerization of APRIL and Trimeric-APRIL CARs





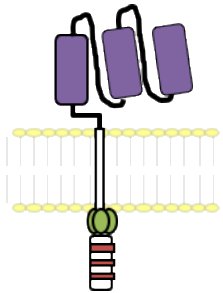




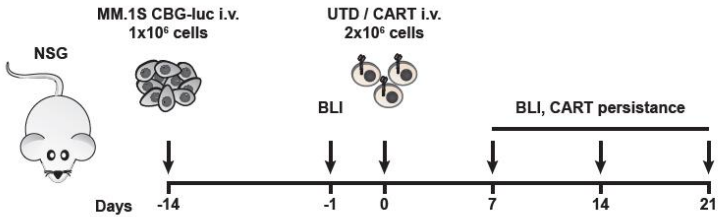


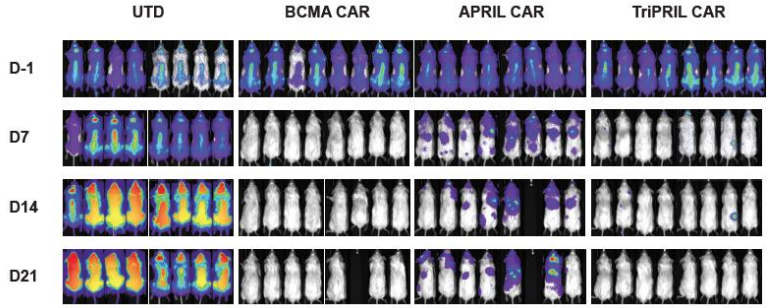
With Wolfgang Schamel

TriPRIL targets BCMA+ or BCMA- myeloma

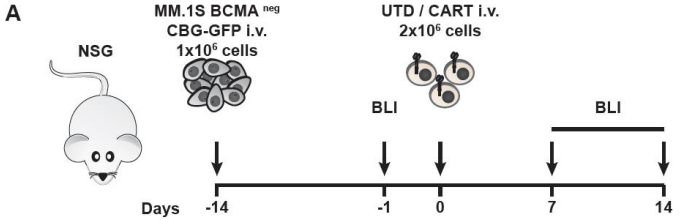


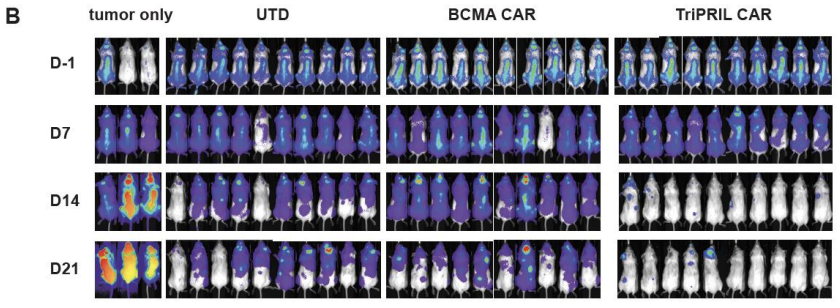
Upfront BCMA+ MM





BCMA-negative MM



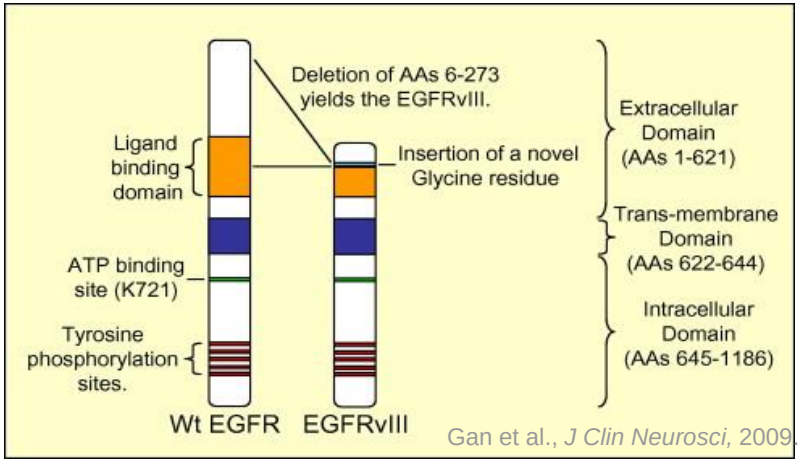


Schmidts et al, Blood Advances, 2019

Challenges for dual targeting CAR T cells in solid tumors

- Targeting tumor heterogeneity is desirable
- Lack of tumor-specific antigens is a challenge
- Would ideally also target or modify the tumor microenvironment

Rationale for EGFRvIII as therapeutic target in GBM



Gan et al., *J Clin Neurosci*, 2009.

