# Current Status of Chimeric Antigen Receptor (CAR) T Cell Therapy

## City of Hope Comprehensive Cancer Center

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# Disclosure

- **Intellectual property and patents in the field of CAR-engineered T-cell therapy.**
- **License agreement with and receive research support from Mustang Therapeutics, Inc.**
- **Conflicts of interest have been reviewed and approved by City of Hope in accordance with its conflict of interest and commitment policy.**
- **There will be discussion about the use of products for non-FDA approved indications.**

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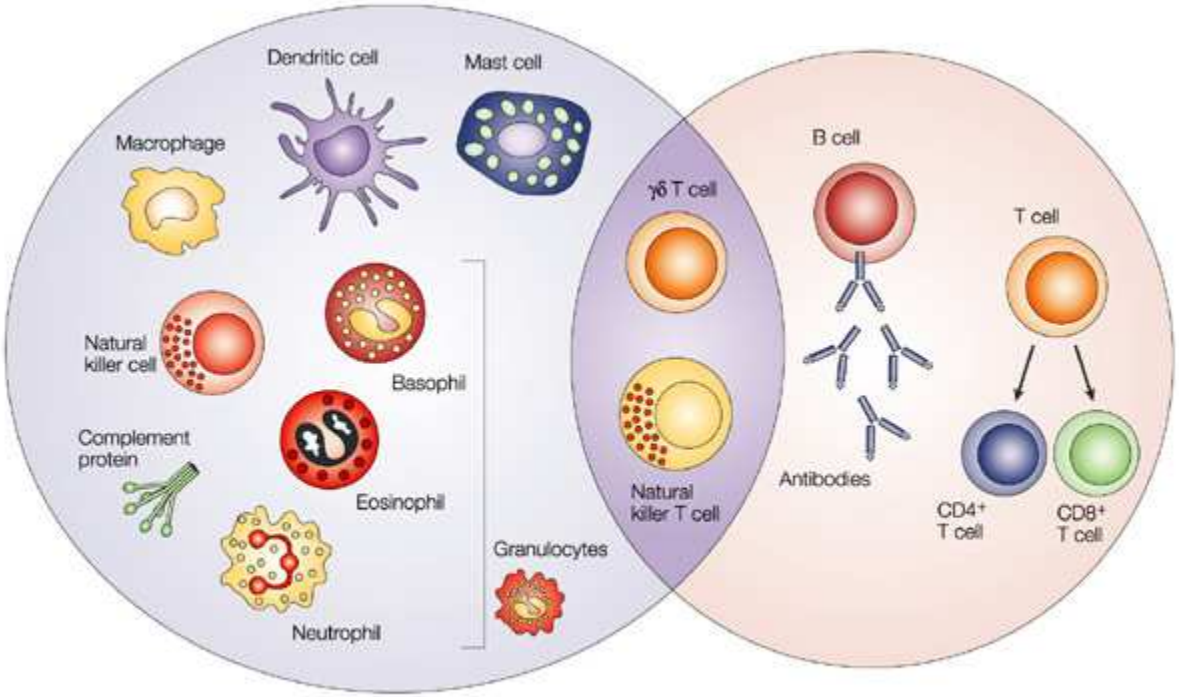
# Human Immune System

Innate Immunity
(rapid response)

Adaptive Immunity
(slow response)




Nature Reviews | Cancer

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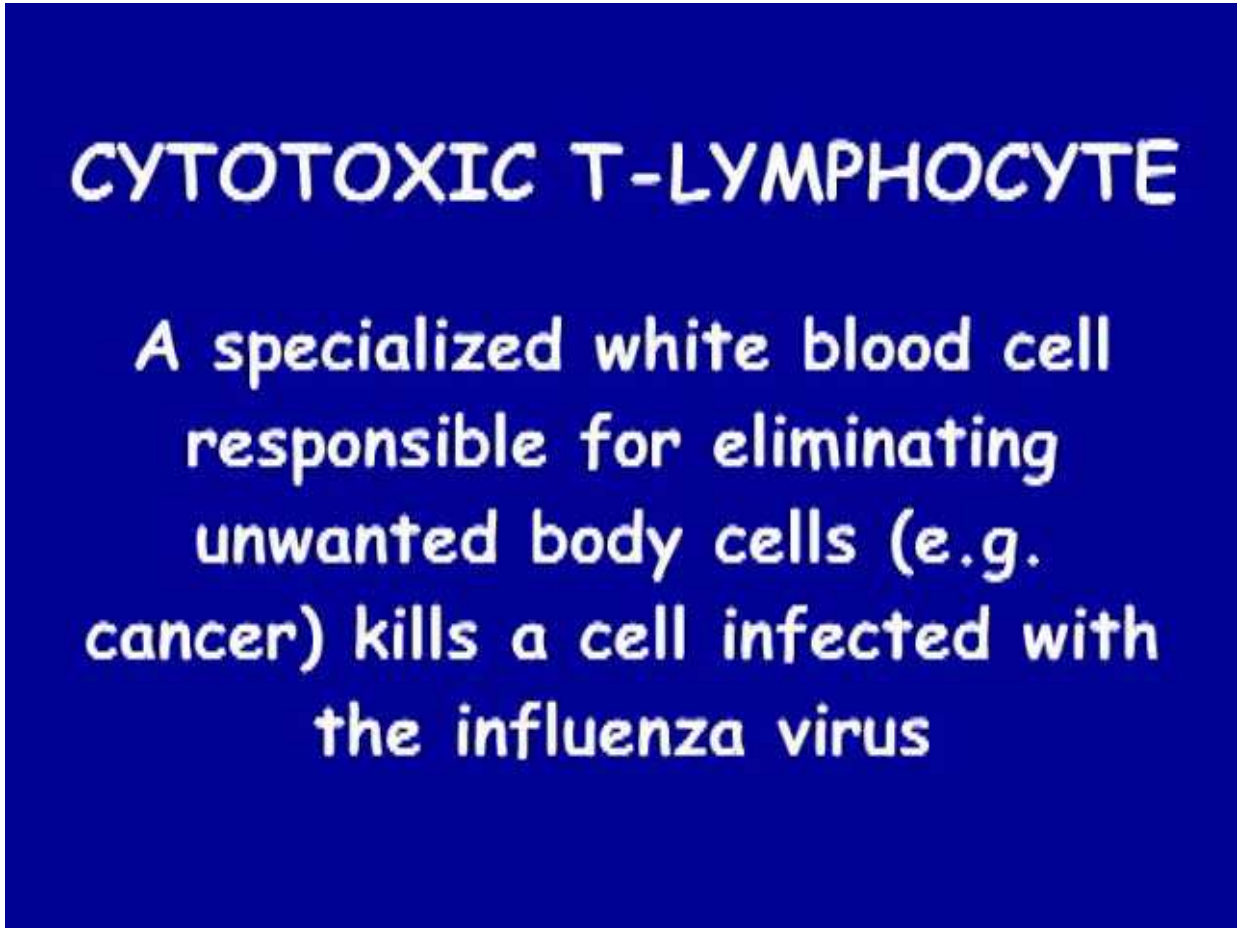
# Harnessing the Potency & Specificity of T Cells for Cancer Therapy

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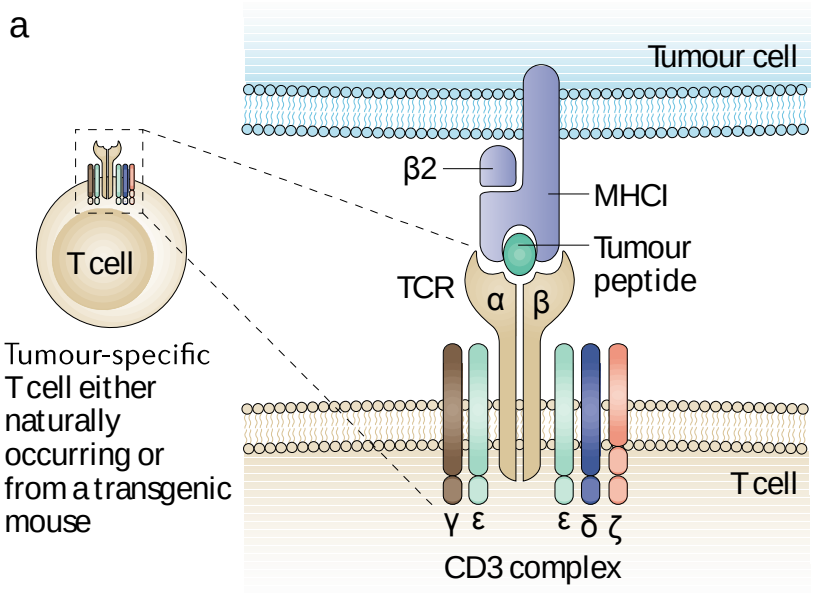
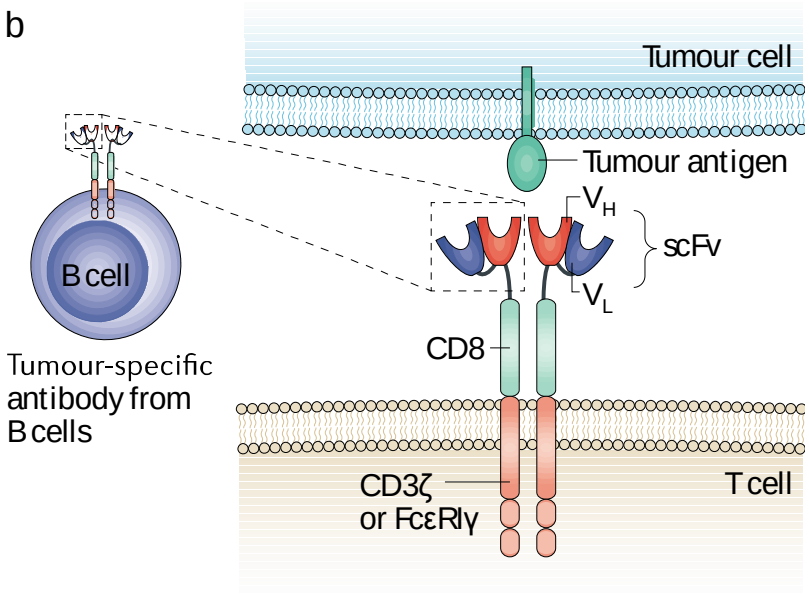
# Engineering T cell Immune Responses

