Immunotherapy for Brain Tumors: Using CAR T-cell Technology for Glioblastoma

Donald M. O’Rourke, M.D.
Associate Professor
Department of Neurosurgery
Abramson Cancer Center
Perlman School of Medicine
University of Pennsylvania
donald.orourke@uphs.upenn.edu
CART cells in GBM

- CART cells in Immuno-Oncology
- EGFRvIII as GBM target: status of clinical trials and CARs for GBM
- EGFRvIII CART Phase I study completed at Penn (n=10)
- New data on GBM TME
- Future efforts to address GBM heterogeneity
Immuno-Oncology: T cell Therapies and Checkpoint Therapies

The Immune Synapse

Checkpoint Therapies

- Cytokine Therapy: IL-2, IFN, IL-7, IL-15, IL-21
- Therapeutic Vaccines: Dendritic cell vaccines, DNA, RNA, Engineered tumor cells

ACT Therapies

- TILs
- CAR T cells
- TCR T Cells

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

**Design of CAR T Cells**

First Generation

- CD4 / CD8z CARs
- scFv CARs
- scFv CD28z CARs
- scFv BBz CARs
- scFv CD27z CARs
- scFv ICOSz CARs

Irving & Weiss, 1991
Letourneur, 1991
Kuwana, 1987
Eshhar, 1993
Roberts, 1995
Finney, 1998
Maher, 2002
Finney, 2003
Imai, 2004
Milone, 2009
Carpenito, 2009
Song, 2012
Guedan, 2014
Duong, 2013

Extracellular

Intracellular

\[ \zeta \]
EGFRvIII CAR Designs: choice of scFv and signaling domain

<table>
<thead>
<tr>
<th>scFv Constructs</th>
<th>hEGFRvIII $K_D$ (nM)</th>
<th>hEGFR ECD $K_D$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine 3C10</td>
<td>25.8</td>
<td>195</td>
</tr>
<tr>
<td>Humanized 3C10 (2173)</td>
<td>101</td>
<td>872</td>
</tr>
</tbody>
</table>
Redirecting the Specificity of T cells– Proposed Mechanism of Action of CAR T cells

- Gene transfer technology is used to stably express CARs on T cells, conferring novel antigen specificity\(^1,2\)

- CTL019 cells can thus be directed against any tumor cell that expresses the CD19 surface antigen

- CTL019 therapy takes advantage of the cytotoxic potential of T cells thereby killing tumor cells in an antigen-dependent manner\(^1,3\)

- Persistent CTL019 cells consist of both effector (cytotoxic) and central memory T cells: a **living drug**\(^3\)

---

<table>
<thead>
<tr>
<th></th>
<th>Small molecule</th>
<th>Protein</th>
<th>Oncolytic vector</th>
<th>Cell Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size API</strong></td>
<td>Usually &lt; 0.2 nm</td>
<td>mAb: 5 nm</td>
<td>&gt; 20 nm (&lt;300 nm)</td>
<td>&gt; 5000 nm</td>
</tr>
<tr>
<td><strong>Stability</strong></td>
<td>High</td>
<td>medium</td>
<td>low</td>
<td>Very low</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>Temp controlled</td>
<td>Difficult - infectious materials</td>
<td>Difficult - genetically modified cells</td>
</tr>
<tr>
<td></td>
<td>easy</td>
<td>established</td>
<td>in progress</td>
<td>in progress</td>
</tr>
<tr>
<td><strong>Handling/distribution</strong></td>
<td>established</td>
<td>Parenteral</td>
<td>Parenteral</td>
<td>Parenteral</td>
</tr>
<tr>
<td></td>
<td>easy</td>
<td>Prefilled syringes</td>
<td>Lyophilisate</td>
<td>Infusion bags</td>
</tr>
<tr>
<td><strong>Regulatory</strong></td>
<td>established</td>
<td>Same product for all patients</td>
<td>Same product for all patients, BL2 required</td>
<td>Short shelf life (d)</td>
</tr>
<tr>
<td><strong>Delivery</strong></td>
<td>Oral</td>
<td>Tablet; capsules</td>
<td>Lyophilisate</td>
<td>Individualized</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>Tablet; capsules</td>
<td>Same product for all patients</td>
<td>Cell processing service required</td>
</tr>
<tr>
<td><strong>Final Market Image</strong></td>
<td>Same product for all patients</td>
<td>Prefilled syringes</td>
<td>Same product for all patients, BL2 required</td>
<td>Usually one treatment only</td>
</tr>
<tr>
<td><strong>Manufacturing specifics</strong></td>
<td>Same product for all patients</td>
<td>Same product for all patients</td>
<td>Same product for all patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Same product for all patients</td>
<td>Same product for all patients</td>
<td>Same product for all patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Same product for all patients</td>
<td>Same product for all patients</td>
<td></td>
</tr>
<tr>
<td><strong>Dosing</strong></td>
<td>Often / chronic</td>
<td>Monthly / chronic</td>
<td>Once to several over lifetime</td>
<td>Usually one treatment only</td>
</tr>
</tbody>
</table>
Health Care Challenges

