Perelman School of Medicine
University of Pennsylvania

GBM CLINICAL TRIALS: EARLY OBSERVATIONS AND FUTURE DIRECTIONS

Cellicon Valley Meeting 2021

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FINANCIAL DISCLOSURES

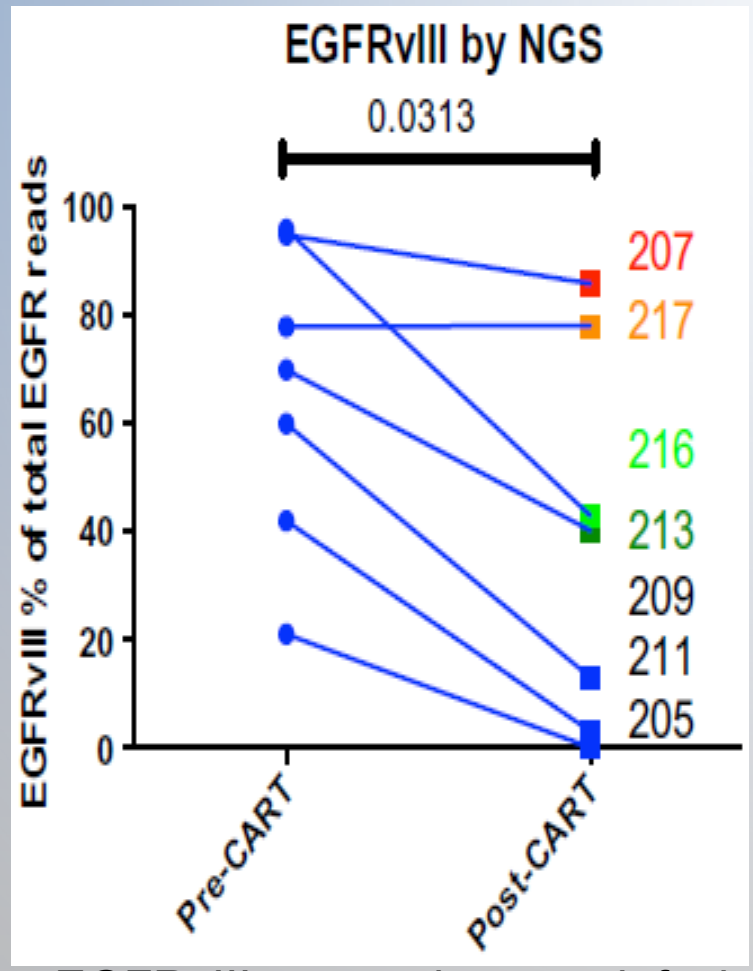
- Clinical Trials using EGFRvIII CAR T cells (PI on both trials; IP on *de novo* GBM trial)
- Sponsored research grant support: Novartis, Tmunity Therapeutics (CAR T cell optimization for GBM)
- Consulting: Implicyte
- Targeted therapy patents: Multiple US/EPO patents on EGFR targeting combined with radiation therapy (licensing royalties)
- Cell therapy patents: IP co-owned by Penn/Novartis (CAR/ICB); additional patents with CAR-T technology including novel scFvs, multivalent CARs, licensed to Tmunity Therapeutics

CHALLENGES AND GOALS

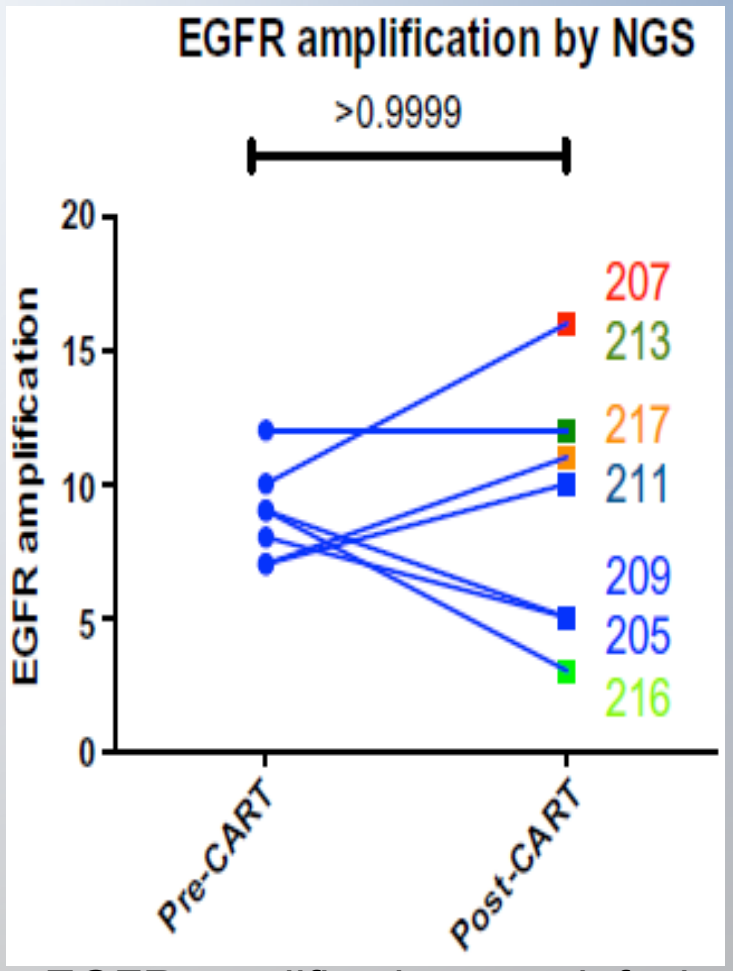
- Immune evolution of GBM and de novo and recurrent phenotypes: different diseases
- Strategies to target glioblastoma heterogeneity
- T cell deficits: anergy, exhaustion and resistance in tumor microenvironment (TME)
- Persistence of CARs in BM, blood, brain
- Preclinical models: GBM organoids (GBOs), canine spontaneous glioblastoma model
- Delivery (CNS)
- Window of Opportunity Trials (neoadjuvant design, human tumor tissue, novel technologies)