## TCR-engineered T cell Therapy




## CAR-engineered T cell Therapy




<u>Advantages CAR T cells</u>

1. Direct attacking of tumor-associated antigens
2. MHC-independent
3. Less opportunity for immune escape

Adapted from Kershaw et al, Nature Reviews Cancer 2013

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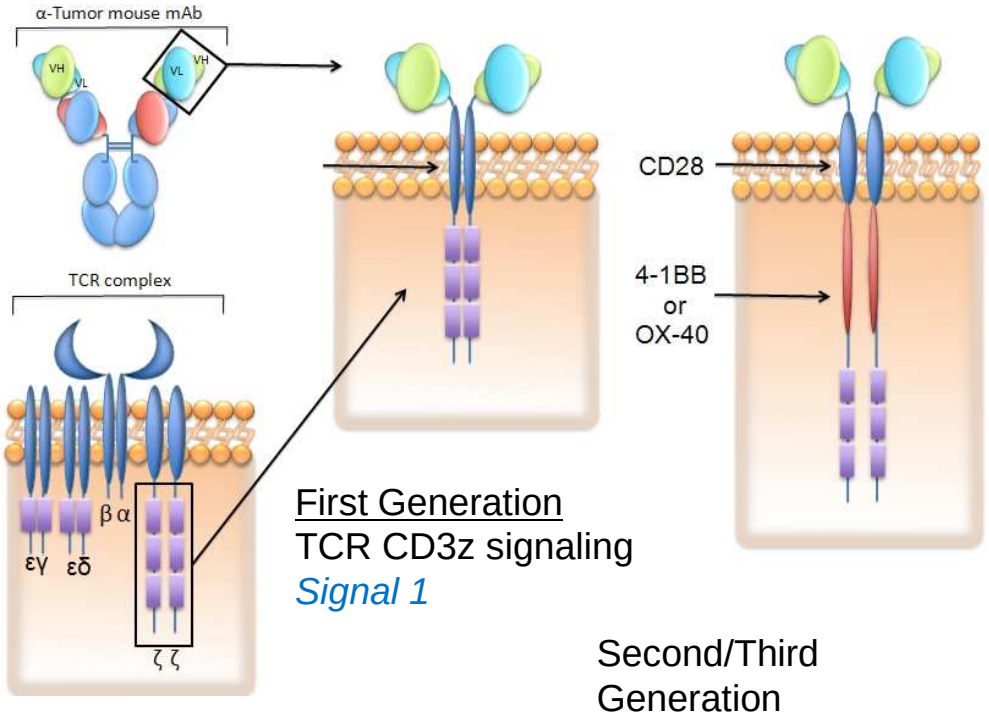
# Chimeric Antigen Receptor (CAR) T Cell Therapy




First Generation
TCR CD3z signaling
*Signal 1*

Second/Third Generation
Costimulatory signaling
CD28, 41BB, OX40, ICOS
*Signal 2*

## Advantages of CARs

- Modular design
- MHC independent
- Functional in both CD4 and CD8 T cells
- Significant numbers of tumor specific T cells can be rapidly generated
- The potential to generate novel long-term antitumor immunity

## Challenges

- Single antigen specificity
- Primarily restricted to extracellular antigens
- On-target and off-target toxicities

Chekmasova & Brentjens; Discovery Today; 2010

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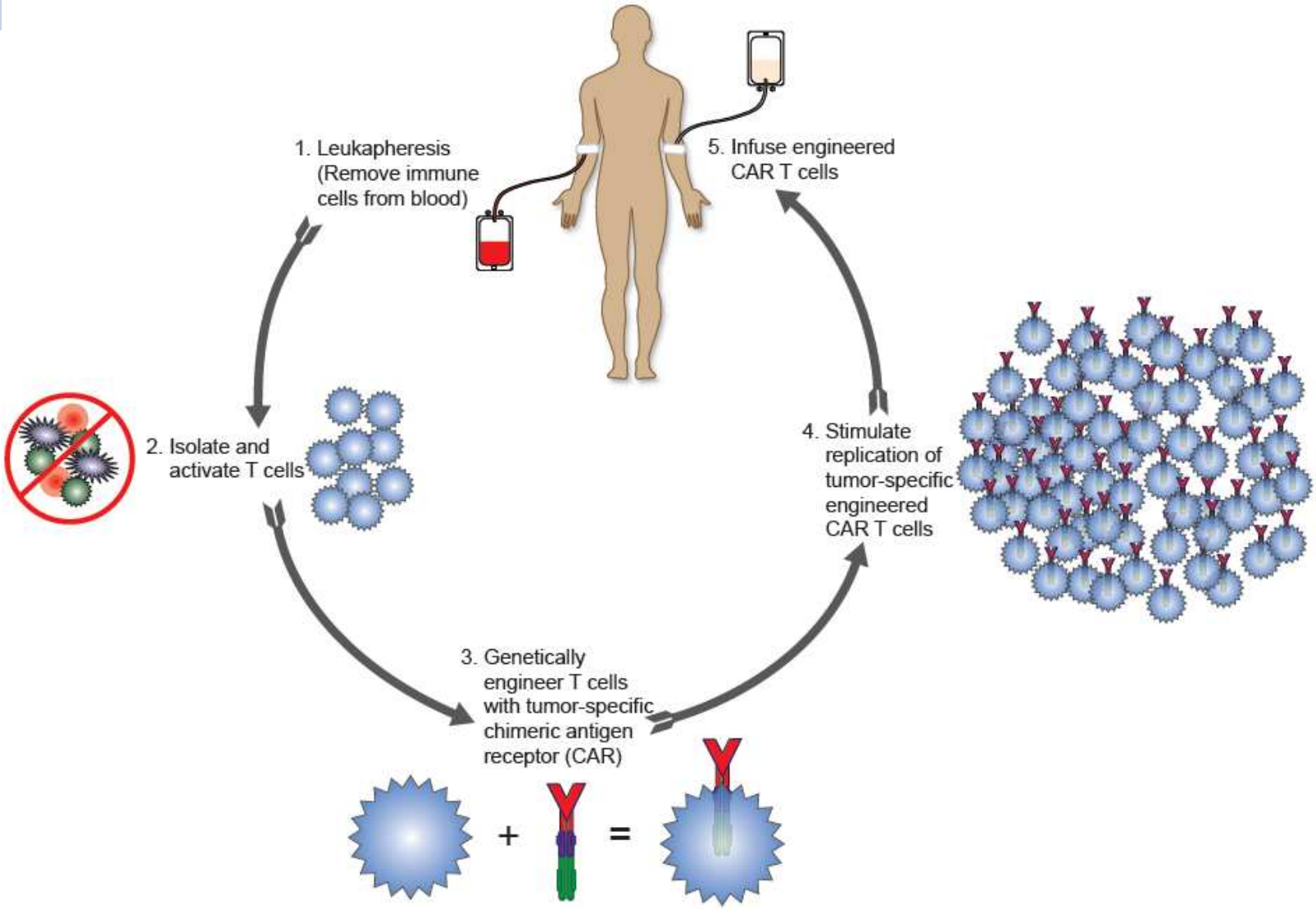
# Adoptive Therapy using CAR-Engineered T cells

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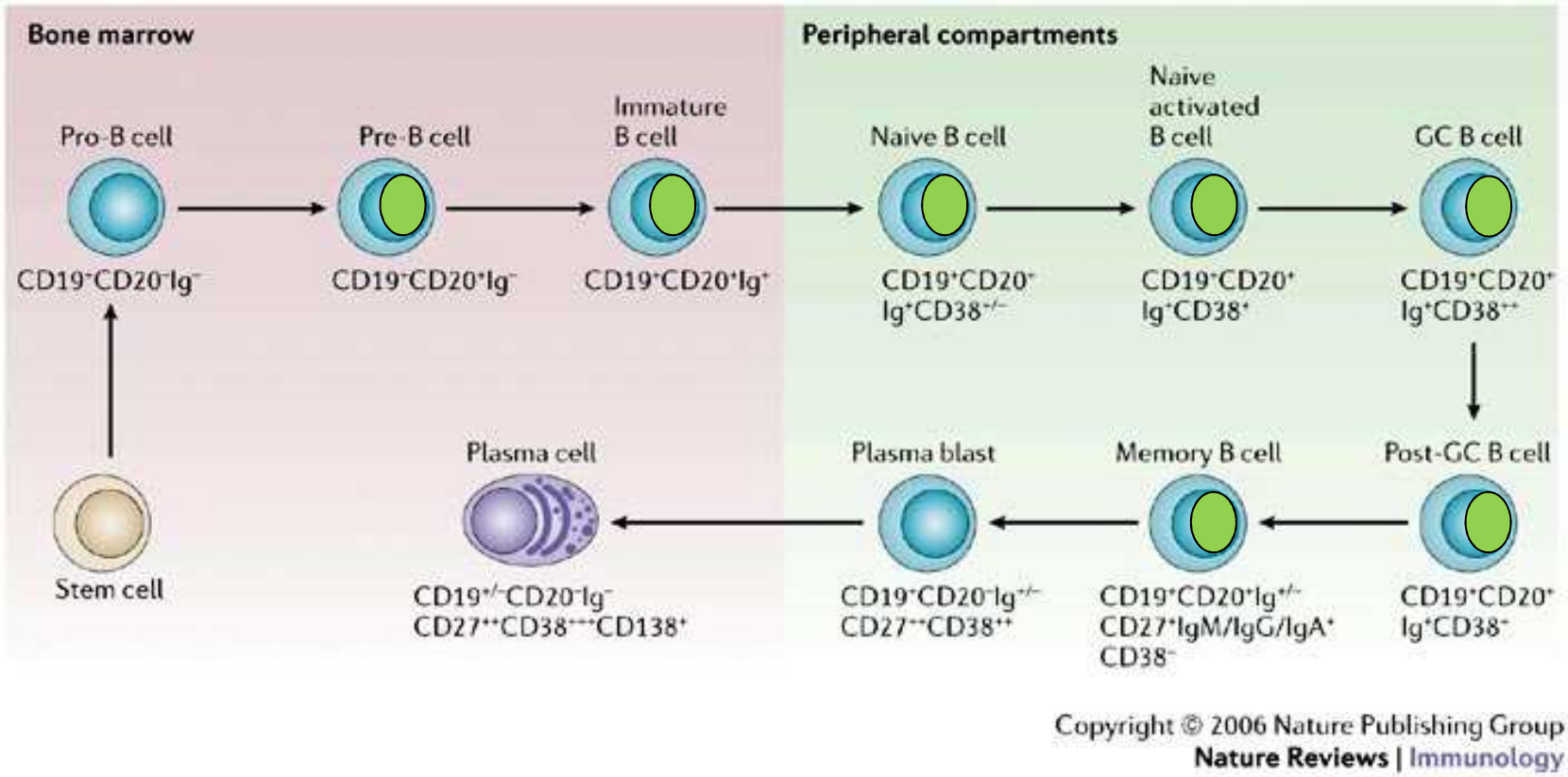
# CD19 & CD20 Expression during B cell development






Edwards *et al. Nature Reviews Immunology* 6, 394–403 (May 2006) | doi:10.1038/nri1838

# CD19CAR

- The first CAR with demonstrated clinical benefit

- It is an ideal immunologic target for B cell malignancies

- 30 CD19CAR clinical trials

- CD19CAR (CTL019) by UPENN received breakthrough therapy designation for adult and pediatric ALL from the FDA in 7/2014

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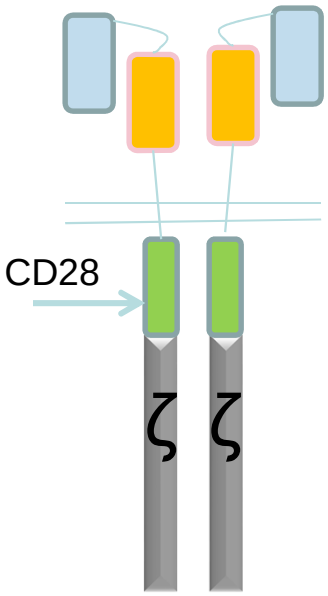
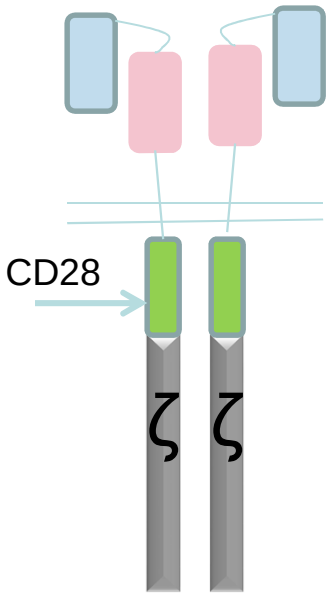
# CD19 CARs