EGFRvIII:

- In-frame deletion of exons 2-7 and generation of novel glycine residue at the junction
- Constitutively active, oncogenic mutation
- Not expressed in normal tissues

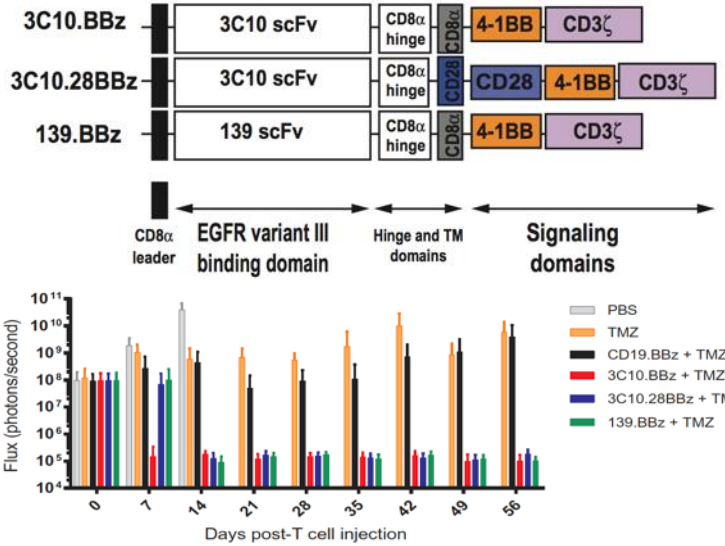
- Expressed in ~30% of GBM cases (but heterogeneous)

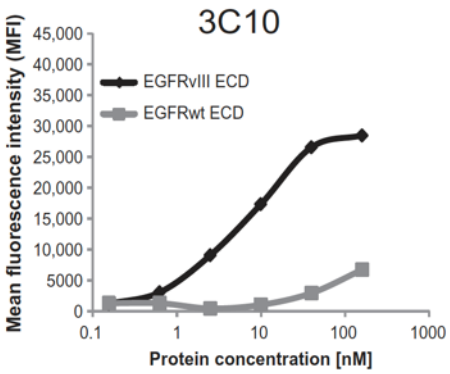
Optimizing anti-EGFRvIII CAR T binding specificity

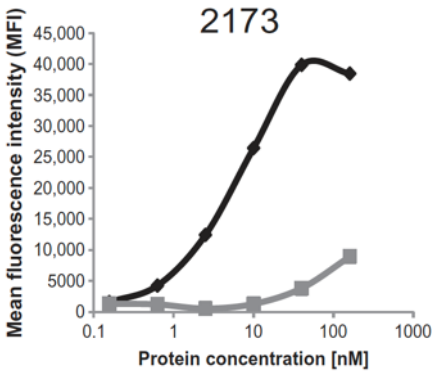
www.ScienceTranslationalMedicine.org 18 February 2015 Vol 7 Issue 275 275ra22

Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma

Laura A. Johnson,[1,2]* John Scholler,[1]* Takayuki Ohkuri,[3] Akemi Kosaka,[3] Prachi R. Patel,[1] Shannon E. McGettigan,[1] Arben K. Nace,[4] Tzvete Dentchev,[4] Pramod Thekkat,[5] Andreas Loew,[5] Alina C. Boesteanu,[1] Alexandria P. Cogdill,[1] Taylor Chen,[1] Joseph A. Fraietta,[1] Christopher C. Kloss,[1] Avery D. Posey Jr.,[1] Boris Engels,[5] Reshma Singh,[5] Tucker Ezell,[5] Neeraja Idamakanti,[5] Melissa H. Ramones,[5] Na Li,[5] Li Zhou,[5] Gabriela Plesa,[1] John T. Seykora,[4] Hideho Okada,[6] Carl H. June,[1,2] Jennifer L. Brogdon,[5] Marcela V. Maus[1,7†]







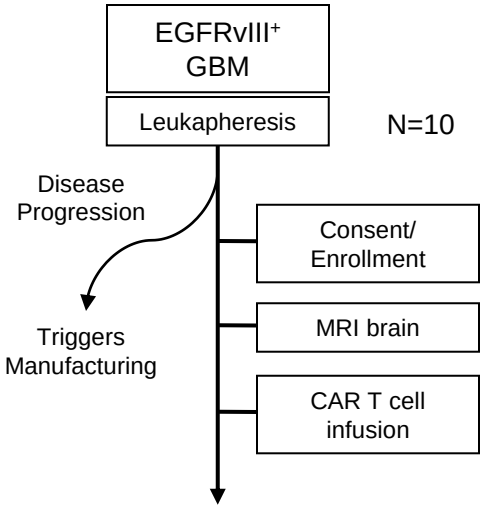
NCT02209376 Phase 1 Study, EGFRvIII CART