TRIAL 1= RECURRENT GBM CAR T TRIAL TARGETING EGFRVIII ONCOPROTEIN #35313

EGFR TARGET HETEROGENEITY-SPECIFIC TARGET EDITING



EGFRvIII expression post-infusion



EGFR amplification post-infusion

Decrease in target antigen (EGFRvIII) was not always mirrored by a decrease in EGFR amplification.

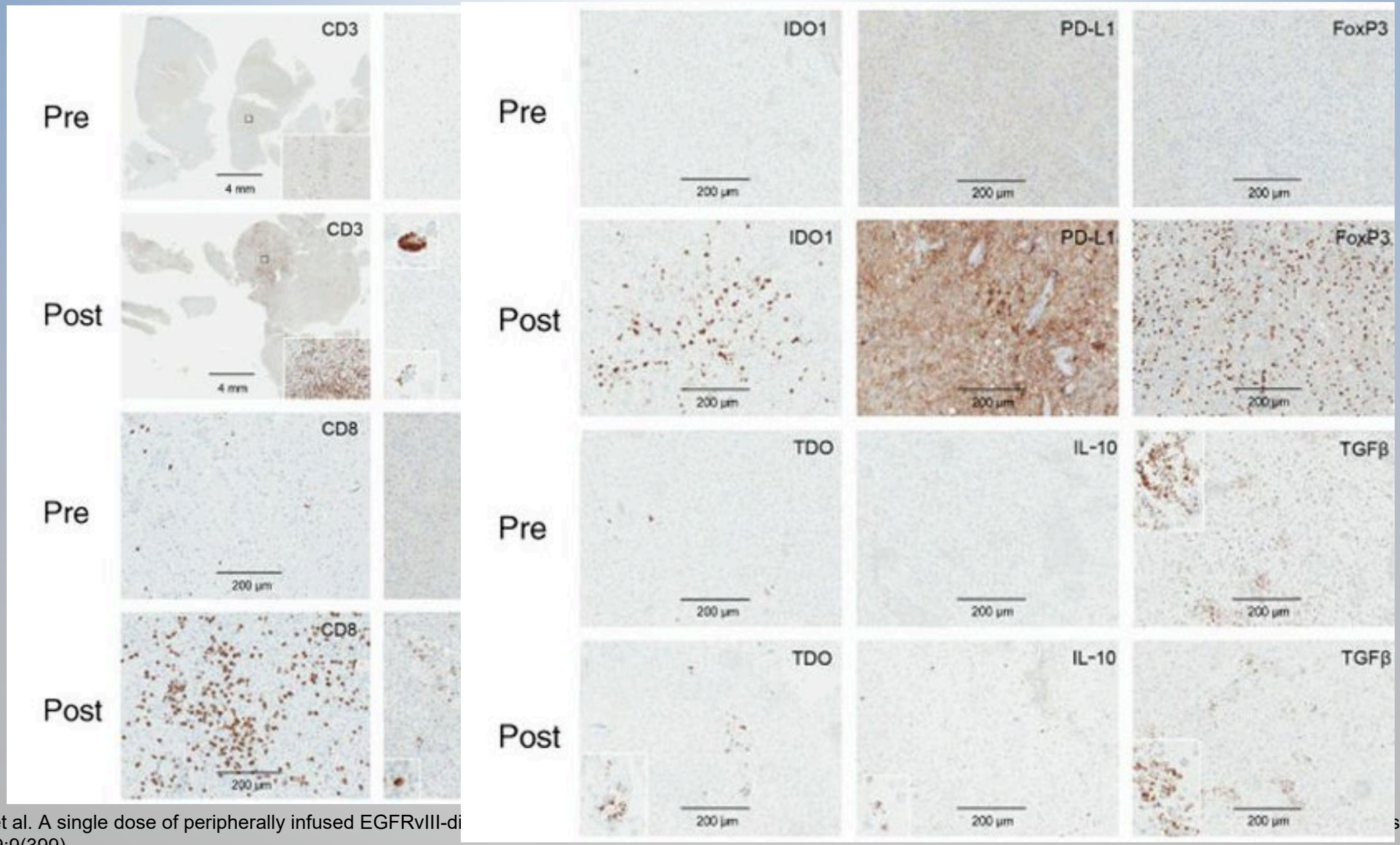
O'Rourke et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med. 2017 Jul 19;9(399).