Issues
- Patient specific “n of 1”
- Blood bank model?
- Central manufacturing?

Levine and June, Nature. 2013
Questions facing the CART field

- Is long term persistence of CAR cells desired?
- Which approaches give durable persistence of CARTs?
- What is the best vector to introduce the CAR: retroviral vector or lentiviral vector?
- What is the optimal T cell type and composition of the infused product?
- *Will CAR T cells work in solid cancers?*

### CARs in Clinical Development

**Commercial CARs:** Autolus/UCL, Bellicum, BioNTech, CBMG, Cardio3→Celyad, CARSgen, Celgene/Bluebird, Cellectis/Servier/Pfizer, Cellular Therapeutics Ltd, Juno/Opus, Kite/Amgen, Mustang/COH, Novartis, Sorrento/Conkwest, Takara, Transposagen/J&J/Janssen, TheraVectys, Unum, Intrexon/Ziopharm

<table>
<thead>
<tr>
<th>Academic Institute (US)</th>
<th>Target(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baylor College of Medicine</td>
<td>CD19, GD-2, Her2, CD30, kappa Ig</td>
</tr>
<tr>
<td>FHCRC</td>
<td>CD19, CD20, ROR1</td>
</tr>
<tr>
<td>MD Anderson Cancer Center (MDACC)</td>
<td>CD19</td>
</tr>
<tr>
<td>Memorial Sloan Kettering</td>
<td>CEA, PSMA</td>
</tr>
<tr>
<td>National Cancer Institute (NCI)</td>
<td>CD19, CD22, CSP4, GD-2, EGFRvIII, mesothelin, VEGFR2</td>
</tr>
<tr>
<td>University of Pennsylvania</td>
<td>CD19, BCMA, Mesothelin, c-Met, EGFRvIII</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Academic Institute (non-US)</th>
<th>Target(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese PLA General Hospital</td>
<td>CD19, CD20, CD33, CD138, HER2</td>
</tr>
<tr>
<td>Christie Hospital NHS Foundation Trust</td>
<td>CD19</td>
</tr>
<tr>
<td>Peter MacCallum Cancer Centre, Australia</td>
<td>LewisY</td>
</tr>
<tr>
<td>University of Zurich</td>
<td>FAP</td>
</tr>
<tr>
<td><em>City of Hope (CA)</em></td>
<td>IL13Ra2</td>
</tr>
</tbody>
</table>
Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy

Christine E. Brown, Ph.D., Darya Alizadeh, Ph.D., Renate Starr, M.S.,
Lihong Weng, M.D., Jamie R. Wagner, B.A., Araceli Naranjo, B.A.,
Julie R. Ostberg, Ph.D., M. Suzette Blanchard, Ph.D., Julie Kilpatrick, M.S.N.,
Jennifer Simpson, B.A., Anita Kurien, M.B.S., Saul J. Priceman, Ph.D.,
Xiuli Wang, M.D., Ph.D., Todd L. Harshbarger, M.D., Massimo D’Apuzzo, M.D.,
Julie A. Ressler, M.D., Michael C. Jensen, M.D., Michael E. Barish, Ph.D.,
Mike Chen, M.D., Ph.D., Jana Portnow, M.D., Stephen J. Forman, M.D.,
and Behnam Badie, M.D.