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma

Donald M. O'Rourke,[1] MacLean P. Nasrallah,[2*] Arati Desai,[3*] Jan J. Melenhorst,[4*] Keith Mansfield,[5*] Jennifer J. D. Morrissette,[6] Maria Martinez-Lage,[2†] Steven Brem,[1] Eileen Maloney,[1] Angela Shen,[7] Randi Isaacs,[5] Suyash Mohan,[8] Gabriela Plesa,[4] Simon F. Lacey,[4] Jean-Marc Navenot,[4] Zhaohui Zheng,[4] Bruce L. Levine,[4] Hideho Okada,[9] Carl H. June,[4] Jennifer L. Brogdon,[5] Marcela V. Maus[10‡]



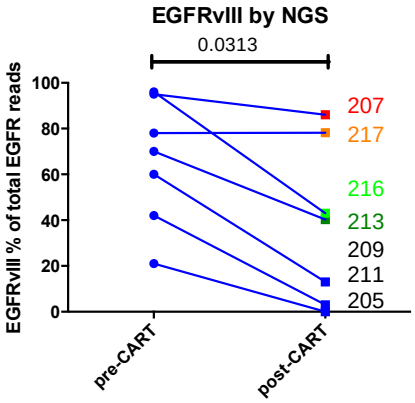
Eligibility

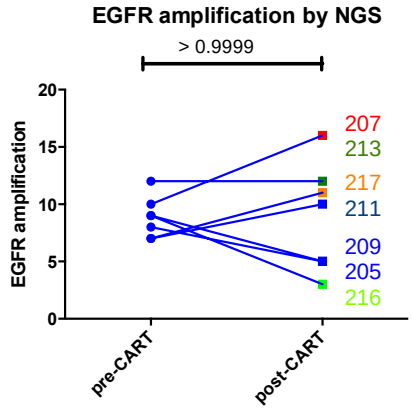
- Confirmed EGFRvIII+ GBM by NGS assay
- Manufacturing of CART triggered by recurrence or disease progression.
- Dexamethasone permitted up to 4MG QD.

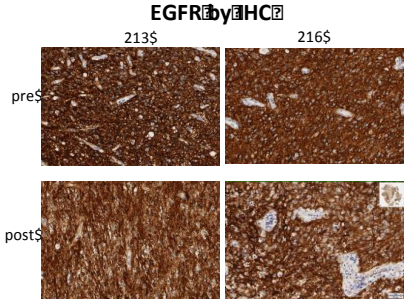
Objectives

- Exploratory study with primary objectives of safety and feasibility ($5x10^8$ CART per patient).

CART-EGFRvIII traffic to tumor, target antigen, but there is antigen heterogeneity and Treg infiltration