Specificity of Antigen Editing after EGFRvIII CART Infusion
EGFR alterations post-infusion

Patient	205	207	209	211	213
Pre-CART	EGFRvIII 21% EGFR p.A289V EGFR amp 9-fold	EGFRvIII 95% EGFR amp 10-fold	EGFRvIII 60% EGFR p.R108K EGFR amp 8-fold	EGFRvIII 42% EGFR p.G598V EGFR amp 7-fold PIK3CA p.E542K	EGFRvIII 70% EGFR p.R108K EGFR amp 12-fold
Post-CART	EGFRvIII negative EGFR amp 5-fold	EGFRvIII 72-95% (multiple areas tested) EGFR amp 16-fold	EGFRvIII 13% EGFR amp 5-fold	EGFRvIII negative EGFR amp 10-fold PIK3CA p.E542K	EGFRvIII 0, 9, 57, 95% EGFR p.R108K EGFR amp 12-fold

EGFR alterations co-occurring with EGFRvIII did not change with 2173 EGFRvIII-CAR T treatment.

GBM TME Adaptive Resistance follows activation with GBM CAR T therapy



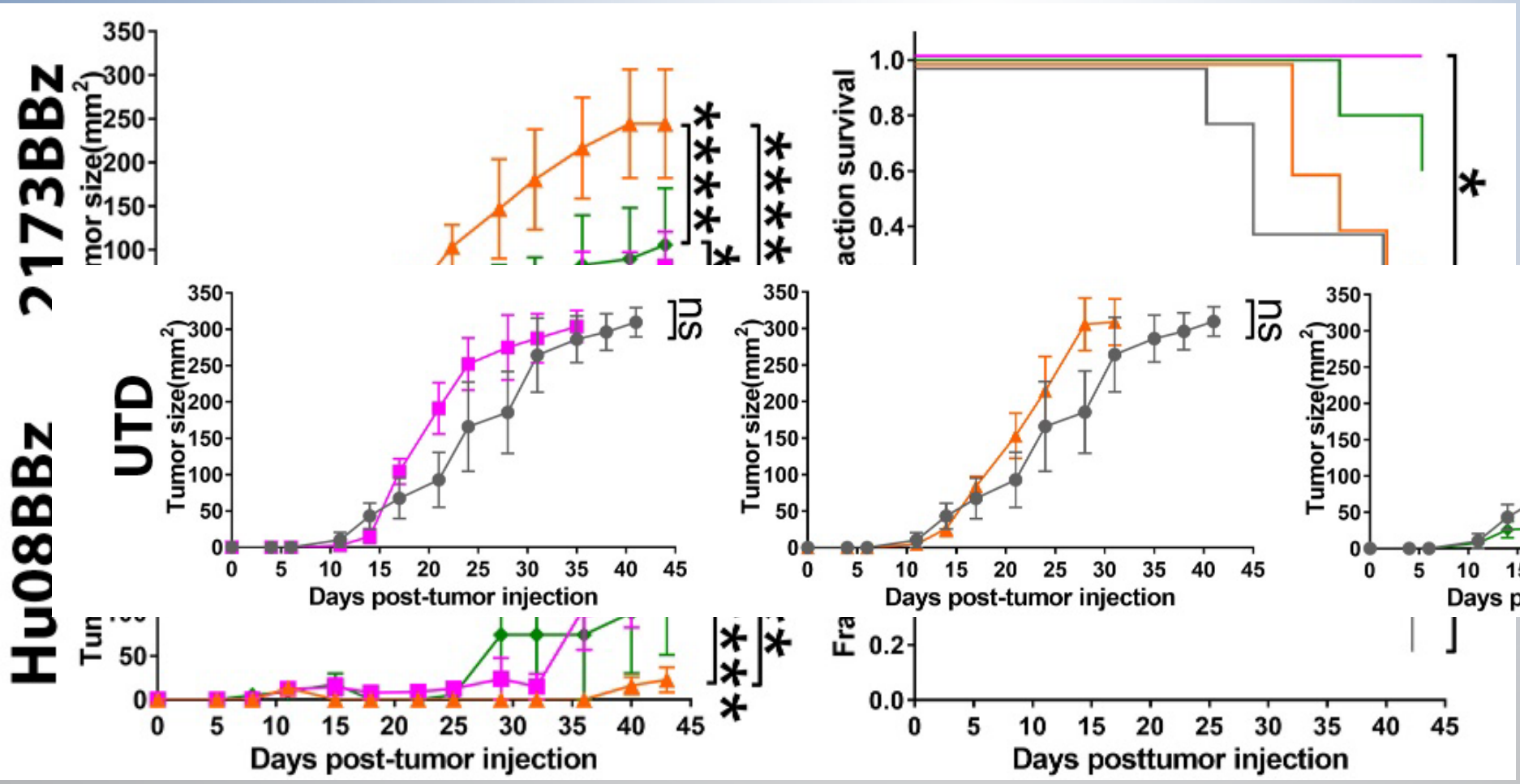
O'Rourke et al. A single dose of peripherally infused EGFRvIII-di stoma. Sci Transl Med. 2017 Jul 19;9(399).