SUMMARY

A patient with recurrent multifocal glioblastoma received chimeric antigen receptor (CAR)—engineered T cells targeting the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13Ra2). Multiple infusions of CAR T cells were administered over 220 days through two intracranial delivery routes — infusions into the resected tumor cavity followed by infusions into the ventricular system. Intracranial infusions of IL13Ra2-targeted CAR T cells were not associated with any toxic effects of grade 3 or higher. After CAR T-cell treatment, regression of all intracranial and spinal tumors was observed, along with corresponding increases in levels of cytokines and immune cells in the cerebrospinal fluid. This clinical response continued for 7.5 months after the initiation of CAR T-cell therapy. (Funded by Gateway for Cancer Research and others; ClinicalTrials.gov number, NCT02208362.)
HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive Glioblastoma: A Phase 1 Dose-Escalation Trial

Nabil Ahmed, MD, MPH; Vita Brawley, BS; Meenakshi Hegde, MD; Kevin Bielamowicz, MD; Mamta Kaira, PhD; Daniel Landi, MD; Catherine Robertson, BS; Tara L. Gray, LVN; Oumar Diouf, MS; Amanda Wakefield, BS; Alexa Ghaddar, DO; Claudia Gerben, MS; Zhengchen Yi, PhD; Aidin Ashoori, BS; Meng-Fen Wu, MS; Hao Liu, PhD; Cliona Rooney, PhD; Gianpietro Dotti, MD; Adrian Ghee, PhD; Jack Su, MD; Yvonne Kew, MD, PhD; David Baskin, MD; Yi-Jonathan Zhang, MD, PhD; Pamela New, MD; Bambi Grizzle, RPh, MS; Milica Stojakovic, PhD; John Hicks, MD, PhD; Suzanne Z. Powell, MD, PhD; Malcolm K. Brenner, MD, PhD; Helen E. Heslop, MD; Robert Grossman, MD, PhD; Winfried S. Weis, PhD; Stephen Gottschalk, MD

**Importance**
Glioblastoma is an incurable tumor, and the therapeutic options for patients are limited.

**Objective**
To determine whether the systemic administration of HER2-specific chimeric antigen receptor (CAR)-modified virus-specific T cells (VSTs) is safe and whether these cells have antglioblastoma activity.

**Design, Setting, and Participants**
In this open-label phase 1 dose-escalation study conducted at Baylor College of Medicine, Houston Methodist Hospital, and Texas Children's Hospital, patients with progressive HER2-positive glioblastoma were enrolled between July 25, 2011, and April 21, 2014. The duration of follow-up was 10 weeks to 29 months (median, 8 months).

**Interventions**
Monotherapy with autologous VSTs specific for cytomegalovirus, Epstein-Barr virus, or adenovirus and genetically modified to express HER2-CARs with a CD28/CD80 signaling endodomain (HER2-CAR VSTs).

**Main Outcomes and Measures**
Primary end points were feasibility and safety. The key secondary end points were T-cell persistence and their antglioblastoma activity.

**Results**
A total of 17 patients (18 females and 9 males; 10 patients ≥18 years [median age, 60 years; range, 30-69 years] and 7 patients <18 years [median age, 14 years; range, 10-17 years]) with progressive HER2-positive glioblastoma received 1 or more infusions of autologous HER2-CAR VSTs (1 × 10^7/m^2 to 1 × 10^10/m^2) without prior lymphodepletion. Infusions were well tolerated, with no dose-limiting toxic effects. HER2-CAR VSTs were detected in the peripheral blood for up to 12 months after the infusion by quantitative real-time polymerase chain reaction. Of 16 evaluable patients (9 adults and 7 children), 1 had a partial response for more than 9 months, 7 had stable disease for 8 weeks to 29 months, and 8 progressed after T-cell infusion. Three patients with stable disease are alive without any evidence of progression during 24 to 29 months of follow-up. For the entire study cohort, median overall survival was 11.3 months (95% CI, 4.1-27.2 months) from the first T-cell infusion and 24.5 months (95% CI, 17.2-34.6 months) from diagnosis.