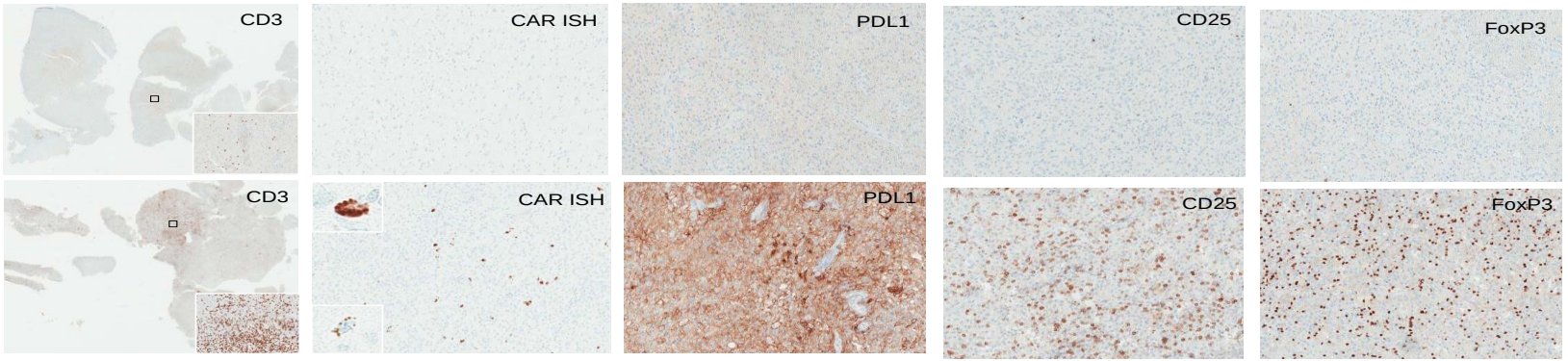






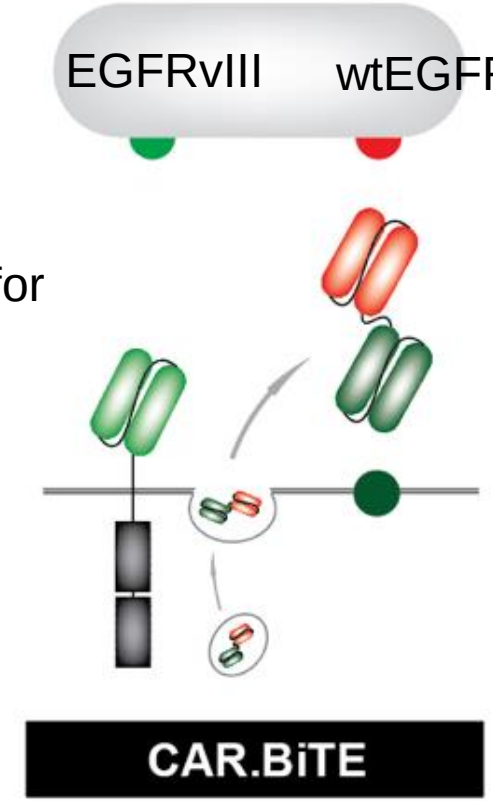
Pre

Post

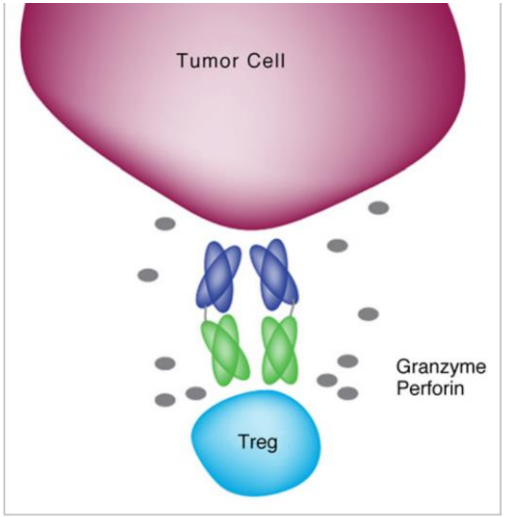


CAR T cells for solid tumors need to overcome heterogeneity and immunosuppressive environment: CAR-BiTE design