EGFRVIII CAR T TRIAL 1 FOR RECURRENT GBM: LESSONS LEARNED

Despite CAR T engraftment and uptake and proliferation *in situ* in GBM, disease progression occurred via two main mechanisms

1. CAR target heterogeneity
   - Tumor escape occurred via antigen loss
   - Although tumors lost EGFRvIII expression, they retained other EGFR-based driver mutations/alterations
2. Tumor microenvironment immunosuppressive response
   - An immunosuppressive wave occurred in response to CAR T cell infusion
   - Distinct feature of baseline and adaptive immunosuppressive GBM TME
   - Cytotoxic immune activity was blunted by this wave

CHECKPOINT BLOCKADE ENHANCED CAR T CELLS FUNCTION IN NSG MICE IN D270 GBM MODEL (YIN *ET AL*, MOL THER 2018).



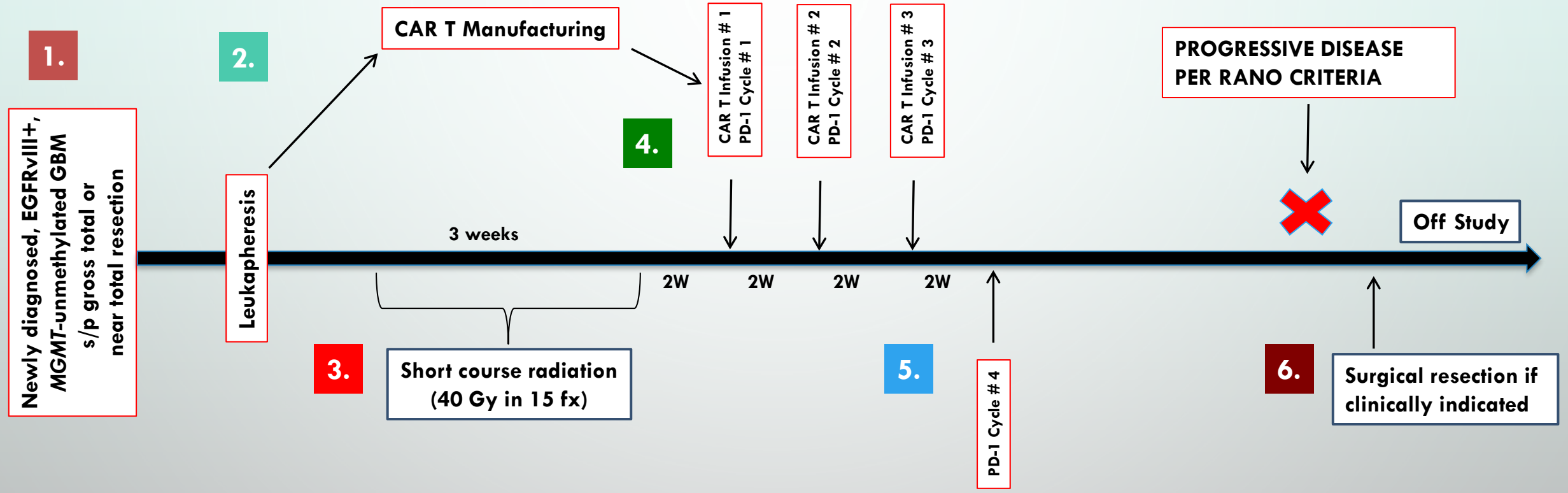
EGFRvIII 2173BBz: PD-1/TIM-3 > CTLA-4
IL13Rα2 Ju08BBz: CTLA-4 > PD-1/CTLA-4

Combination therapy of CAR T cells and immune checkpoint blockade can be optimized based on the CAR target.

TRIAL 2 = *DE NOVO* GBM TRIAL: MULTIPLE EGFRVIII CAR T INFUSIONS (3) + CONCURRENT PEMBROLIZUMAB (4)