**Conclusions and Relevance**
Infusion of autologous HER2-CAR VSTs is safe and can be associated with clinical benefit for patients with progressive glioblastoma. Further evaluation of HER2-CAR VSTs in a phase 2b study is warranted as a single agent or in combination with other immunomodulatory approaches for glioblastoma.
CAR T cell design

Humanized Anti-EGFRvIII scFv

4-1BB
CD3ζ

Design chosen on basis of Pre-clinical data published Science Translational Medicine Feb 18, 2015. Johnson et al

GMP Manufacturing process as established in Penn CVPF
Adoptive T cell Therapy of Cancer

Ideally, will recapitulate the end result of a vaccine to induce T cell-immunity

- Large number of potent antigen specific T cells
- Expansion in vivo in response to antigen encounter
- Potent anti tumor activity
- Contraction and long-term persistence
- Ability to respond to challenge
Two Approaches to Bypass Central Tolerance and Generate Potent T Cells to Self Antigens

Transfer of “potent’ TCR
- Secreted, surface and intracellular antigens are candidates
- Native and Physiologic TCR complex
- Antigens expressed at very low levels might be excellent candidates if processed/presented efficiently
- MHC restricted recognition of tumor cells

Transfer of CAR (Chimeric Antigen Receptors)
- MHC unrestricted recognition of tumor cells
- Limited to cell surface epitopes
- T cells may be able to migrate into areas not accessible to antibody
- Complement independent
Advantages of adoptive T cell transfer therapy

1. “instant vaccine”: infuse large numbers of selected cells with high avidity for tumor antigens (EGFRvIII is tumor-specific)

2. Administer cells activated ex-vivo to exhibit anti-tumor effector function: Expansion ex vivo and in vivo

3. Potentially identify exact cell subpopulations and effector functions required for cancer regression in vivo

4. Transferred cells can be modified genetically prior to infusion

5. Host can be manipulated prior to cell transfer to provide altered environment for transferred cells
**EGFRvIII as a CAR Target for GBM**

**Pros and Cons as a CAR Target**

- **Pro:** tumor specific mutation so that on-target, off-tumor toxicity is unlikely; oncogenic driver confers poor prognosis

- **Con:** EGFRvIII is a subclonal mutation: tumor heterogeneity

**Celldex data: Re-Act vs. Act IV?**

Celldex Re-Act Interim data, EGFRvIII peptide vaccine, recurrent GBM, SNO 2014, 2015, ASCO 2015

OS: Bev-Naïve Relapsed GBM (Group 1)

Median (95% CI)
- rindopepimut + bevacizumab (n=35) 12.0 (9.7, --)
- control + bevacizumab (n=37) 8.8 (6.8, 11.4)

Patients have not yet experienced progression of disease on study treatment

HR = 0.47 (0.25, 0.91)  
p = 0.0208
Celldex Act IV: Peptide vaccination with RINDOPEPIMUT (EGFRvIII) for newly diagnosed GBM

**A**

MRD (< 2cm)

ITT

SRD (> 2cm)

**B**

**C**

Trend to Efficacy with bulky Disease (p=0.066)

Lancet Oncology (In review)
**Protocol Design and Consort Diagram for EGFRvIII CART Study: NCT02209376**

**A**
- EGFRvIII+ glioblastoma
  - leukapheresis
  - Progression of Disease
    - Screen/consent for Step 1
      - Triggers manufacturing
        - Consent for Treatment/Enrollment Step 2
          - MRI brain
            - CAR T cell infusion Day 0
              - Follow-up Days 1,3,7,10,14,21
                - Day 28 MRI brain Research studies
  - Follow-up every 4 weeks until month 6
  - Follow-up every 2 months until 2 years