One tumor-specific target for the CAR

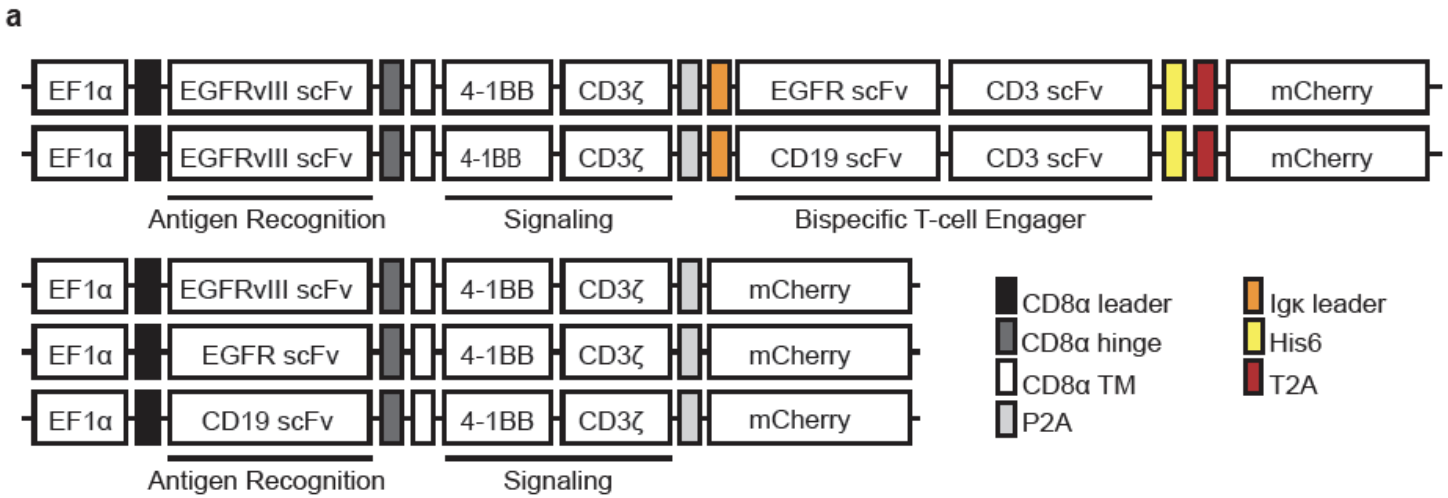


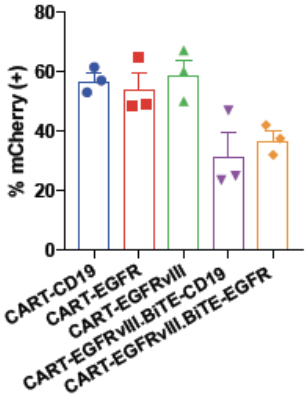
BiTE can target the "undruggable"
- Local site
- Rapid clearance
- Can re-direct Tregs



Choi et al, CIR 2013

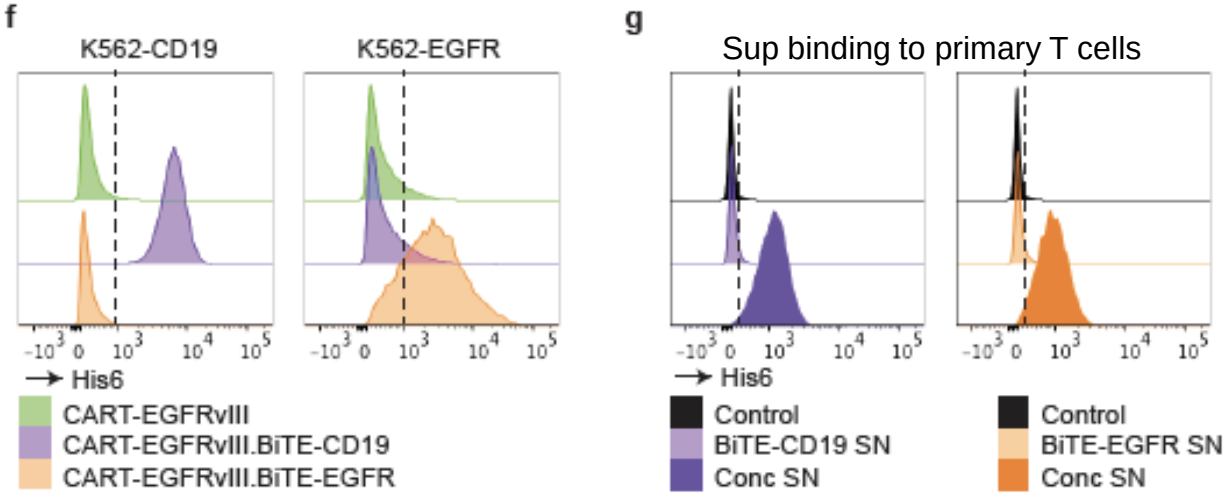
Constructs: bicistronic LV to express CAR-BiTEs