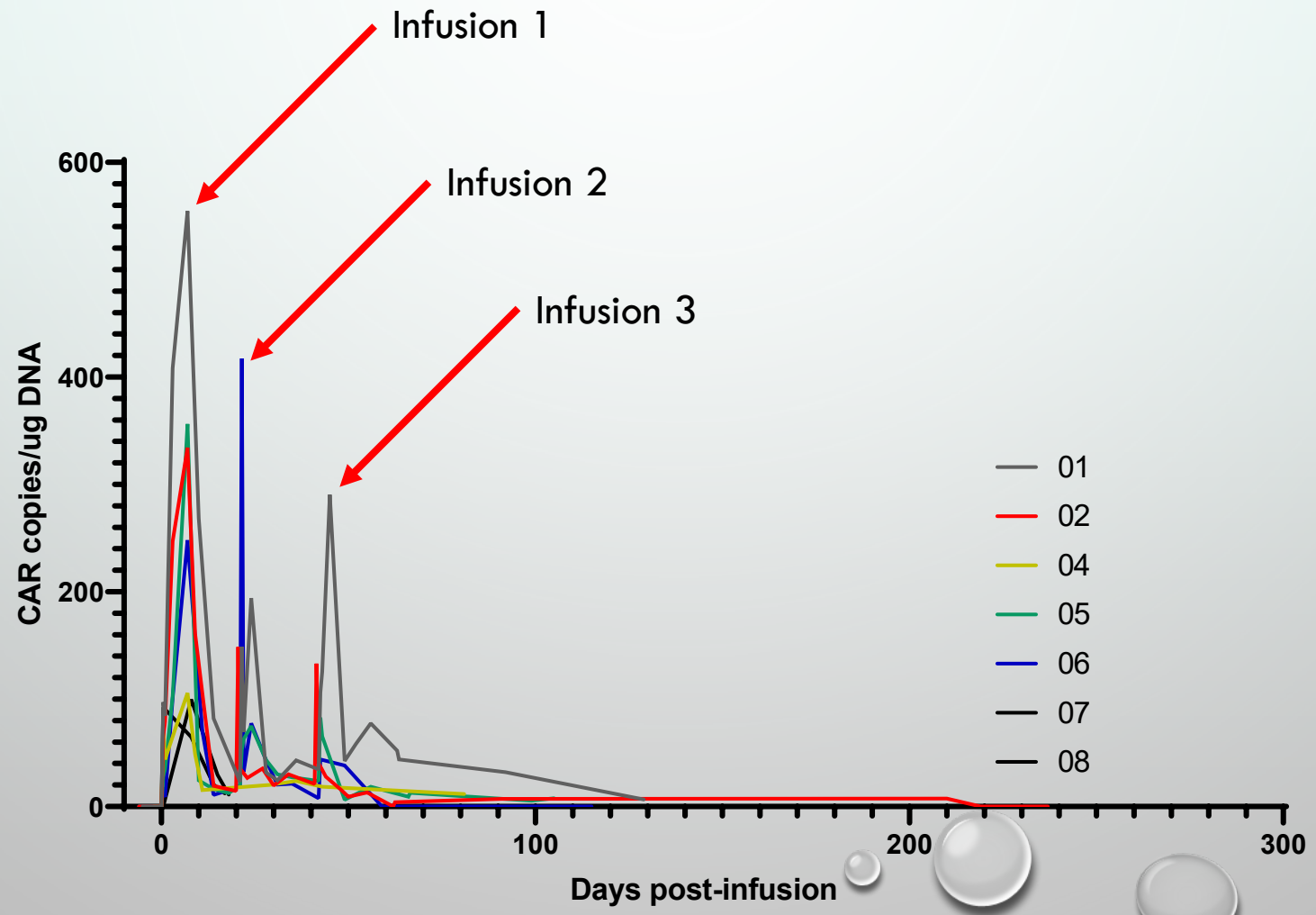
#13318

STUDY SCHEMA:
NEW TRIAL COHORT, 2019 NCT03726515
PHASE I STUDY OF EGFRVIII (2173)-DIRECTED CAR T CELLS IN COMBINATION WITH PD-1 INHIBITION (PEMBROLIZUMAB) IN PATIENTS WITH *NEWLY DIAGNOSED*, *MGMT*-UNMETHYLATED GLIOBLASTOMA