**B**
- 17 EGFRvIII+ subjects consented on Step 1
  - 3 clinical decline
    - 14 apheresed
      - 3 pending progression/manufacturing
        - 1 withdrawn
          - 10 consented to Step 2
            - 10 subjects infused
<table>
<thead>
<tr>
<th>ID Number</th>
<th>Gender</th>
<th>Age at Time of CAR-T Infusion</th>
<th>Time from Initial Resection to CAR-T Infusion</th>
<th>Line of treatment at CAR-T infusion</th>
<th>KPS at Time of CAR-T Infusion</th>
<th>Steroid Dose at Time of CAR-T Infusion</th>
<th>EGFR vili Expression</th>
<th>Treatments Prior to Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>201</td>
<td>M</td>
<td>60</td>
<td>218 days</td>
<td>4</td>
<td>90</td>
<td>dexamethasone 4 mg/day</td>
<td>93%</td>
<td>XRT/TMZ + 1 cycle TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: CCNU x 1 dose + Lomustine x 2 doses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: carboplatin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4th line: CAR-T infusion</td>
</tr>
<tr>
<td>202</td>
<td>M</td>
<td>74</td>
<td>642 days</td>
<td>4</td>
<td>90</td>
<td>0</td>
<td>6%</td>
<td>XRT/TMZ + 3 cycles TMZ, ACT-IV: 5 Vaccines Received</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (6% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: Surgery (6% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4th line: CAR-T infusion</td>
</tr>
<tr>
<td>204</td>
<td>F</td>
<td>54</td>
<td>179 days</td>
<td>2</td>
<td>70</td>
<td>0</td>
<td>72%</td>
<td>XRT/TMZ + 1 cycle TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: CAR-T Infusion</td>
</tr>
<tr>
<td>205</td>
<td>F</td>
<td>64</td>
<td>676 days</td>
<td>3</td>
<td>100</td>
<td>0</td>
<td>21%</td>
<td>XRT/TMZ + 16 cycles TMZ, ACT-IV: 8 Vaccines received</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (21% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: CAR-T Infusion</td>
</tr>
<tr>
<td>207</td>
<td>M</td>
<td>76</td>
<td>462 days</td>
<td>4</td>
<td>80</td>
<td>0</td>
<td>95%</td>
<td>XRT/TMZ + 7 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (95% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: CCNU &amp; Avastin (2 cycles)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4th line: CAR-T Infusion</td>
</tr>
<tr>
<td>209</td>
<td>F</td>
<td>59</td>
<td>227 days</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>60%</td>
<td>XRT/TMZ + 3 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: CAR-T Infusion</td>
</tr>
<tr>
<td>211</td>
<td>F</td>
<td>57</td>
<td>291 days</td>
<td>3</td>
<td>60</td>
<td>0</td>
<td>42%</td>
<td>XRT/TMZ + 4 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (42% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: CAR-T Infusion</td>
</tr>
<tr>
<td>213</td>
<td>F</td>
<td>51</td>
<td>326 days</td>
<td>3</td>
<td>80</td>
<td>dexamethasone 4 mg/day</td>
<td>70%</td>
<td>XRT/TMZ + 7 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: DC-Vax: Total 5 vaccines received</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: CAR-T Infusion</td>
</tr>
<tr>
<td>216</td>
<td>M</td>
<td>45</td>
<td>682 days</td>
<td>3</td>
<td>90</td>
<td>0</td>
<td>96%</td>
<td>XRT/TMZ + 12 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (96% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: CAR-T Infusion</td>
</tr>
<tr>
<td>217</td>
<td>M</td>
<td>66</td>
<td>390 days</td>
<td>4</td>
<td>80</td>
<td>0</td>
<td>80%</td>
<td>XRT/TMZ + 6 cycles TMZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd line: Surgery (80% EgrfVIII)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd line: Lomustine x 1 dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4th line: CAR-T Infusion</td>
</tr>
</tbody>
</table>
CART engraftment and persistence in blood occurs in GBM

Flow Cytometry
Pre-treatment biopsies of recurrent GBM

**Patient 31213-211, T-cell infiltrated region**

CD3CD8 positive T cells are present but lack evidence of activation (IFN-γ, CD25 and CD134) and cytotoxic (granzyme b) phenotype
Post EGFRvIII-CAR treatment biopsies of GBM (day +6 CART infusion)

Patient 31213-211, T-cell infiltrated region

Multifocal CAR cell infiltrates associated with increased expression of IFN-γ, CD25 and CD134 and tumor cell EGFRvIII expression
Post EGFRvIII-CAR treatment biopsies of recurrent GBM (CART + 120 days)