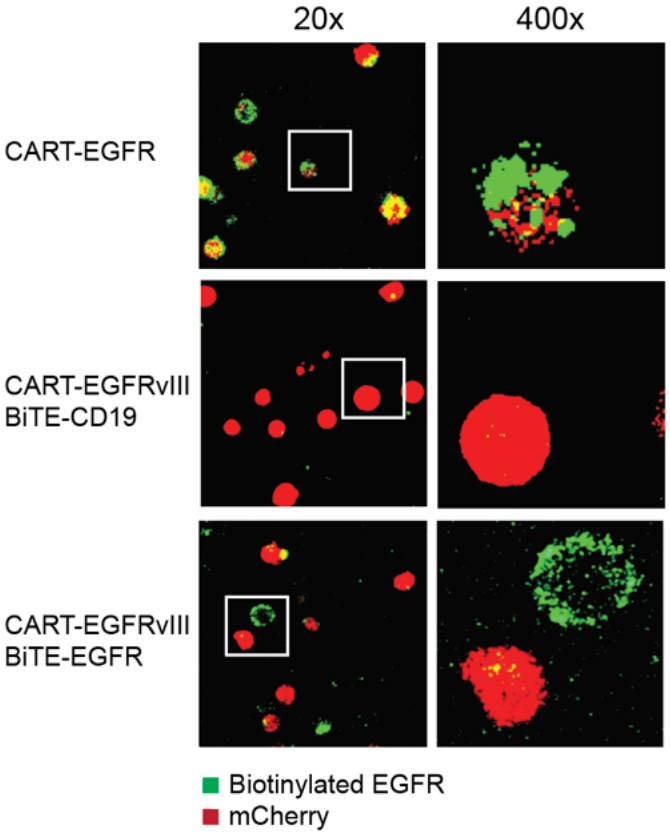
Choi et al, Nature Biotechnology 2019

BiTEs are detectable on cell surfaces and concentrated supernatants in vitro



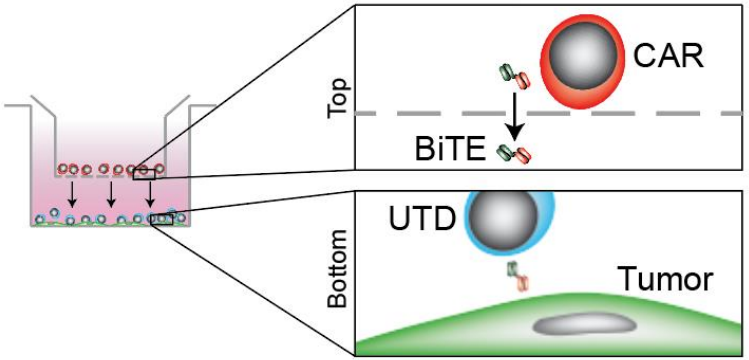
Choi et al, Nature Biotechnology 2019

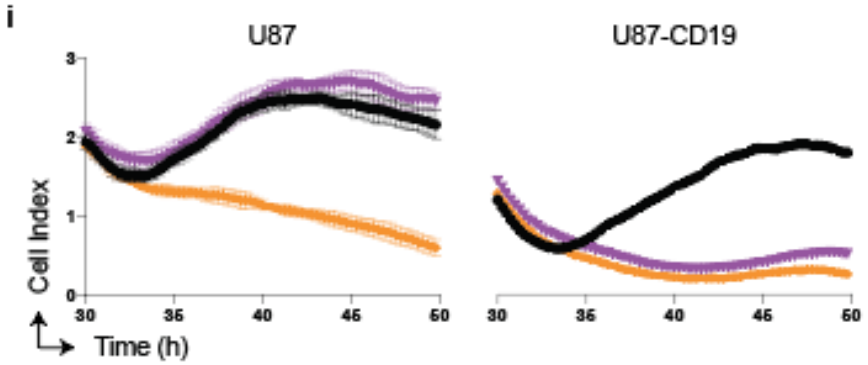
BiTEs (green) bind to both CAR+ (red) and bystander T cells



Choi et al, Nature Biotechnology 2019

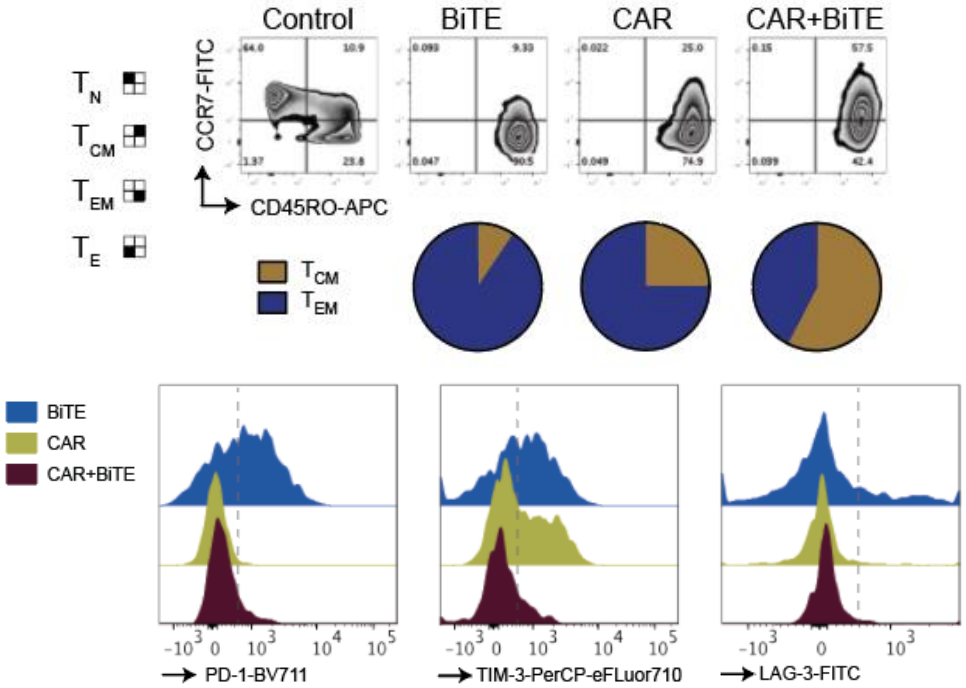
Secreted BiTEs bind CART but also redirect bystander T cells