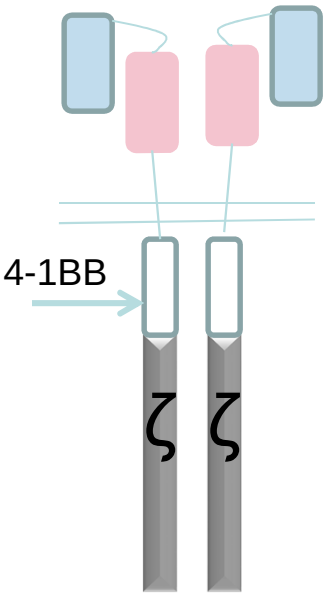
CD19-28 CAR (MSKCC)



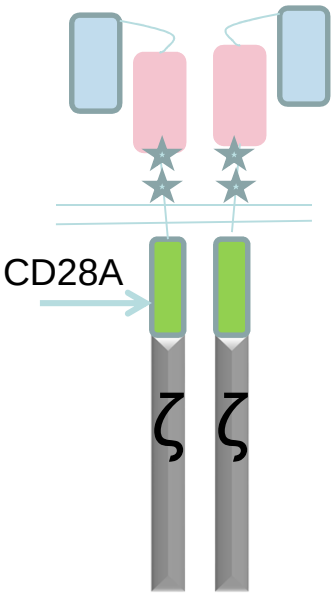

CD19-28 CAR (NCI)




CD19-BB CAR (UPENN)




CD19-28 CAR (COH)

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# Trial Design (example)

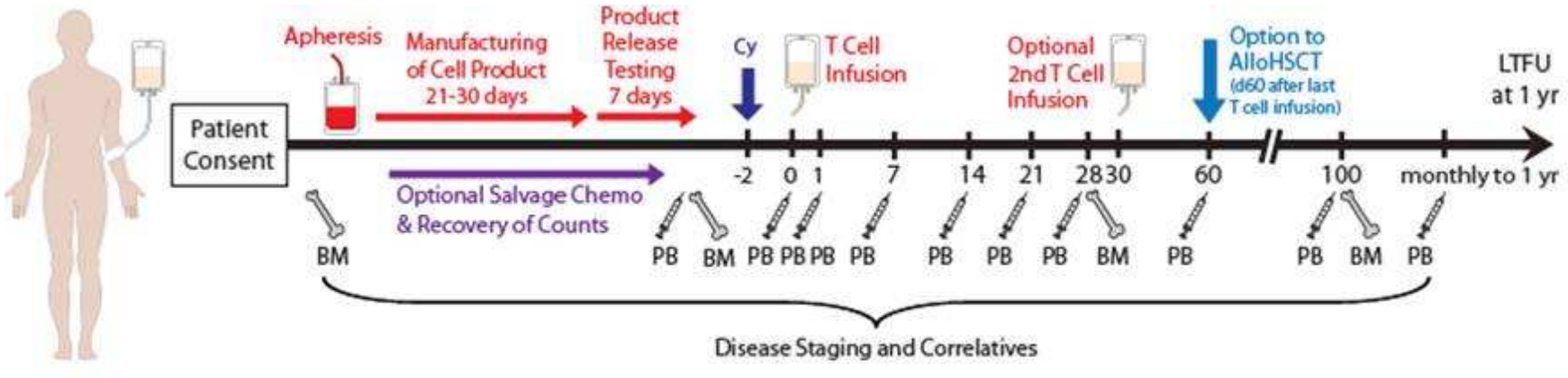


**Figure S1. T cell product manufacturing and patient treatment plan.** Cy=cyclophosphamide, AlloHSCT-allogeneic hematopoietic stem cell transplantation, BM=bone marrow aspiration/biopsy, PB=peripheral blood for correlative assays, LTFU=long term follow up

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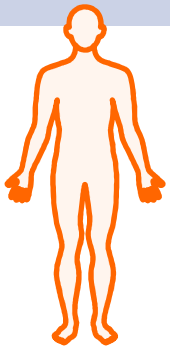
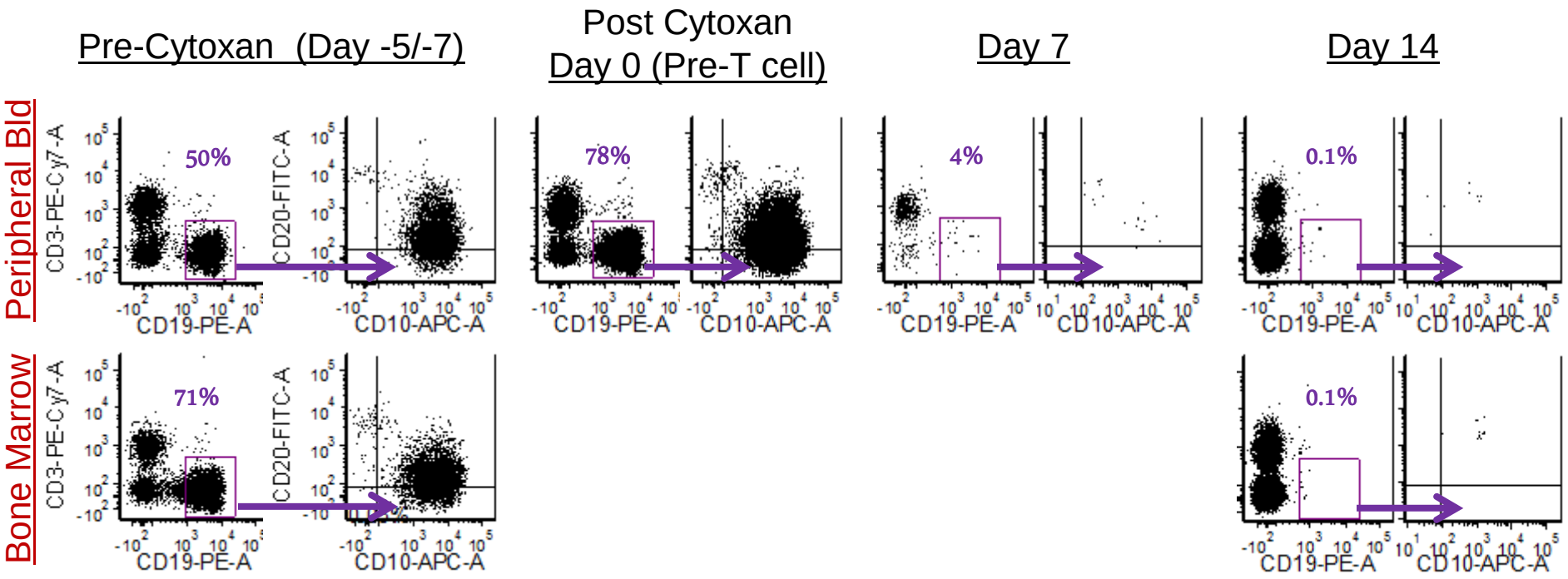
# Clinical Potential of CD19-CAR T Cell Therapy



- **UPN084:** 37 yr; female
- Relapse ALL; BCR-ABL positive
- Post 3x hyper-CVAD (Cytoxan) chemotherapy;
  Imatinib/Dasatinib/Ponatinib;
  2x Allo-transplant (double cord blood); Blinatumomab
- Treated with CD19-CAR T cells
  - Day -3 &-2: 1.5 gm/m2/d Cytoxan
  - Day 0: 200M CD19-CAR $T_{CM}$



# CD19CAR T Cell Trials

| CAR T cells | 19-28z MSKCC | FMC63-28z NCI | FMC63-BBz UPENN |
|---|---|---|---|
| Number of pts | 16 | 21 | 25 (ped); 5 (adult) |
| Ages | 50 (18 - >60) | 5 - 25 | 11 (5-22); 47 (26-60) |
| Prior allo | 4 (25%) | 8 (38%) | 18 (72%); 0 (6 mos) |
| refractory | 14 (88%) | 8 (38%) | 0; 3 (60%) |
| MRD-ve | 2 | 0 | 5 (20%); 0 |
| MRD+ve | 5 | 5 | 0; 1 |
| Blasts > 50% | 6 | 8 (50 to 96%) | n/a |
| CSF | 2 | 2, CNS-2 (asx) | 2, CNS-2 (asx) |

Davila ML et al. Science Trans Med 2014;
Lee DW et al. Lancet 2014; Oct 10
Maude SL et al. NEJM 2014; Oct 16

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# CD19 CAR T Cell ALL Trials

| CAR T cells | 19-28z MSKCC | FMC63-28z NCI | FMC63-BBz UPENN |
|---|---|---|---|
| Lymphodepletion | Cy 1.5 – 3 g/m2 | Flu 25mg/m2/d d-4, -3, -2,<br>Cy 500mg/m2 d-2 | D-2 to -6, cy/vp, flu/Cy, Cy, clof, vp/arc<br>CVAD-B, CVAD-A<br>none (3, cytopenia) |
| T cell expansion | n/a | 11 days | 8 – 12 days |
| feasibility | 15/16 | 19/21 | N/A |
| T cell dose | 3 x $10^6$/kg | 1 to 3 x $10^6$/kg | 1-10 x 107TC/kg /1 - 3 days; |
| T cell persistence | 30 days ( 0 – 120) | Up to 58 days | 24 months (n=1) |
| CAR in CSF | | 11/17 | 17/19 |

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# CD19CAR T Cell ALL Trials

| Study | N (Evaluable) | CR (MRD -) | Time to CR (day) | Proceed to Allo | Outcome p allo |
|---|---|---|---|---|---|
| Penn ped | 39 | 92% | <28 | 3/12 | 3/3 CR 4-15 mo |
| Penn adult | 12 | 66% | <28 | 4/12 | 3/4 CR alive |
| NIH (Lee) | 21 (ped +YA) | 67% | <28 | 10/13 | 10/10 CR |
| MSK (Park) | 33 (27) | 89% (88%) | 22.5 | 10/20 | 8/10 CR 3 - 8 mo<br>1 CD19-ve relapse |
| FHCRC | 15 (13) | 82% | n/a | 0 | |