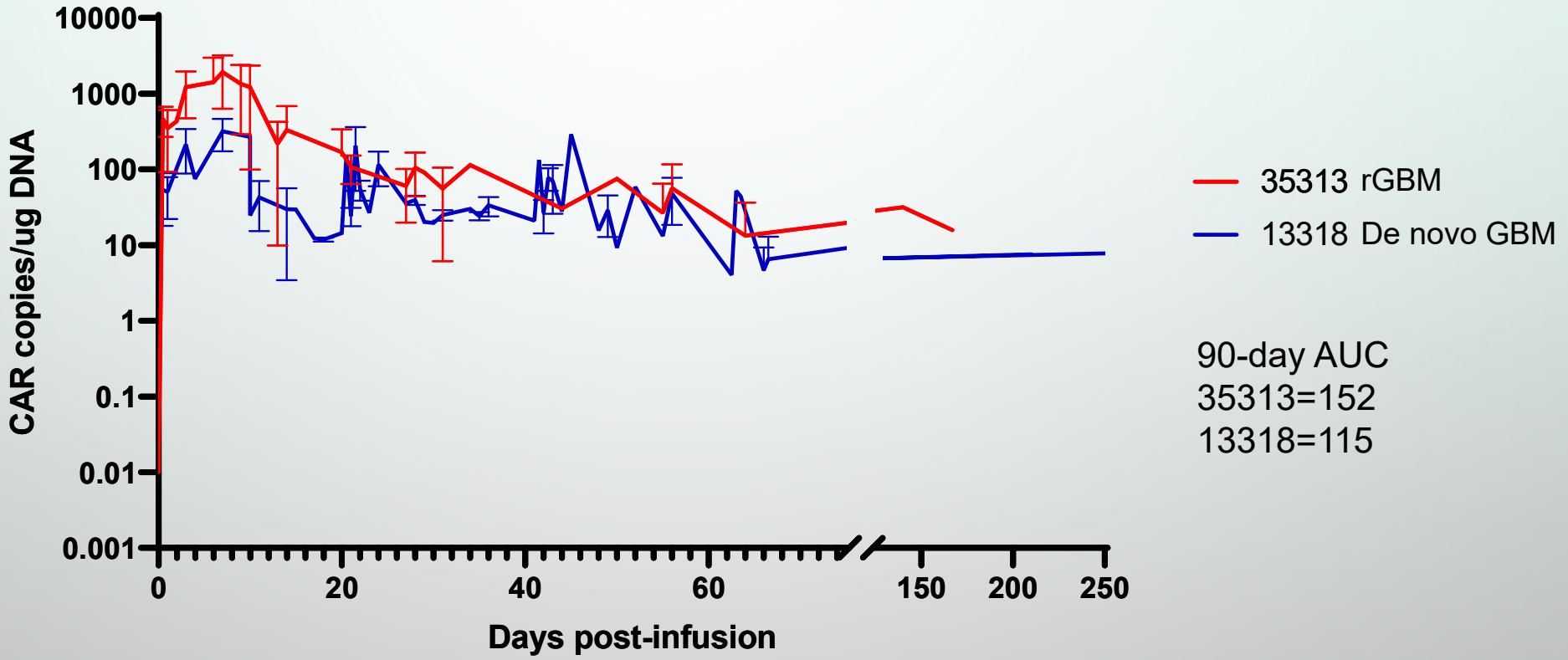


PI: D. O'Rourke, Co-I: Stephen Bagley, Arati Desai

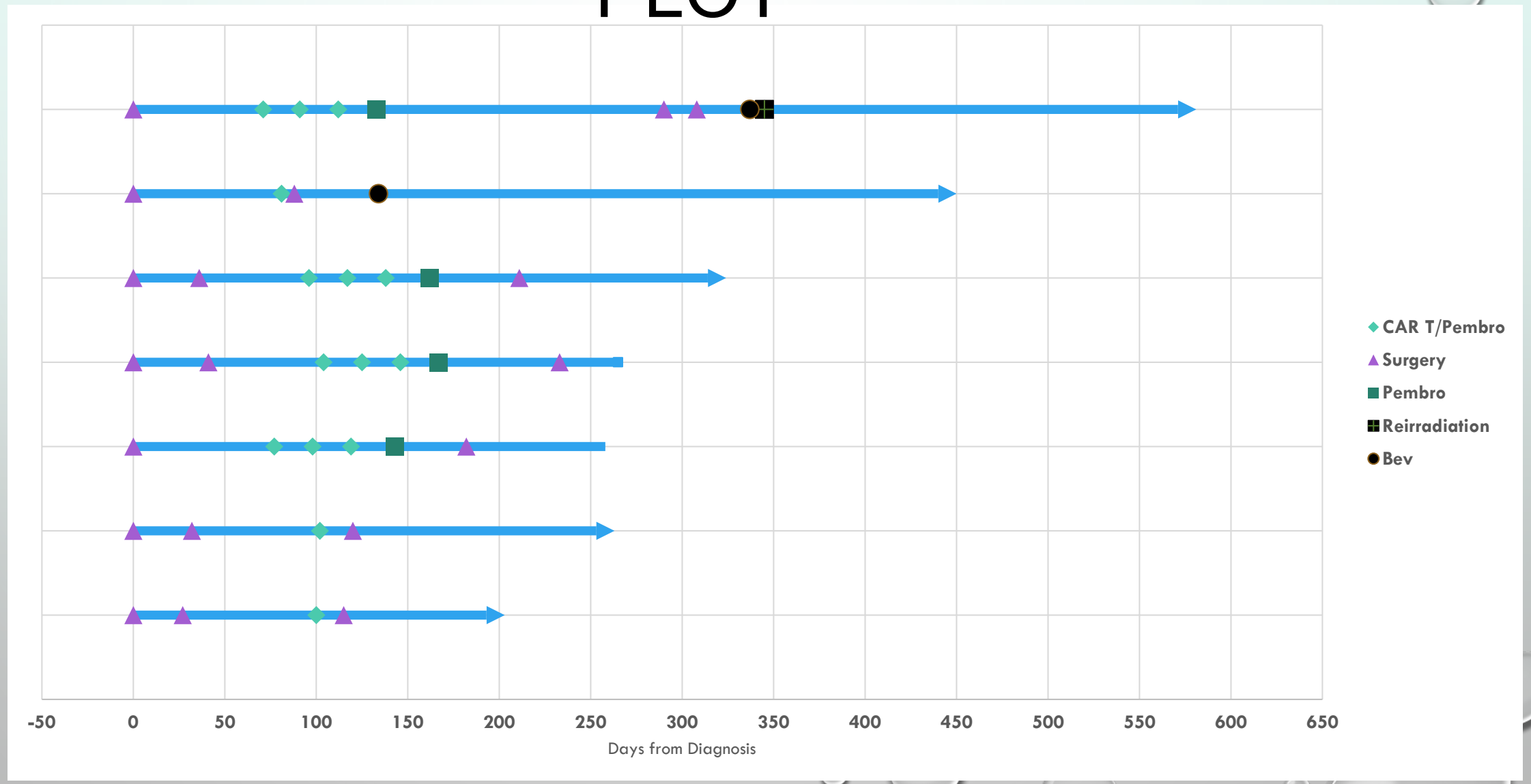
QPCR OF CAR T CELLS TO EVALUATE PERIPHERAL BLOOD ENGRAFTMENT



ENGRAFTMENT: COMPARISON OF 35313 AND 13318 TRIALS



DE NOVO GBM COHORT: SWIMMER'S PLOT

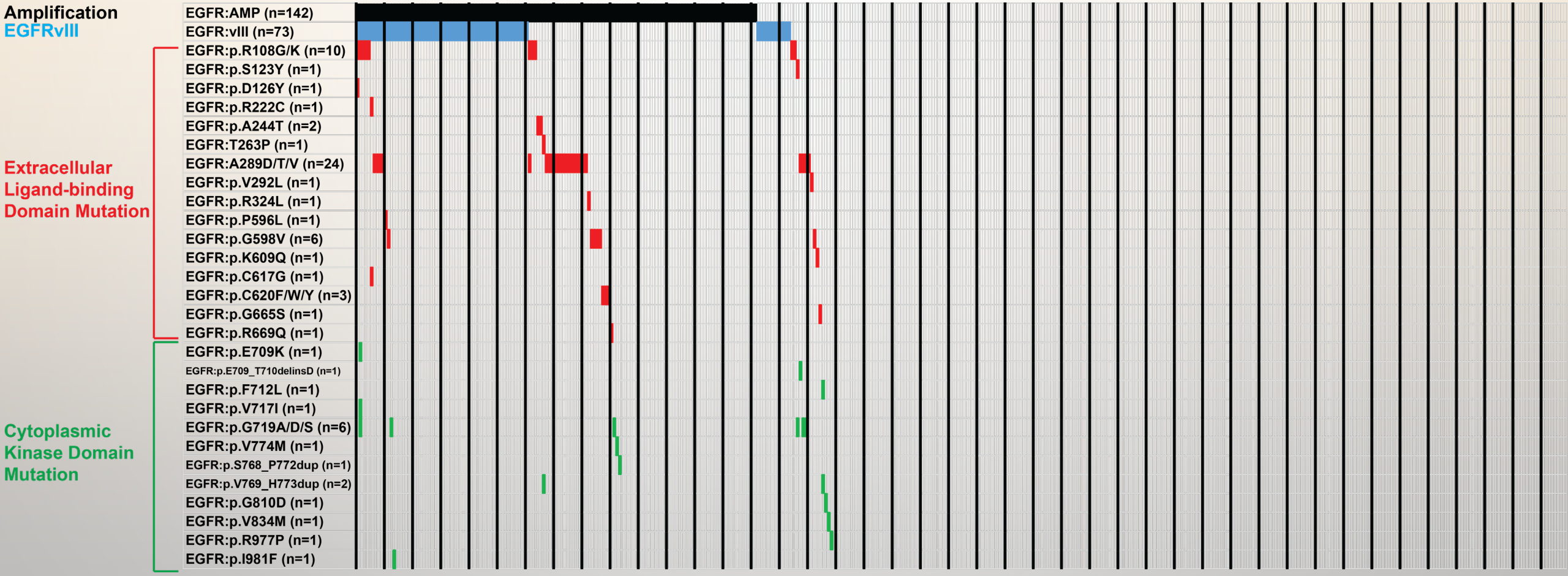


TRIAL 2: MULTIPLE INFUSIONS OF EGFRVIII CAR T/PD-1 BLOCKADE: LESSONS LEARNED

1. Engraftment kinetics similar to Trial 1 (rGBM) but amplitude reduced
