T cell therapy moving into new territory

Marcela V. Maus, MD, PhD

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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 CD3ζ
CD19-directed chimeric antigen receptor T cells in the clinic


*Defined ratio of CD4:CD8
Cell Therapy: Collection, Manufacturing, Administration

1. Leukapheresis
2. T-cell activation/transduction
3. Modified T-cell expansion
4. Chemotherapy
5. Modified T-cell infusion
**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

<table>
<thead>
<tr>
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<th>ZUMA-1</th>
<th>JULIET</th>
<th>TRANSCEND CORE</th>
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<tr>
<td>ORR (%)</td>
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<td>52</td>
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<td>CR (%)</td>
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<td>6m ORR (%)</td>
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<td>37*</td>
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<tr>
<td>6m CR (%)</td>
<td>36</td>
<td>30*</td>
<td>41</td>
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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

DON’T GIVE HIM ANYMORE WORK TO DO!

CAN’T YOU SEE HE’S EXHAUSTED!

PD-1
TIM-3
LAG3

Dzu-Doodles
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

Gill ASH 2018
Mechanisms of Resistance

- Poor T cell fitness (lack of proliferation and/or persistence)
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  - Allogeneic source?
- Target antigen loss
  - Splice variants
  - Reversion to myeloid phenotype
  - Mutations
  - B cells transduced with CARs and masking
  - Trogocytosis of CD19
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
Frameshift mutations in CD19 with loss of TM domain

Orlando et al, Nature Med, 2018

Fig. 1 | Wild-type (WT) CD19 and the predicted mutated CD19 protein structures for the CD19−/− 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.
CAR-transduced B-ALL cells "mask" the CD19 antigen

Ruela, 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
CAR 37 in B and T cell lymphoma

**Figure 4**

A. Jeko-1 CBG-GFP → UTDCAR T i.v. 2×10⁶ cells → BLI D-1 D0 D7 D14 D21 D28 D35

B. Untransduced CAR-37 H-L → CAR-19

D28 D35

Flux

UTD CAR-37 H-L CAR-19

UTD/CAR T i.v. 3×10⁶ cells → flow D7 D14 D21 D28 D35

NOD/SCID

Flux

UTD CAR-37 H-L CAR-19

0 7 14 21 28 35
0 14 21 28 35
0 7 14 21 28 35

Scarfo et al, Blood, 2018
MGH Team and parallel streams enable rapid translation

Preclinical Pharm/Tox Studies
- In vitro
- In vivo
- Confirmatory

Clinical Protocol Design
- T cell CMC manufacturing process validations
- Initial run
- Eng runs
- Validation

Establish correlative PK/PD assays

Phase I Clinical Trial Setup
- SRC/IRB/IBC review
- Release
- Site activation

Lentiviral vector manufacturing
- Lenti manufacturing waitlist and contract
- Release

IND Preparation and Submission to FDA
- Pharm/tox studies completed
- GMP vector Ready but not fully released
- Protocol submission
- IND submission
- FDA IND approval
- FPFV

Jan 2018
- CAR selection completed

Jan 2019
- GMP vector released
- Protocol submission
- IND submission
- FDA IND approval
- FPFV

We are here (May 5, 2019)

CONFIRMATORY JAN 2018 CAR SELECTION COMPLETED
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 malignancies.

• **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%

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

Schmidts et al, Blood Advances, in press

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

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

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

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

3C10 BBz  3C10 scFv  CD8 hinge  4-1BB  CD3ζ
3C10.28BBz  3C10 scFv  CD8 hinge  CD28  4-1BB  CD3ζ
139 BBz  139 scFv  CD8 hinge  4-1BB  CD3ζ

Mean fluorescence intensity (MFI)

Protein concentration [nM]

Mean fluorescence intensity (MFI)

Protein concentration [nM]
**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

**Graphs:**
- EGFRvIII by NGS
- EGFR amplification by NGS

**Images:**
- Pre-treatment biopsy
- Post-treatment biopsy (13 days)
- Pre-treatment biopsy
- Post-treatment biopsy (13 days)

**Figure B:**
- IDO1
- PDL1
- FoxP3
- TGF-β
- Pre-treatment biopsy
- Post-treatment biopsy (13 days)

**Tables:**
- EGFRvIII % of total EGFR reads
- EGFR amplification by NGS
- EGFR by IHC

**Notes:**
- EGFRvIII by NGS: 0.0313
- EGFR amplification by NGS: > 0.9999
- EGFR by IHC: 213S, 216S
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)**
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