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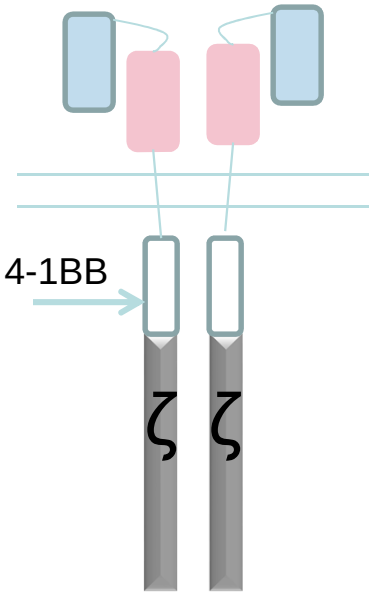
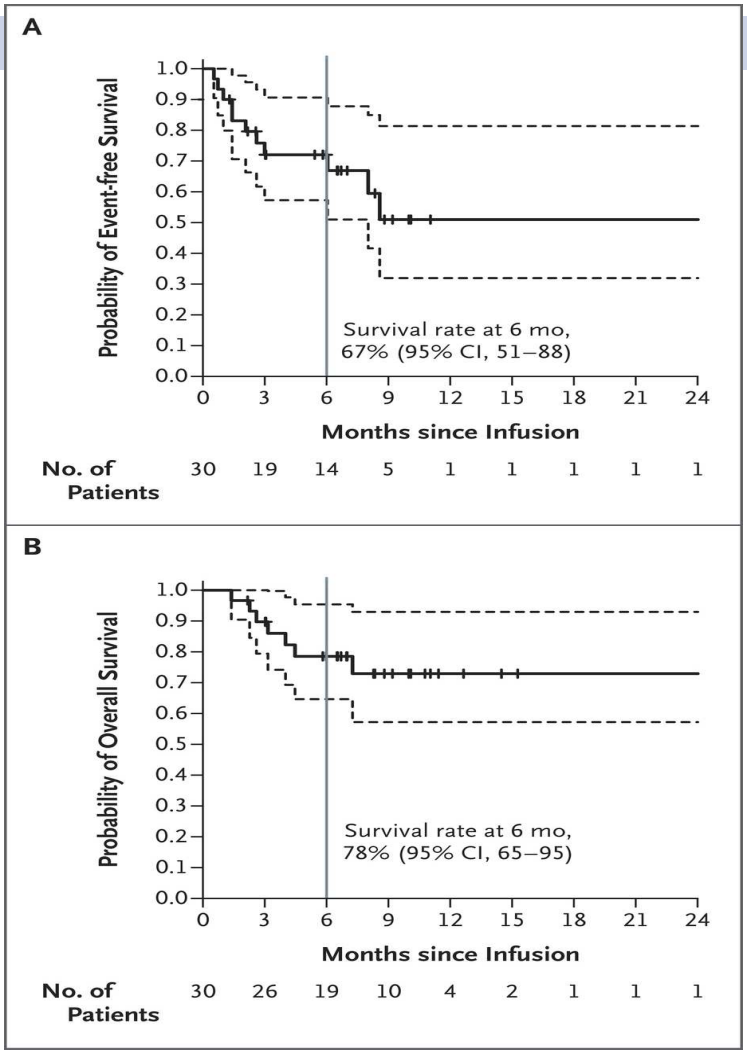
# Probability of Event-free and Overall Survival at 6 Months.

CD19-BB CAR
(UPENN)




N = 30
- at 1 month,
  - 27 (90%) CR
    - 22/27 MRD - ve
    - 3/27 MRD +ve
    - 2 not available

- at median f/u 7 months
  - 19 remains in CR
    - 15 no further Tx

**Maude SL et al. N Engl J Med 2014;371:1507-1517**

The NEW ENGLAND JOURNAL of MEDICINE

# Summary

- ✓ CD19CAR T cell mediates potent anti-leukemic effect
- ✓ Response does not correlate with CD19 expression density
- ✓ High CNS penetration
- ✓ Post treatment relapse are due to
  - early loss of CD19CAR T cells
  - generation of CD19-variants.

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# B Cell Aplasia

On target, off tumor effect

CD19 expresses throughout normal B cell development

Clinical outcome

-indicator for CD19CAR T cell persistence and response

-hypogammaglobulinemia (serum IgG < 400 or 500 mg/dl)

-No significant increase of severe infection

Management

-IVIG replacement if IgG less than 400 mg/dl

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# Cytokine Release Syndrome

- A constellation of inflammatory symptoms from cytokine elevations.
- Association with T cell activation and proliferation in T cell-engaging therapies.
- Association with clinical benefit.
- CRS-related death reported after Blinatumomab and CAR T treatment.

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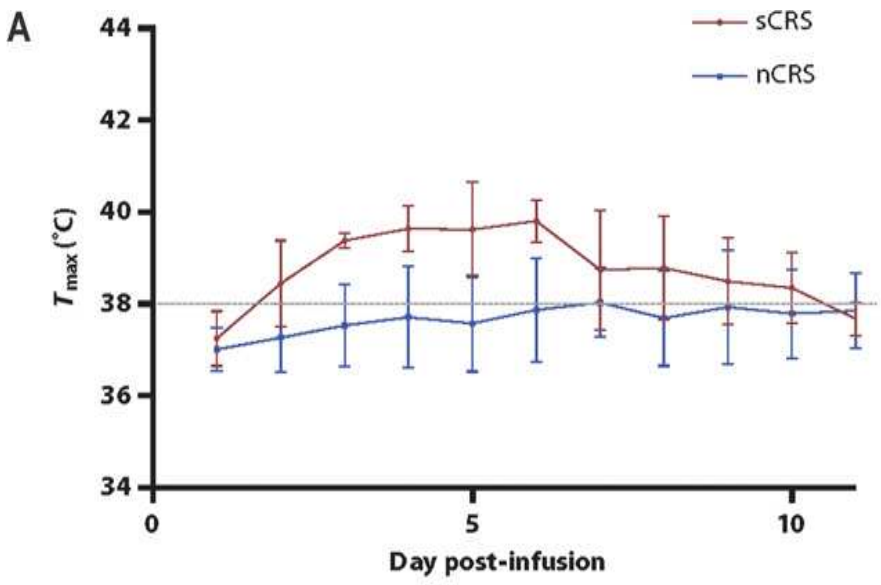
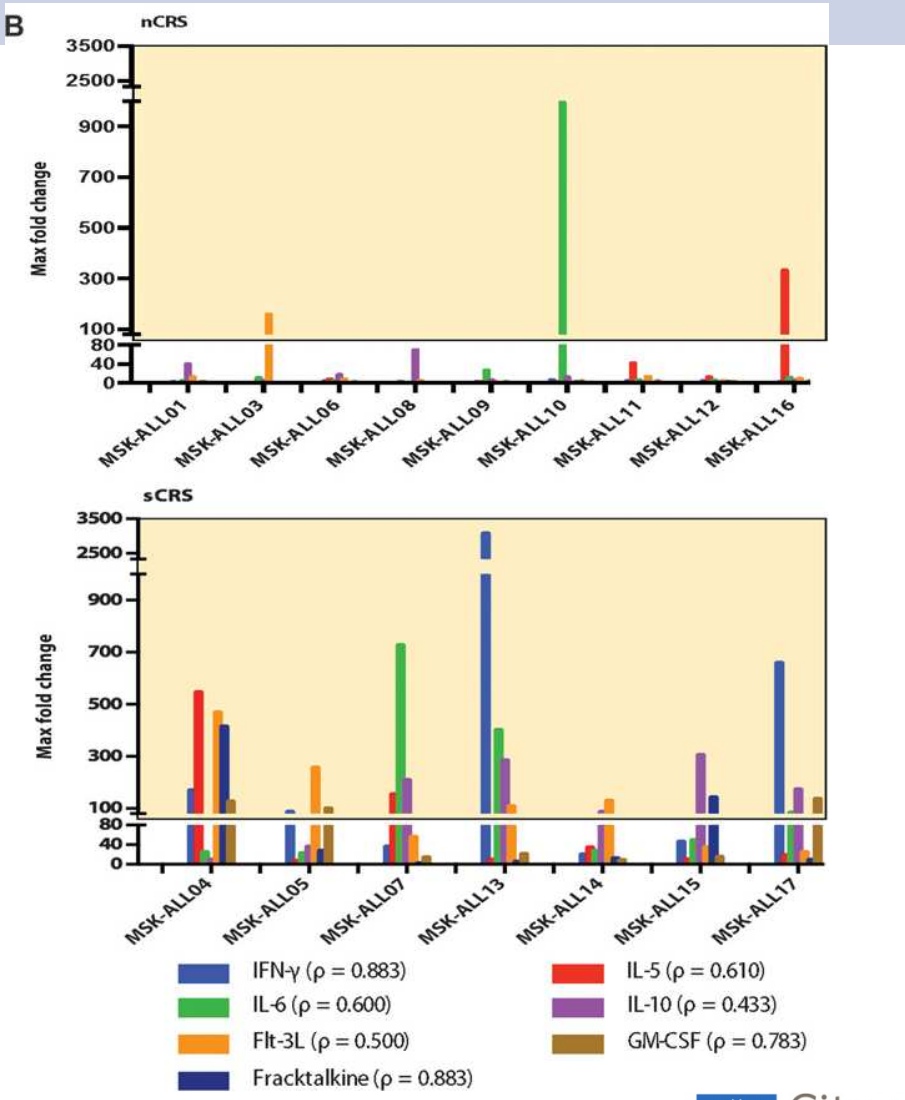
# Fig. 1. Characteristics of the CRS.(A) Average max temperatures on days 1 to 11 after CAR T cell infusion in patients with sCRS compared to nCRS patients




IFN-γ

IL-6

IL-10

Science Translational Medicine AAAS

Published by AAAS

M. L. Davila et al., Sci Transl Med 2014;6:224ra25

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# CRP is a Good Biomarker for CRS Syndrome