2. CART/PD-1 mechanism is unclear as well as question of possible hyper-progression
3. T cell function and composition of apheresis and infusion products
4. CAR target heterogeneity
   - Tumor escape occurred via antigen loss
   - Although tumors lost EGFRvIII expression, they retained other EGFR-based driver mutations/alterations
5. Tumor microenvironment and response to multiple CART/PD1 infusions-under evaluation

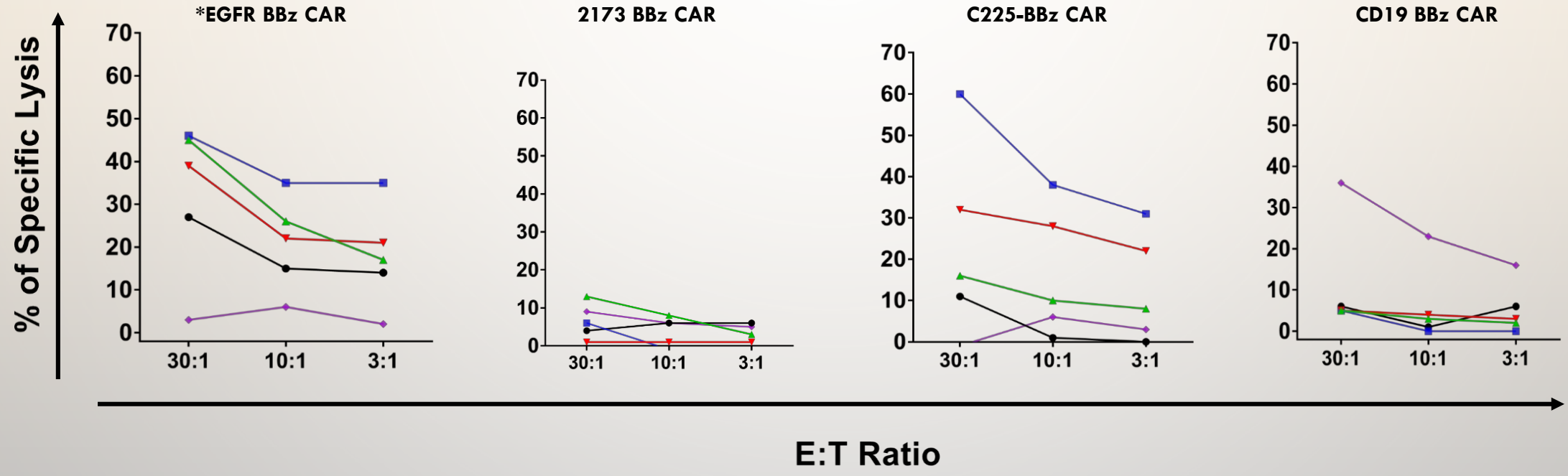
NEXT GOAL: ADDRESSING TUMOR AND TARGET HETEROGENEITY THROUGH CROSS-REACTIVE SCFV