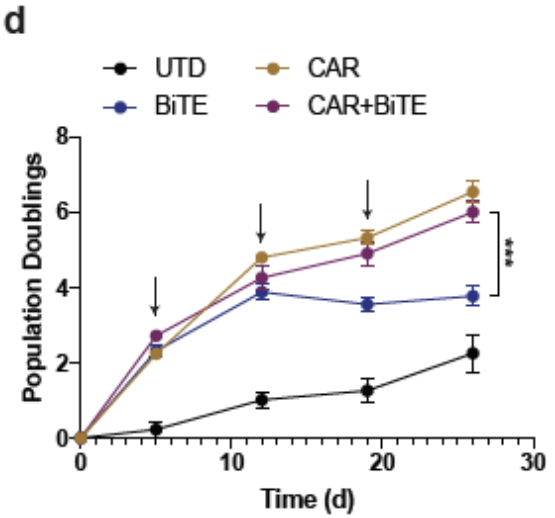




Choi et al, Nature Biotechnology 2019

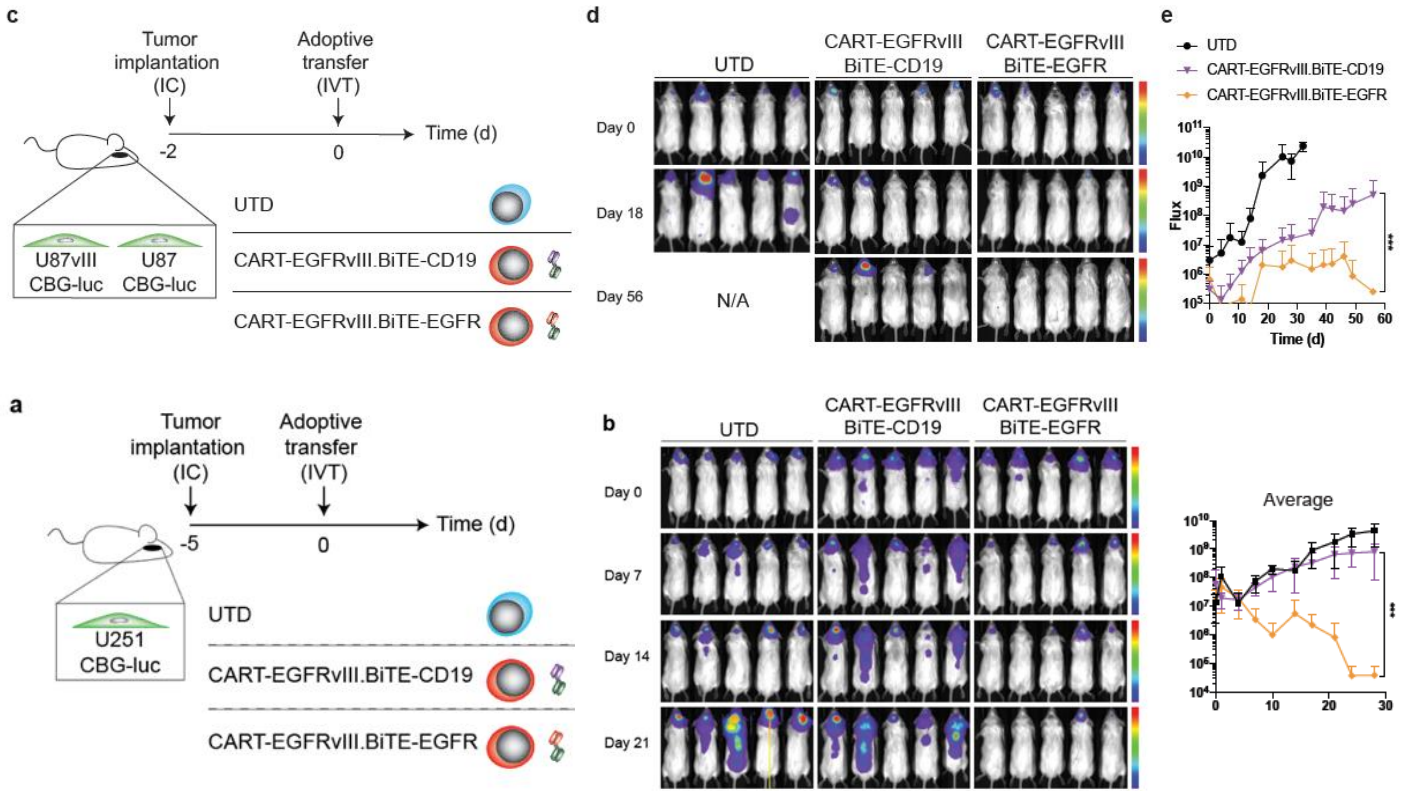
CAR-BiTEs have more Tcm, less exhausted phenotype and sustained expansion in vitro