200mg/l
In CPOE

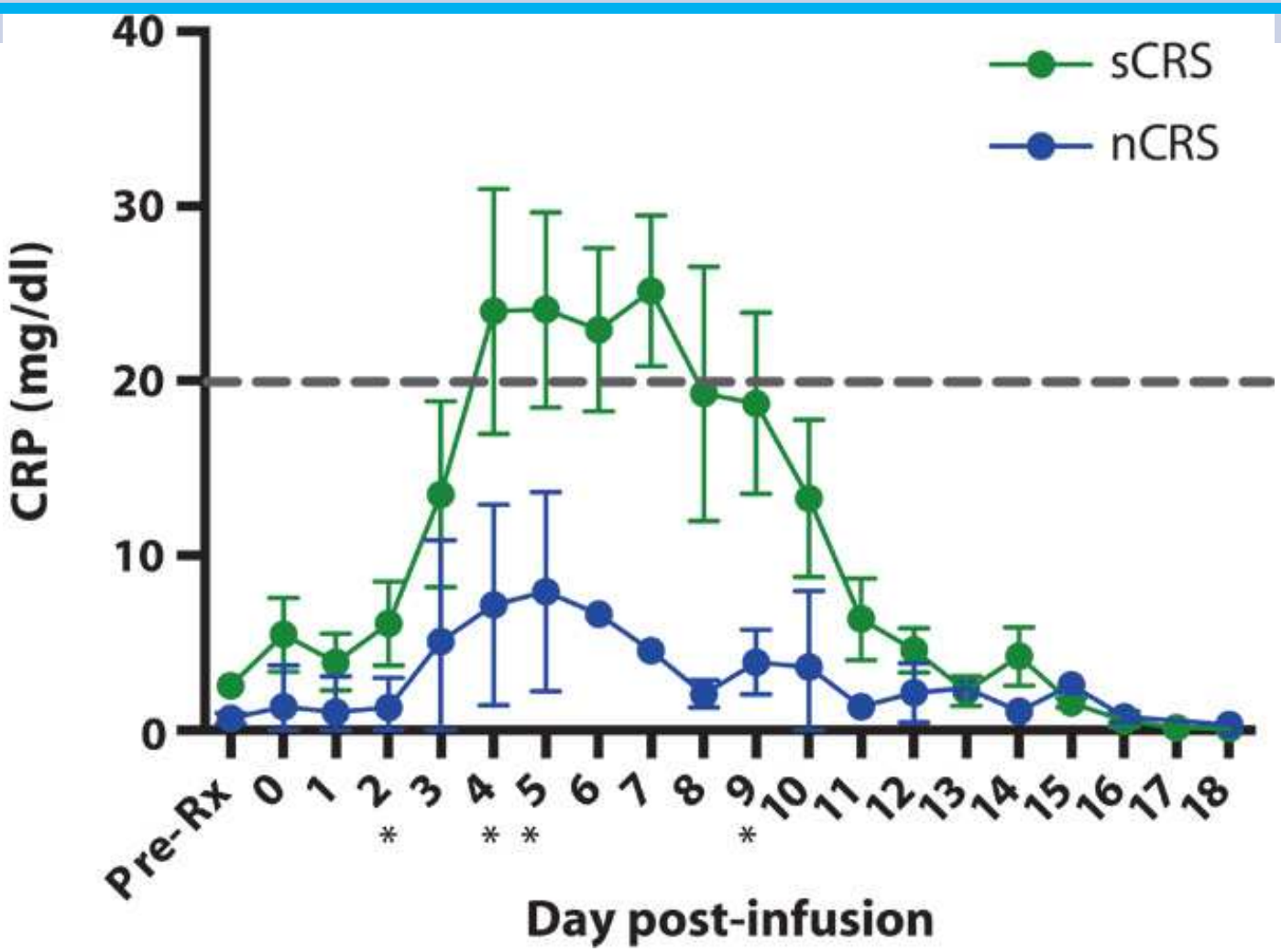


**Fig. 4. CRP levels in patients infused with 19-28z CAR T cells.**

Science Translational Medicine AAAS



M. L. Davila et al., Sci Transl Med 2014;6:224ra25

# Clinical Signs and Symptoms Associated with CRS

| Organ system | Symptoms |
|---|---|
| Constitutional | Fever ± rigors, malaise, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, headache |
| Skin | Rash |
| Gastrointestinal | Nausea, vomiting, diarrhea |
| Respiratory | Tachypnea, hypoxemia |
| Cardiovascular | Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late) |
| Coagulation | Elevated D-dimer, hypofibrinogenemia ± bleeding |
| Renal | Azotemia |
| Hepatic | Transaminitis, hyperbilirubinemia |
| Neurologic | Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, seizures |

Lee DW et al. Blood 2014

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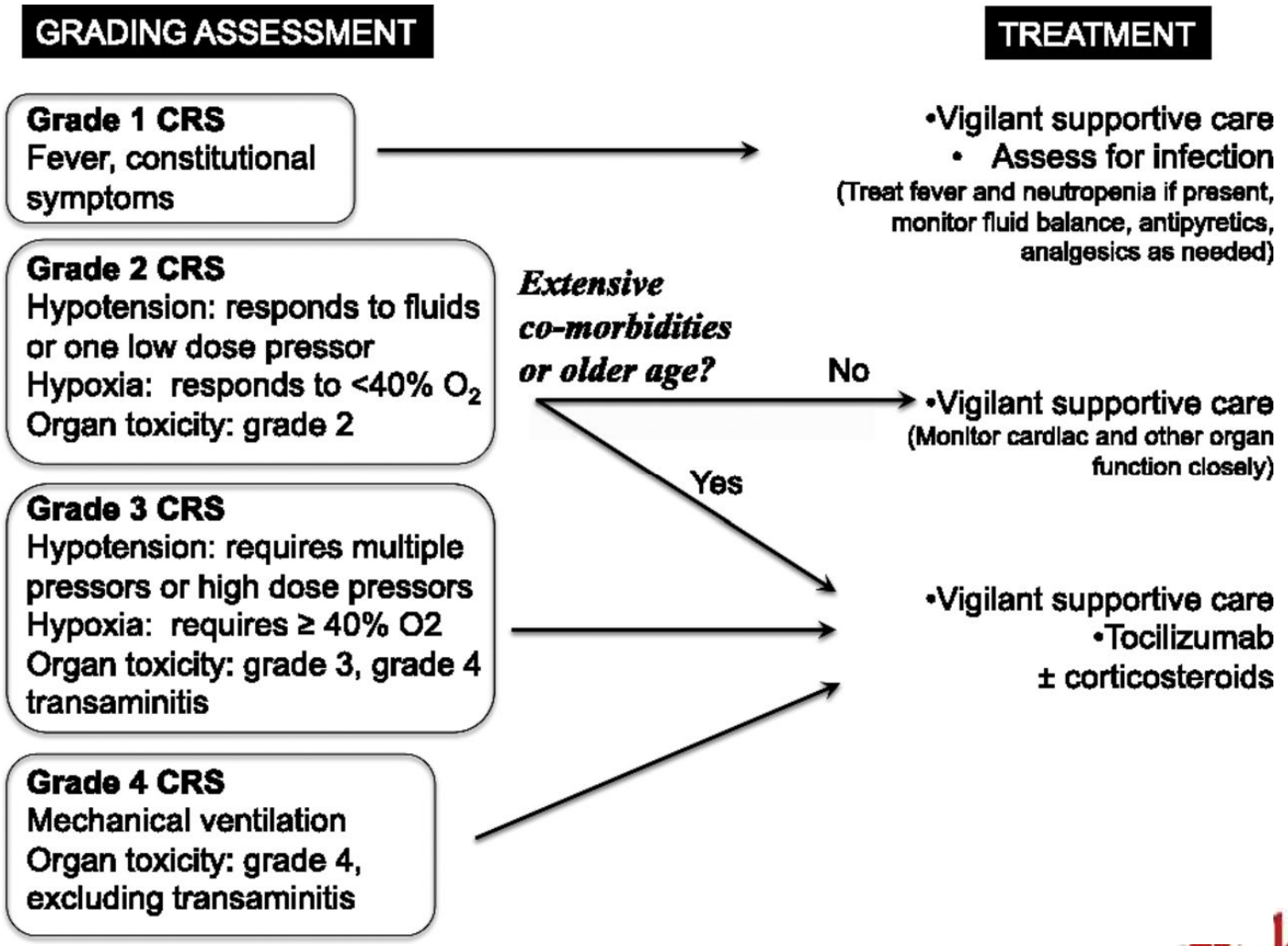
# Treatment Algorithm for Management of CRS Based on the Revised CRS Grading System.






Lee D W et al. Blood 2014;124:188-195

blood

# Lessons Learned from CD19-CAR T cell Therapy

❖**CD19-CAR T cells mediate impressive clinical efficacy against CD19-malignancies across institutions, CAR designs, manufacturing platforms and trial designs.**

- Complete response rates are reported for 66-90% of patients with ALL (Davila et al, STM, 2014; Maude et al. NEJM. 2014; Lee et al. 2015.

❖**CAR T cell antitumor activity is often associated with toxicities that if not properly managed are life-threatening.**

- Cytokine release syndrome (CRS) and neurotoxicity: clinically managed with corticosteroids and anticytokine therapy (i.e. tocilizumab).
- B cell Aplasia: clinically managed with intravenous immunoglobulin (IVIG).

❖***Not all patients respond to CD19-CAR therapy and the long-term durability is still being evaluated*.**

- Differences in response rates between leukemia versus lymphoma highlight tumor-specific challenges.
- Relapse of CD19 antigen negative ALL is observed.
- Therapeutic efficacy correlates with level of CAR T cell persistence and expansion.

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# *Engineering CD123-specific CAR T cells for the Treatment of Acute Myeloid Leukemia*


## Research Team

**Armen Mardiros**
Ravi Bhatia
Cedric Dos Santos
Tinisha McDonald

## Regulatory Team

**Jamie Wagner**
Anita Kurien
Julie Ostberg
Sandra Thomas
COH OIDRA

## Clinical Team

**Elizabeth Budde**
Stephen Forman

# Background

- The interleukin-3 receptor α chain (CD123) is over-expressed on AML cells compared to normal adult bone marrow (Jordan CT et al *Leukemia* 2000)

- Two phase I trials (CSL360 and SL-401) have been completed (NCT00401739 and NCT00397579)

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# CD123 expression on AML patients

| AML Sample ID | Age/Sex | Cytogenetics | Clinical status | Sample Type | CD123 (RFI) | CD34+ selected %CD123+ |
|---|---|---|---|---|---|---|
| 179 | 74/M | Intermediate-risk t(1;7), t(14;15) | Relapsed | PB | 428.32 | 99.22 |
| 373 | 47/M | Poor-risk, Complex abnormalities in 3 cell lines | Relapsed | PB | 1052.83 | 99.66 |
| 493 | 46/F | Intermediate-risk Trisomy 8 | Relapsed | PB | 23.98 | 76.80 |
| 519 | 44/F | del(17p), dic (11;7), clonal loss of TP53/17p13.1 | Relapsed | PB | 63.18 | 97.40 |
| 545 | 58/M | Intermediate-risk t(3;6), del(7) | Induction failure | PB | 52.73 | 99.32 |
| 559 | 59/M | Complex abnormalities, Massive hyperdiploidy | Relapsed | Apheresis | 9.30 | 45.0 |
| 605 | 55/M | Normal | Persistent | PB | 58.48 | 99.91 |
| 722 | 22/M | Intermediate risk t(14;21), del(9q) | Untreated | PB | 33.53 | 92.74 |
| 813 | 48/F | Complex abnormalities, Trisomy 8, Trisomy 21, add(17) | Untreated | PB | 37.19 | 90.93 |