**Patient 31213-209, T cell infiltrated region**

Multifocal CD3 T cell infiltrates negative for EGFRvIII CAR cells and lacking expression of activation markers.
Specific editing of EGFRvIII antigen by activated T cells
## Specificity of Antigen Editing after EGFRvIII CART Infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>205</th>
<th>207</th>
<th>209</th>
<th>211</th>
<th>213</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Post-CART</strong></td>
<td>EGFRvIII negative EGFR amp 5-fold</td>
<td>EGFRvIII 72-95% (multiple areas tested) EGFR amp 16-fold</td>
<td>EGFRvIII 13% EGFR amp 5-fold</td>
<td>EGFRvIII negative EGFR amp 10-fold PIK3CA p.E542K</td>
<td>EGFRvIII 0, 9, 57, 95% EGFR p.R108K EGFR amp 12-fold</td>
</tr>
</tbody>
</table>

*Other mutations largely preserved in GBM clones?*
Subject 216 day +13

A

Pre

Post

CD3

CAR ISH

IFN-γ ISH

CD3

CAR ISH

IFN-γ ISH

CD8

GRZMB

CD25

Pre

Post

CD8

GRZMB

CD25
Expression data of EGFRvIII and CART sequences in GBM tumor tissue pre- and post-infusion.
In situ assessment of immunosuppressive molecules in the tumor microenvironment (TME) before and after CAR T cell infusion.
Induction of Immunosuppressive Tumor Microenvironment (TME) after CART Infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>211</th>
<th>213</th>
<th>217</th>
<th>216</th>
<th>207</th>
<th>205</th>
<th>209</th>
<th>211</th>
<th>213</th>
<th>217</th>
<th>216</th>
<th>207</th>
<th>205</th>
<th>209</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>-43</td>
<td>-326</td>
<td>-85</td>
<td>-81</td>
<td>-124</td>
<td>-63</td>
<td>-209</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>34</td>
<td>55</td>
<td>120</td>
</tr>
<tr>
<td>CAR ISH</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CD4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CD8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CD25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Granzyme b</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>FoxP3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IDO1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IL-10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TDO</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PD-L1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>PD1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>NP</td>
<td>NP</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>NP</td>
<td>NP</td>
<td>2</td>
</tr>
<tr>
<td>TGF-β</td>
<td>NP</td>
<td>NP</td>
<td>2</td>
<td>2</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>
T cell receptor-beta CDR3 deep sequencing analysis of T cells: Pre-CART and Post-CART brain and Infusion Product

D

Patient 205

2,827

17,928

Patient 209

593

41

Patient 211

10,545

585

1,052

E

7,912

2,068

16,745

3,226

800

1,768

61,327

77,794

78,092
Immunohistochemical co-localization of CD3/FoxP3 and CD8/Ki67.

Analysis of brain tumor samples performed pre-and post-CART-EGFRvIII infusion.

~Increased in proliferating CD8+ T cells post-infusion
~Increased Tregs post-infusion
~Kinetics of these events? Co-occurring?
Overlap in clonal T cells between pre-infusion tumor, CART infusion product, and post-infusion tumor.

**Implications for distribution of T cell clones?**

- Up to 25% of Post-CART clones in brain were present in infusion product
- Approximately 5% of clones in infusion product are seen in post-CART brain
- Infusion products share more clones with Post-CART brain
Clonality of Infusion Products: no clonal dominance

<table>
<thead>
<tr>
<th>Patient</th>
<th>Entropy</th>
<th>Clonality</th>
<th>Max Frequency (%)</th>
<th>Gene Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>15.341</td>
<td>0.040</td>
<td>0.51419</td>
<td>121,744</td>
</tr>
<tr>
<td>209</td>
<td>15.680</td>
<td>0.036</td>
<td>0.53756</td>
<td>133,753</td>
</tr>
<tr>
<td>211</td>
<td>16.188</td>
<td>0.006</td>
<td>0.05178</td>
<td>112,021</td>
</tr>
<tr>
<td>216</td>
<td>16.550</td>
<td>0.012</td>
<td>0.50127</td>
<td>155,006</td>
</tr>
<tr>
<td>217</td>
<td>16.461</td>
<td>0.013</td>
<td>0.24846</td>
<td>155,761</td>
</tr>
</tbody>
</table>
Conclusions