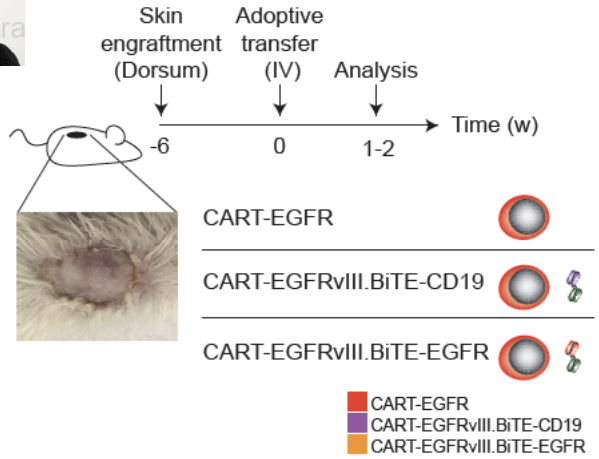
Choi et al, Nature Biotechnology 2019

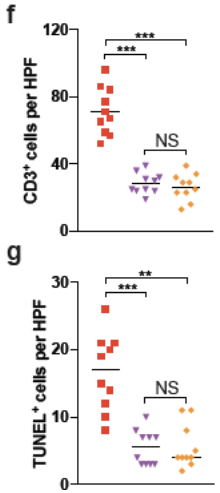
CAR-BiTEs induce responses in EGFRvIII- and mixed GBM in vivo

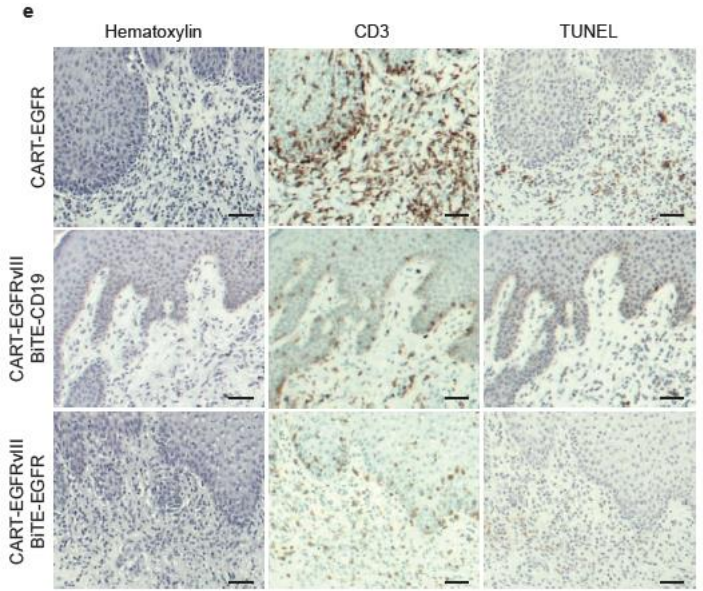


Choi et al, Nature Biotechnology 2019

No evidence of EGFR (BiTE)-related toxicity in skin graft model







Choi et al, Nature Biotechnology 2019

Conclusions: Overcoming resistance and toxicity

- Multitargeting
  - There are multiple ways to target more than one antigen with a CAR T cell
  - Using one vector/one cell population may be most effective (and most cost-effective)
  - Creative approaches are needed depending on expression profile of each antigen
- T cell exhaustion (drug combinations, gene editing)
- Toxicity (adding cytokine blockade beyond IL-6R)

Acknowledgements

Maus Lab

Science CAR T team
Irene Scarfo
Mark Leick
Stefanie Bailey
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Becca Larson
Korneel Grauwet
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Fred Preffer

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Yi-bin Chen

Manufacturing
Jerry Ritz

Collaborators
Hiroaki Wakimoto
David Weinstock
Joren Madsen
Daniel Irimia
Ben Ebert

NIH National Cancer Institute
Gabrielle's Angel Foundation for Cancer Research
The V Foundation for Cancer Research
Damon Runyon Cancer Research Foundation
MGH Massachusetts General Hospital
Stand Up To Cancer