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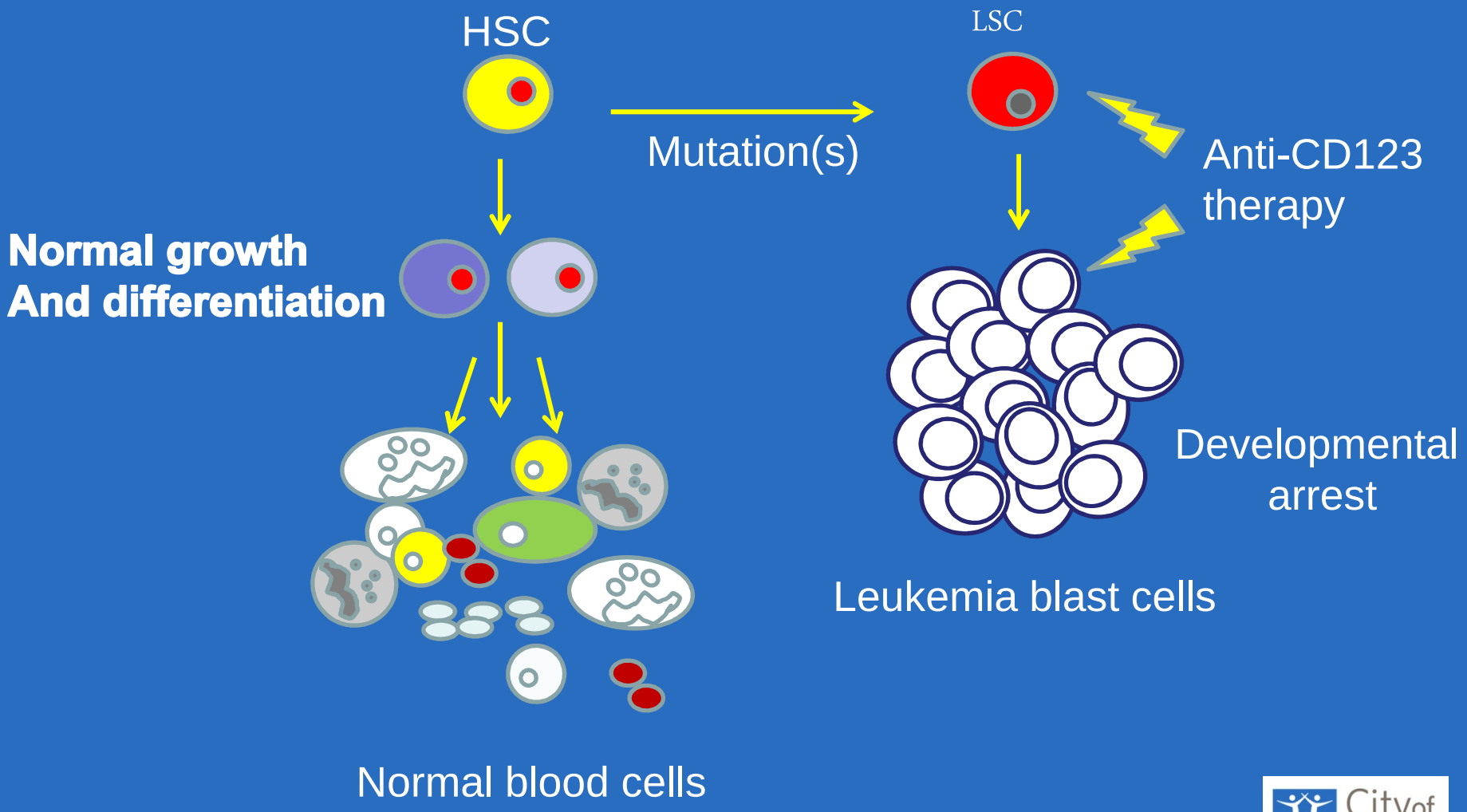
# Therapeutic Concept



# Phase I Clinical Trial with CD123-specific CAR T cells to Initiate in 2015

| COH/ Activation | Patient Population | Agent | T cells Dose | Planned Enrollment |
|---|---|---|---|---|
| **13272/ Sum '14** | Relapsed/Refractory AML | Autologous CD123R(EQ)28ζ/EGFRt$^{+}$ Bulk T cells | 50 to ≤800x10$^{6}$ CAR+ cells | 18 |

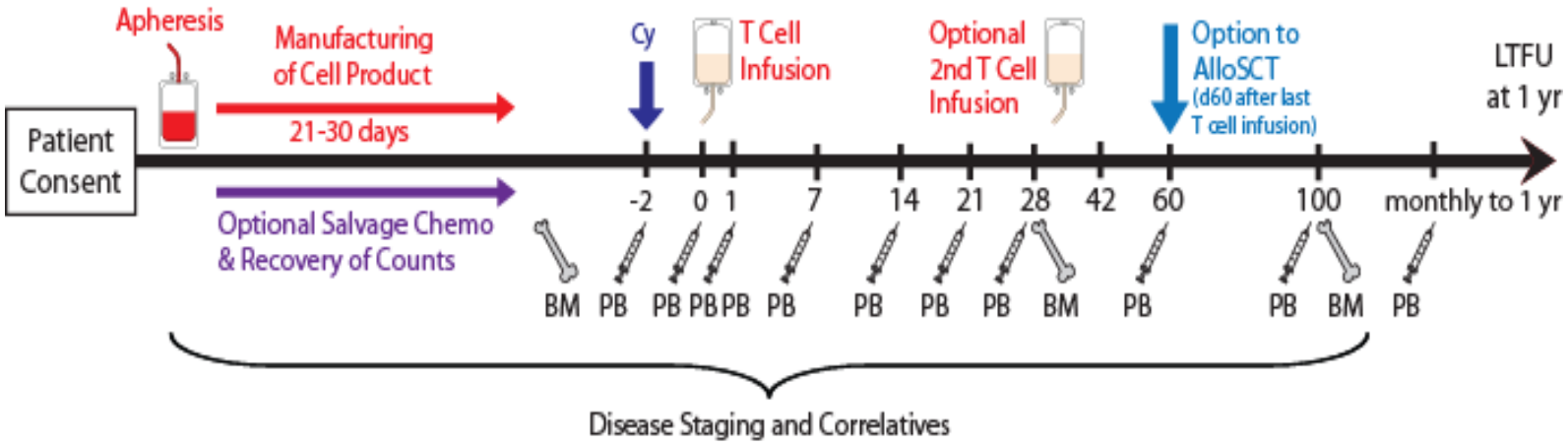



**Figure S.1. T cell product manufacturing and patient treatment plan.** Cy=cyclophosphamide, Rtx=rituximab, BM=bone marrow aspiration/biopsy, PB=peripheral blood for correlative assays, LTFU=long term follow up

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# Multiple Myeloma (MM)

- Multiple myeloma (MM), a plasma cell malignancy, accounts for slightly more than 10 percent of hematologic malignancies in the United States

- Current therapies for MM often induce remission, but nearly all patients eventually relapse. Over 11,000 people die from this disease/year.

- T-cell mediated anti-tumor therapies using genetically modify T cells with specific chimeric antigen receptors (CARs) have non-overlapping activity, toxicity and tumor resistance profiles compared to conventional chemotherapeutic agents.

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# CAR Re-directed T Cell Therapy for Multiple Myeloma

| | CS-1 | CD44v6 | BCMA |
|---|---|---|---|
| Known as | (SLAM) | Variant 6 of CD44 | necrosis factor receptor superfamily member 17 (TNFRSF17) or CD269 |
| Function | the signaling lymphocyte activation | cell adhesion and signaling, tumor invasion, and tumor dissemination (stem cell nich) | B cell development and maturation by binding B cell activating factor (BAFF) and a proliferation ligand |
| Antigen expression | Normal plasma cells and MM; Low on NK cells | AML, MM; low on monocytes and keratinocytes | Normal plasma cells and MM |
| Targeted therapy with mAb | Yes | Yes | No |
| CAR study | Pre-clinical | Pre-clinical | Phase I trial |

Which one is the best target?

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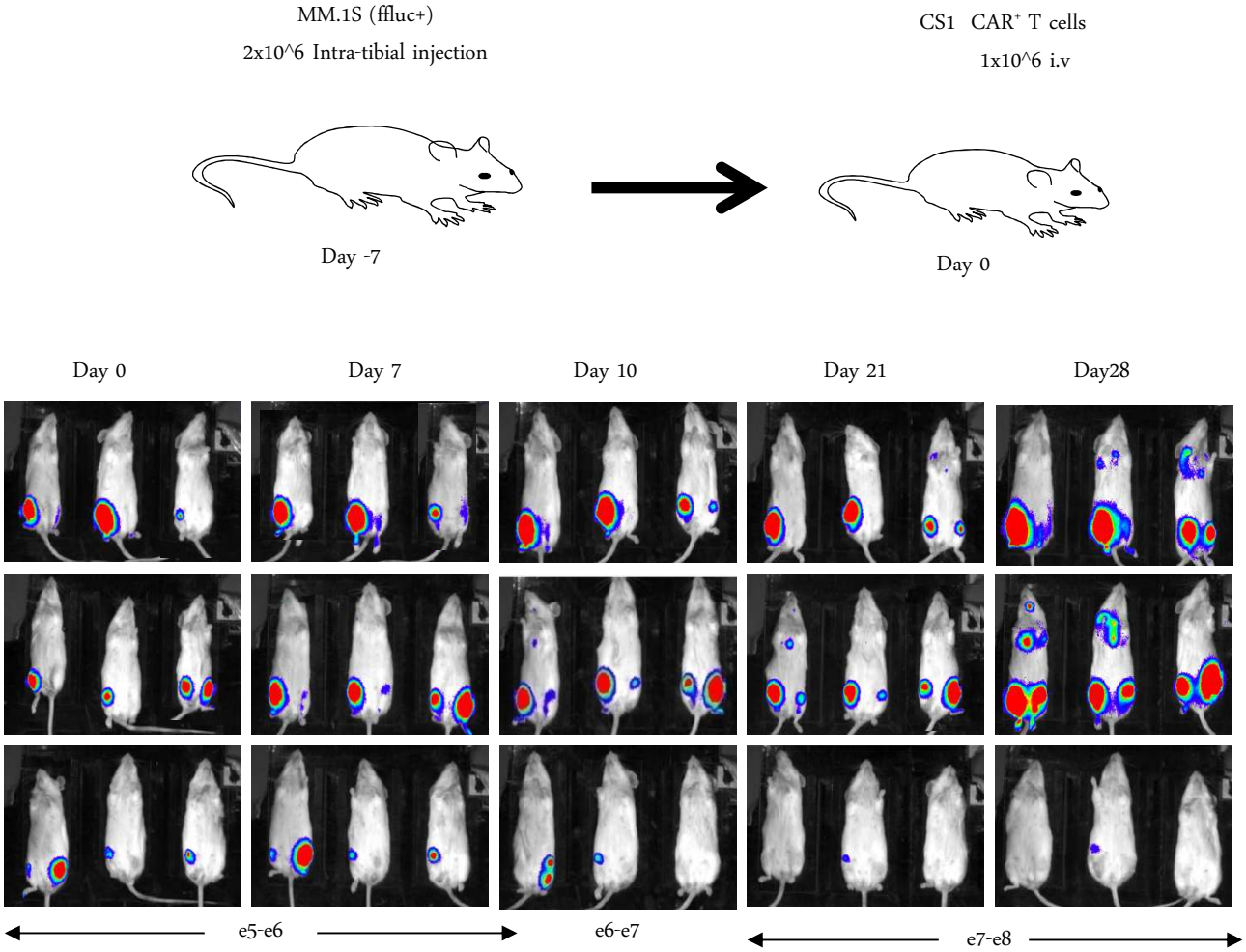
# In vivo antitumor efficacy of $T_{CM}$ derived CS-1 CAR T cells

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# Do CAR T cells offer promise for treatment of solid cancers?