- Screening for EGFRvIII and CART-EGFRvIII manufacturing is feasible for GBM patients and infusion is safe.
- There is no EGFR-directed toxicity in 10/10 patients (unlike cetuximab).
- Mechanism of Action established:
  - CART-EGFRvIII expand in blood and traffic to brain and become activated/proliferate.
  - CART-EGFRvIII detectable in GBM cells.
  - EGFRvIII antigen loss (NGS analysis) in GBM cells.
- Induction of new T cell infiltrates by IHC in tumor resection specimens. This may suggest antigenic spreading or bystander T cells.
- Induction of large number of additional T cell clonotypes following CART-EGFRvIII infusion: Phenotype of these cells?
- **Hypothesis:** Early T cell activation followed by Adaptive Immunosuppression in TME. Current efforts focused on targeting TME and tumor heterogeneity.
Current Status of CART Immunotherapy for GBM at Penn/ACC

Summary
• First Penn cohort (n=10) (First in Human) completed for recurrent GBM, (O'Rourke et al., Science Translational Medicine) (In press).

Results
• EGFRvIII CART cells cross blood brain barrier and proliferate in situ.

• EGFRvIII CART cells become activated in EGFRvIII GBM cells within one week and reduce levels of EGFRvIII antigen in viable GBM cells.

Issues to resolve
• CART activation and T cell clonotype expansion is accompanied by robust increase in immunosuppressive tumor microenvironment (TME).

• Tumor heterogeneity and co-expression of additional EGFR mutations (60%).
EGFR mutational landscape in GBM: Penn Cohort, 2016

Amplification
EGFRvIII

Extracellular
Ligand-Binding Domain
Mutation

Cytoplasmic
Kinase Domain
Mutation

- EGFR:AMP (N = 81)
- EGFR:vIII (N = 35)
- EGFR:p.R108G (N = 1)
- EGFR:p.R108K (N = 9)
- EGFR:p.S123Y (N = 1)
- EGFR:p.A289D (N = 2)
- EGFR:p.A289T (N = 5)
- EGFR:p.A289V (N = 10)
- EGFR:p.G598V (N = 5)
- EGFR:p.C620Y (N = 1)
- EGFR:p.E709K (N = 1)
- EGFR:p.F712L (N = 1)
- EGFR:p.V717I (N = 1)
- EGFR:p.G719D (N = 2)
- EGFR:p.G719S (N = 1)
- EGFR:p.S768_P772dup (N = 1)
- EGFR:p.V769_H773dup (N = 1)
- EGFR:p.N771_H773dup (N = 1)
- EGFR:p.N771_P772insH (N = 1)
- EGFR:p.V774_C775InsNAHV (N = 1)
- EGFR:p.V774M (N = 1)
- EGFR:p.T790M (N = 1)
- EGFR:p.G810D (N = 1)
- EGFR:p.L858R (N = 1)
Oncogenic mutations in EGFR ectodomain in GBM
Activating GBM-linked EGFR Extracellular mutations localize to critical ‘hinge’ points in ligand-dependent conformational rearrangement.

These mutations also sensitive EGFR to inhibition by lapatinib.

How do these mutations cause ligand-independent activation of EGFR?

There are analogous transforming mutations of ErbB3
(Baiswal, et al. 2013, Cancer Cell)
### A)