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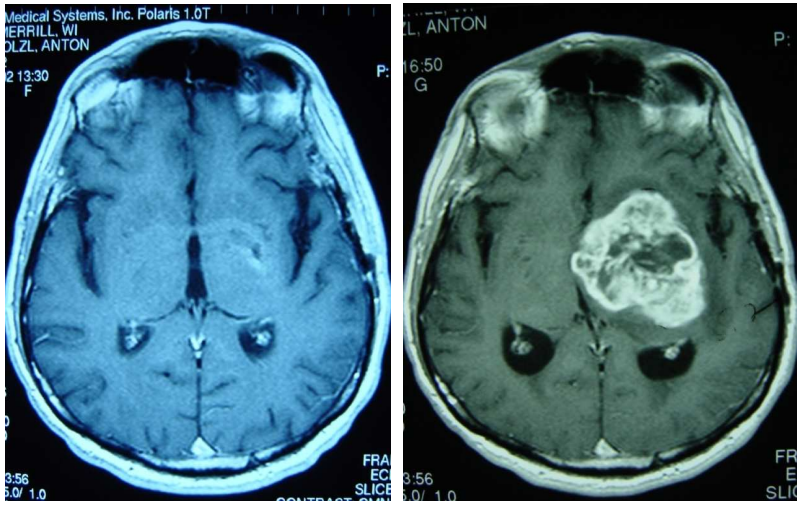
# CAR T cell Therapy for the Treatment of Glioblastoma

**Glioblastoma (GBM) is the most aggressive primary brain tumor, and one of the least curable of all human cancers with a 5-year survival rate of <10%.**

**Dec 2002** **March 2003**



## Challenges for GBM Therapy

- **Invasiveness**
- **Heterogeneity**
- **Blood-brain barrier prevents penetration of many chemotherapies**
- **Standard therapies (surgery, radiation, and chemotherapy) are ineffective and may cause injury of this critical and highly sensitive organ**

*Can CAR T cell therapy offer promise over standard therapy by reprogramming an antitumor immune response?*

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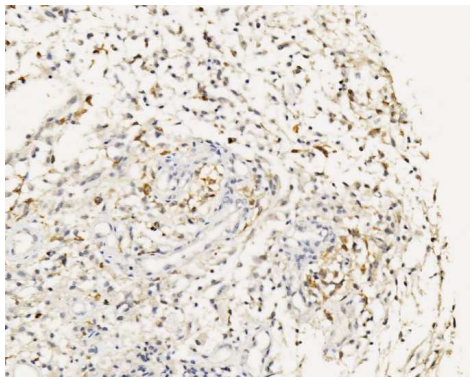
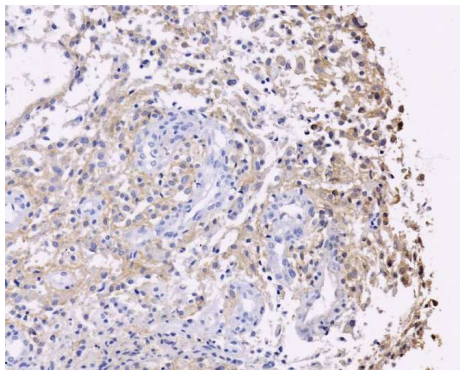
# Glioma Antigen Selection for CAR T Cell Therapy

| Target | GBM | Normal Brain | Clinical | Clinical Details |
|---|---|---|---|---|
| IL13Rα2 | +++ | -- | COH; NCT02208362 | IL13BBζ-CAR Tcm<br>i.c. delivery |
| EGFR | ++++ | ++ | -- | |
| EGFRvIII | ++++ | -- | NCI; NCT01454596<br>UPenn; NCT02209376 | CD28-41BBζ-CAR ; i.v. delivery<br>41BBζ-CAR ; i.v. delivery |
| HER2 | +++ | -- | Baylor; NCT02442297 | CD28ζ-CAR CMV+ T cells<br>i.v. delivery |
| EphA2 | ++ | -- | China; NCT02575261 | Unknown |

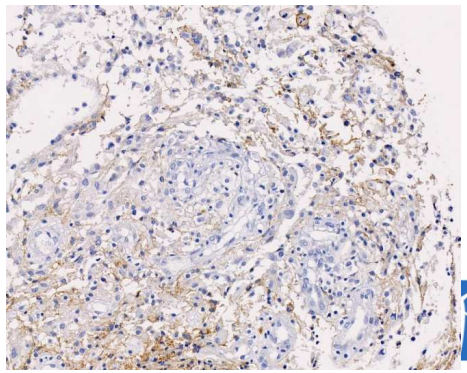
IL13Rα2



Her2



EGFR



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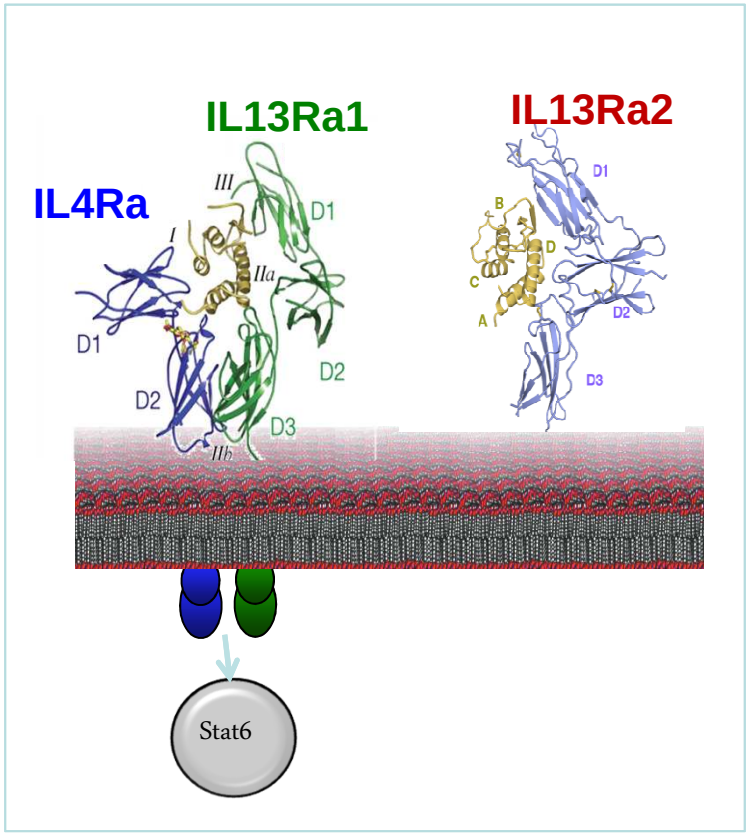
# IL13Rα2: An Immunotherapeutic Target for GBM

- IL13Rα2 is a high-affinity IL-13 receptor.
- IL13Rα2 is over-expressed by the majority of high-grade glioma.
- IL13Rα2 is also expressed by other solid tumors (ovarian, colorectal, pancreatic, kidney etc)
- IL13Rα2 is associated with tumor invasion and metastasis, and poor patient survival.
- IL13Rα2 displays low/negative expression on normal tissue expression, except for testis.
- IL13Rα2 is distinct from a second IL-13 receptor, IL13Rα1, which requires heterodimerization with IL-4Rα for high affinity binding of IL-13.

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# Phase I Clinical Trials Utilizing CD8$^+$ IL13-zetakine$^+$ T cells for the Treatment of Recurrent Glioblastoma

*Behnam Badie, M.D.; Clinical PI*

**GBM1 -- BB-IND 10109 / IRB 1020:**
**Patient Specific Autologous CAR T cell**

- **Patients:** 3
- **Infusions**: 12 @ 1x10$^7$-10$^8$
- **CAR:** 1$^{st}$ generation IL13-zetakine CAR
- **T cell:** CD8$^+$ CTL clone
- **SAEs:** headache, transient neurological worsening

**GBM2 -- BB-IND 14194 / IRB 07082**
**"Off-the-Shelf" Allogeneic CAR T cell**

- **Patients:** 6
- **Infusions**: 4 @ 1x10$^8$ , 2500-500 IU IL-2;
- **CAR:** 1$^{st}$ generation IL13-zetakine CAR
- **T cell:** Steroid resistant CD8$^+$ CTL line
- **SAEs:** dehydration, stroke

Collaboration Sangamo BioSciences

## *Lessons Learned*

- Feasibility of engineering autologous and allogeneic CAR T cells for GBM therapy.
- Safety of IL13Rα2-specific CAR T cells for GBM therapy.
- Tolerability of up to 12 T cell infusions at cell doses of 1x10$^8$ and co-administered of IL-2 i.c.
- Evidence for transient anti-tumor activity.

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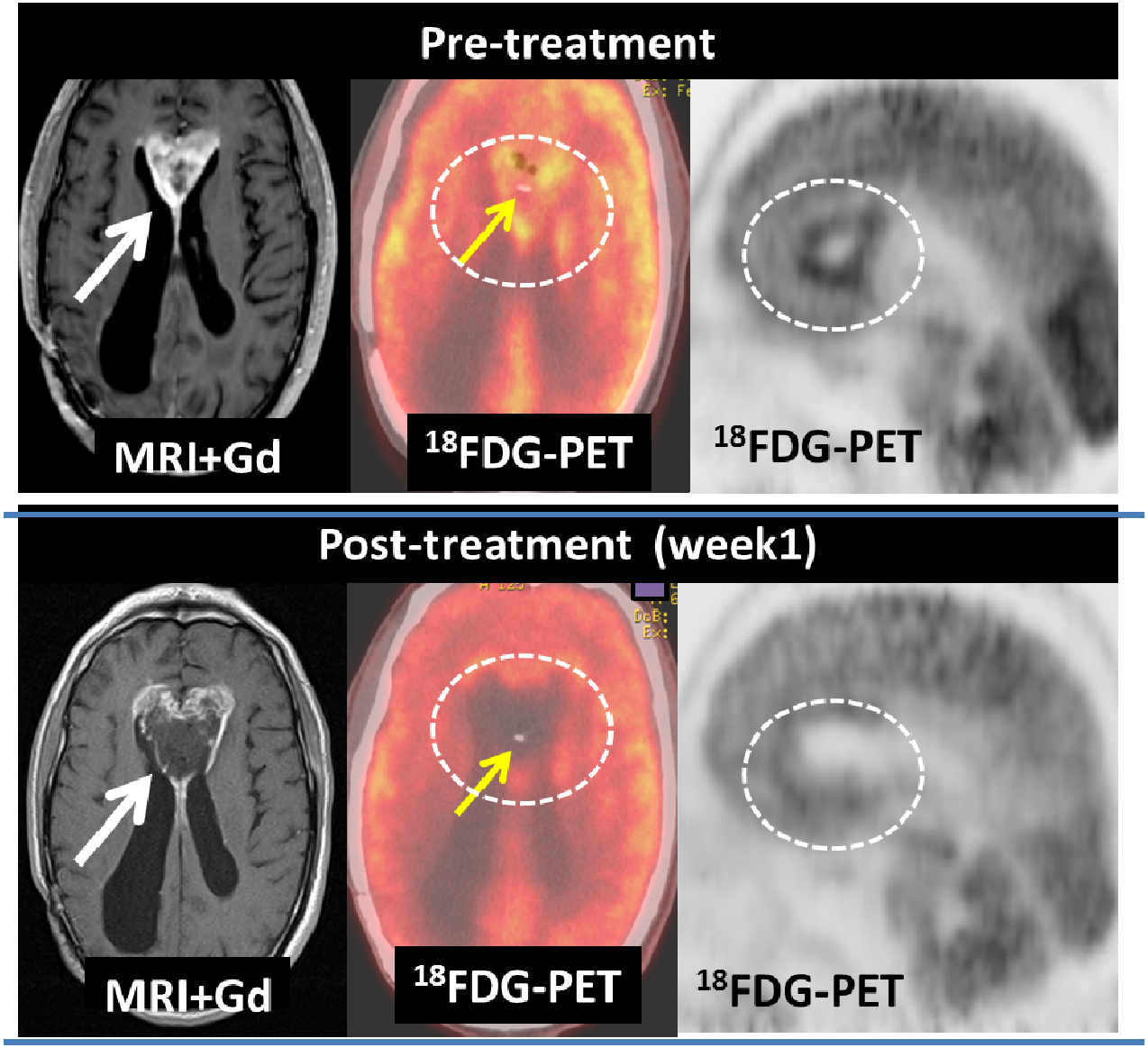
# Transient Regression of a GBM Lesion Following First-Generation Allogeneic CD8$^{+}$ IL13ζ$^{+}$ T cell Therapy