<table>
<thead>
<tr>
<th>Clonotype</th>
<th>Fab Clones</th>
<th>VH gene</th>
<th>D gene</th>
<th>JH gene</th>
<th>HC-CDR3</th>
<th>VL gene</th>
<th>JL gene</th>
<th>LC-CDR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3, 4, 9, 17, 19</td>
<td>Ory V1S34*01</td>
<td>Ory D6-1*01</td>
<td>Ory J6-01*</td>
<td>ARDLLFGMDF</td>
<td>Ory LV5S9*01</td>
<td>Ory LJ5-01*</td>
<td>FTAAHTGSSLHV</td>
</tr>
<tr>
<td>2</td>
<td>7, 8, 13, 14</td>
<td>Ory V1S69*01</td>
<td>Ory D4-2*01</td>
<td>Ory J2-01*</td>
<td>ARYGIGWYALSV</td>
<td>Ory LV3S2*01</td>
<td>Ory LJ5-01*</td>
<td>QLWSSTGAV</td>
</tr>
<tr>
<td>3</td>
<td>1, 18, 20</td>
<td>Ory V1S44*01</td>
<td>Ory D4-1*01</td>
<td>Ory J4-02*</td>
<td>ARSGGGVYFDV</td>
<td>Ory LV3S2*01</td>
<td>Ory LJ5-01*</td>
<td>QLWSSTGAV</td>
</tr>
</tbody>
</table>

### B)

![Graph showing OD 405-490](image)

**Legend:**
- PBS
- HIS-VHH
- R108K
- wtEGFR
- EGFRvIII
- domain 3 S492R

---

Don Siegel, Antibody discovery using phage display technology
Questions

- EGFRvIII as target? Other oncogenic EGFRs
- Define and further enhance TILs in GBMs using CART cells?
- Checkpoint inhibitors— which one? combination?
- Combinatorial or bispecific CART constructs?
- Antibody (scFv) optimization?
- Which patient population? Minimal residual disease vs. bulky disease?
- Combination therapy: kinase inhibition, cytotoxic therapy
- GBM subtypes: Sensitivity to Immunotherapy?
# CART EGFRvIII GBM Phase I Trial Team

## Penn Clinical Site
- Donald O’Rourke
- Arati Desai
- Eileen Maloney
- Steven Brem
- Suzanne Frangos

## Penn Pathology
- Jennifer Morrissette
- Maria Martinez-Lage
- MacLean Nasrallah
- Laura Johnson (CCI)

## Penn Neuroradiology
- Suyash Mohan
- Sumei Wang
- Gaurav Verma

## Penn Sponsor Team
- Carl June
- *Marcela Maus (MGH)*
- Liz Hexner
- Emma Meagher
- Regina Young
- Gabriela Plesa
- Pamela Shaw
- Amy Marshall
- Cynthia Desir

## Penn CVPF
- Zoe Zheng
- Bruce Levine
- Alex Malyhkin

## Penn TCSL
- Simon Lacey
- Jos Melenhorst

## Medical Oversight
- Tara Gangadhar
- DSMB team
- Hideho Okada
- Susan Chang

## Funding
- NCI K08 16639
- Novartis
GBM CART Antibody Discovery Team

Penn/ ACC Brain Cancer Immunotherapy Initiative

Basic/Preclinical

- Don L. Siegel, Ph.D, M.D., Director, Division of Transfusion Medicine & Therapeutic Pathology, Medical Director for Cellular Therapies
- Michael Milone, M.D., Ph.D, Pathology & Laboratory Medicine

Translational/Clinical

- Gerald Linette, M.D, Ph.D., Parker Institute, CCI
- Jos Melenhorst, Ph.D, Penn Institute for Immunology
- Beatriz Carreno, Ph.D, Parker Institute
- Additional Members, CART Study Team, multi-disciplinary
Immunophenotype HEAT MAP: ISH: each dot= 3 pixels; IHC: each dot= 1 pixel
Heat Map of Signal Density

Foxp3

CD25
Clinical Endpoints and Survival in Phase I Cohort

C

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Duration on study</th>
<th>Total follow-up</th>
<th>Death</th>
<th>Surgical resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>209</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>205</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>216</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>217</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>213</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>204</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>202</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>211</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>207</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time in Days

D

Overall Survival

Median OS: 251 days
Who to treat and when to treat: recent progress

- IDH1 R132H glioma → PARP inhibitors, epigenetic therapies
- IDH1wt glioma → immunotherapy (checkpoint inhibitors)
- RNAseq classifier to identify strategies for each specific GBM subtype
- CART cells: IL13Ra2, HER2, EGFRvIII?
- Immunotherapy combinations?
- When to treat: initial diagnosis, recurrence, MRD, bulk disease?