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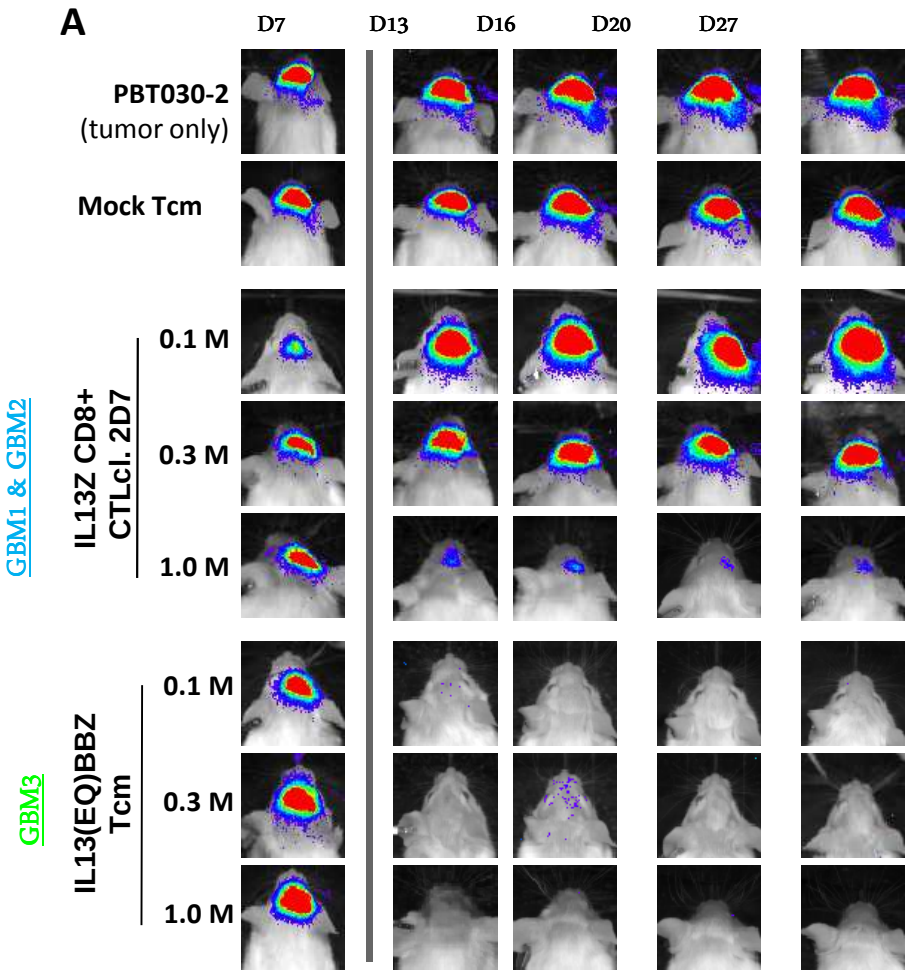
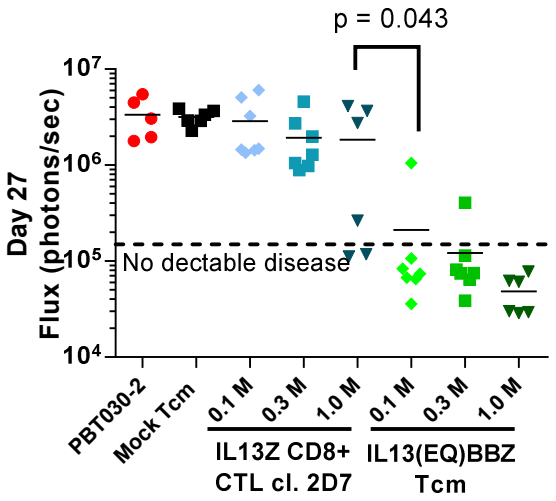
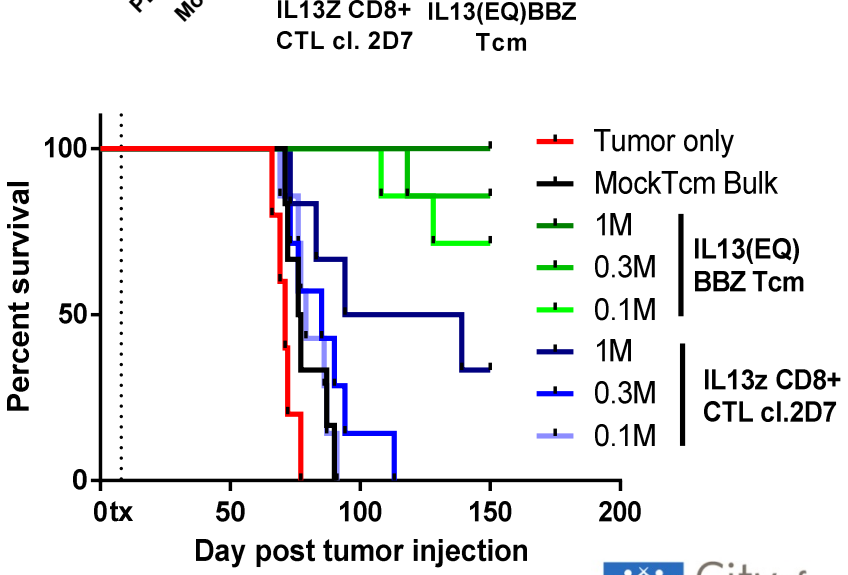
# Next-Generation IL13BBz CAR $T_{CM}$ Are More Potent than First-Generation CD8+ IL13z T cells

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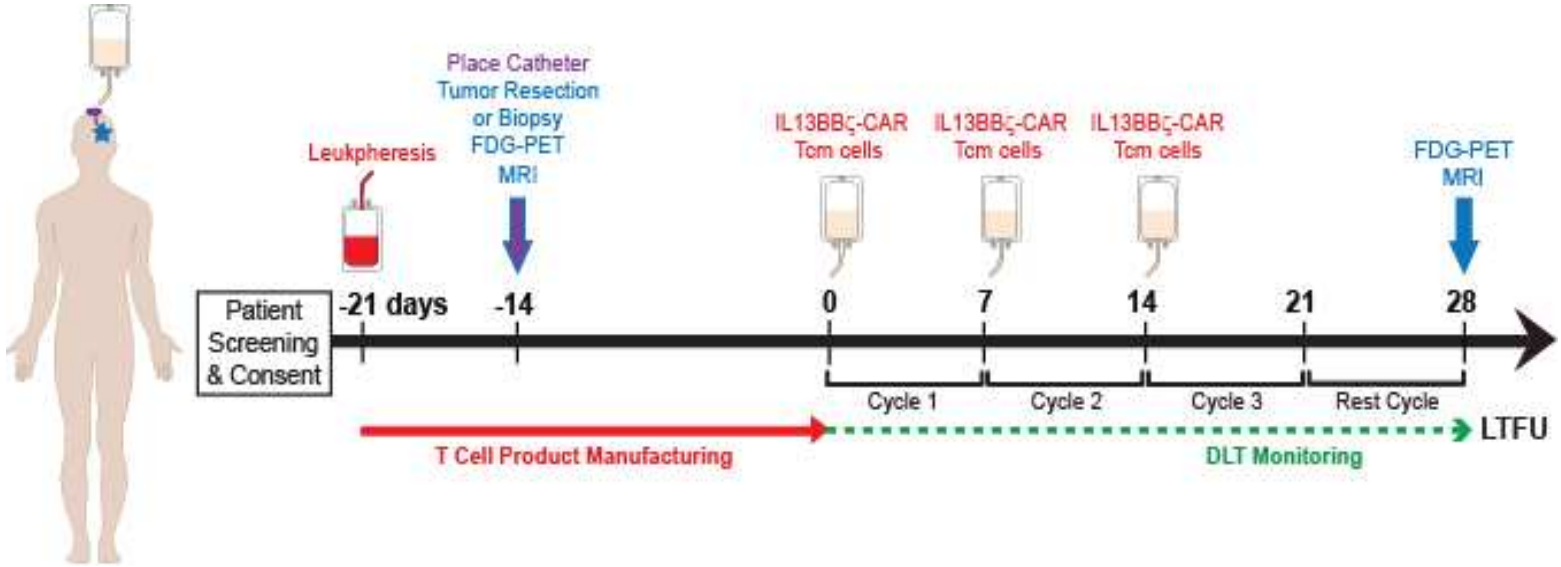
# Phase I Clinical Trial Evaluating Next-Generation IL13Rα2-specific CAR T cells

**Clinical PI: Dr. Behnam Badie**

| Patient Population | Investigational Agent | T cells Dose | Planned Enrollment | Study Objectives |
|---|---|---|---|---|
| Relapsed/Refractory GBM<br>Arm 1: resectable<br>Arm 2: Non-resectable | Autologous IL13(EQ)BBζ/CD19tCD4+ CD8+ $T_{CM}$ | 2 to 50x$10^6$ CAR T cells | 12/arm | •Assess the feasibility and safety of IL13(EQ)BBζ/CD19t+ $T_{CM}$ for research participants with recurrent/refractory malignant glioma<br><br>•Determine maximum tolerated dose (MTD) and a recommended phase II dosing plan (RP2D) for each strata |




**Funding:** Gateway for Cancer Research; R01-FD005129-01

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# Major hurdles for the development of solid cancer CAR T cells

**(1) Tumor-antigen expression (outside of CD19) is not wholly restricted to tumors, but also found at *lower* levels on normal tissue**

- Potential for increased off-tumor, on-target effects

**(2) The solid tumor microenvironment is uniquely immune-suppressive**

- Blocks T cell infiltration, activation/function, and proliferation
- Hampers overall efficacy of endogenous T cell immunity and adoptive T cell therapies

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# Solid Cancer CAR T Cell Therapy

- **Can CAR T Cells Be Tumor-Antigen Expression Selective?**
  - Optimize CAR constructs/components (i.e. co-stimulatory domains, non-signaling linker domains, scFv's, etc.) to develop truly expression-selective CARs
  - Goal: Target over-expressed tumor antigens, while avoiding tissue with "normal" low antigen expression

- **Can we design rational combinations using CAR T cells with blockade of immunosuppression?**
  - Develop therapies to target the suppressive microenvironment

    i.e. CpG-*STAT3* siRNA, PD-1/PD-L1 checkpoint inhibitors, MDSC and Treg-specific inhibitors

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# Future Implications for Developing CAR T Cells for Cancer

✓ *Address Tumor Heterogeneity*

Can we overcome tumor heterogeneity by BOXING IN antigen escape with multi-targeting CAR T cells

- *PSCA, Her2, IL13Rα2, etc.*

✓ *Overcome Suppressive Microenvironment*

Can we neutralize the fibro-inflammatory stromal microenvironment to augment CAR T cell therapy

- *Target STAT3-mediated inflammation*
- *Combine with other therapies (MDSC, CAFs)*

✓ *Tumor specificity (off-tumor effects)*

- Determine the optimal starting T cell population
- CAR domain optimization for differential target selectivity
- Suicide gene approach to manage potential toxicity

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# COH T Cell Therapy Research Program

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