EGFR mutational landscape demonstrates heterogeneity



Penn Brain Tumor Tissue Bank Cohort

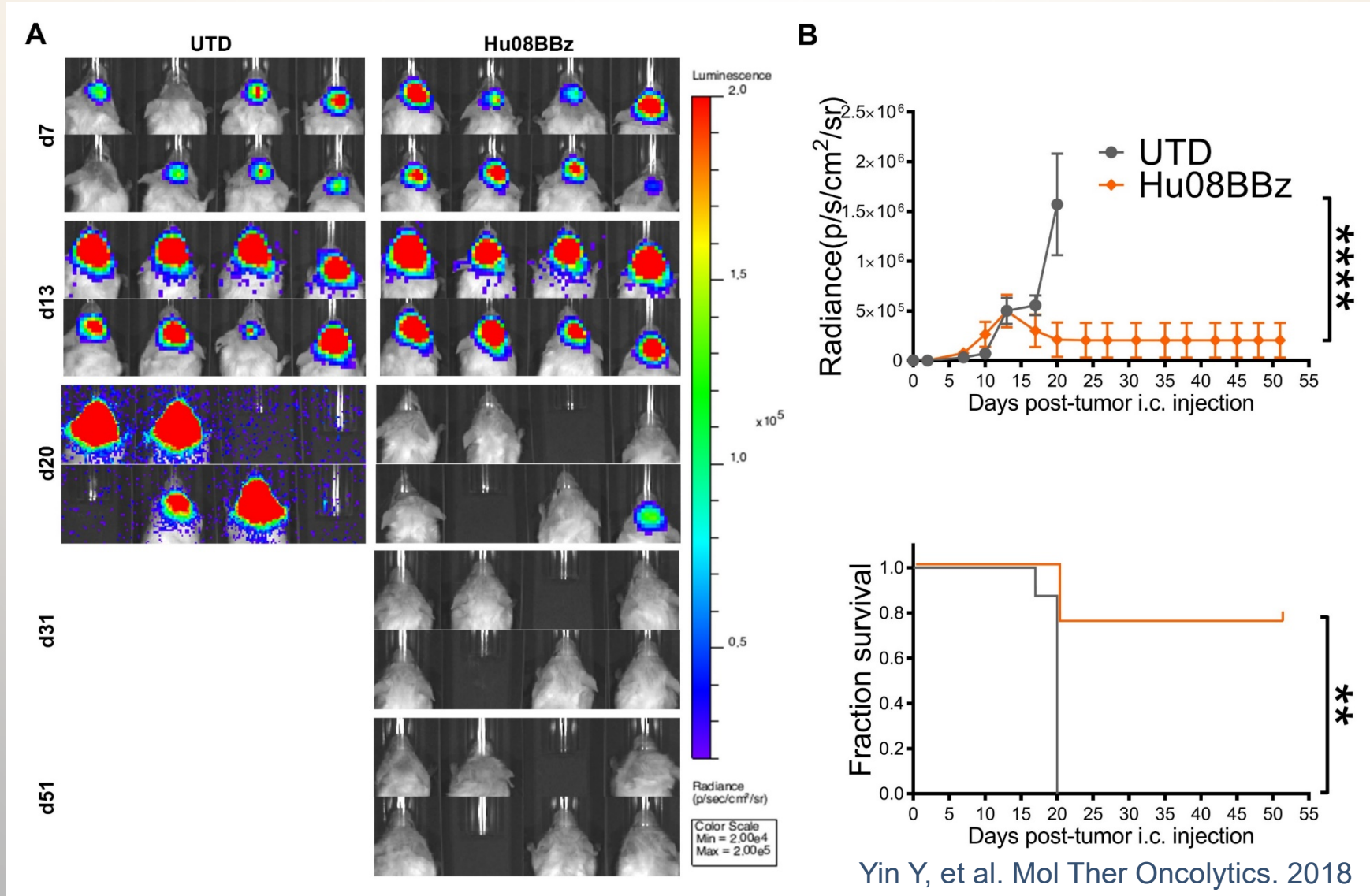
Cross-reactivity of EGFR CAR against EGFR mutants



Binder et al, "Epidermal Growth Factor Receptor Extracellular Domain Mutations in Glioblastoma Present Opportunities for Clinical Imaging and Therapeutic Development." Cancer Cell 2018 Jul 9;34(1):163-177.e7.

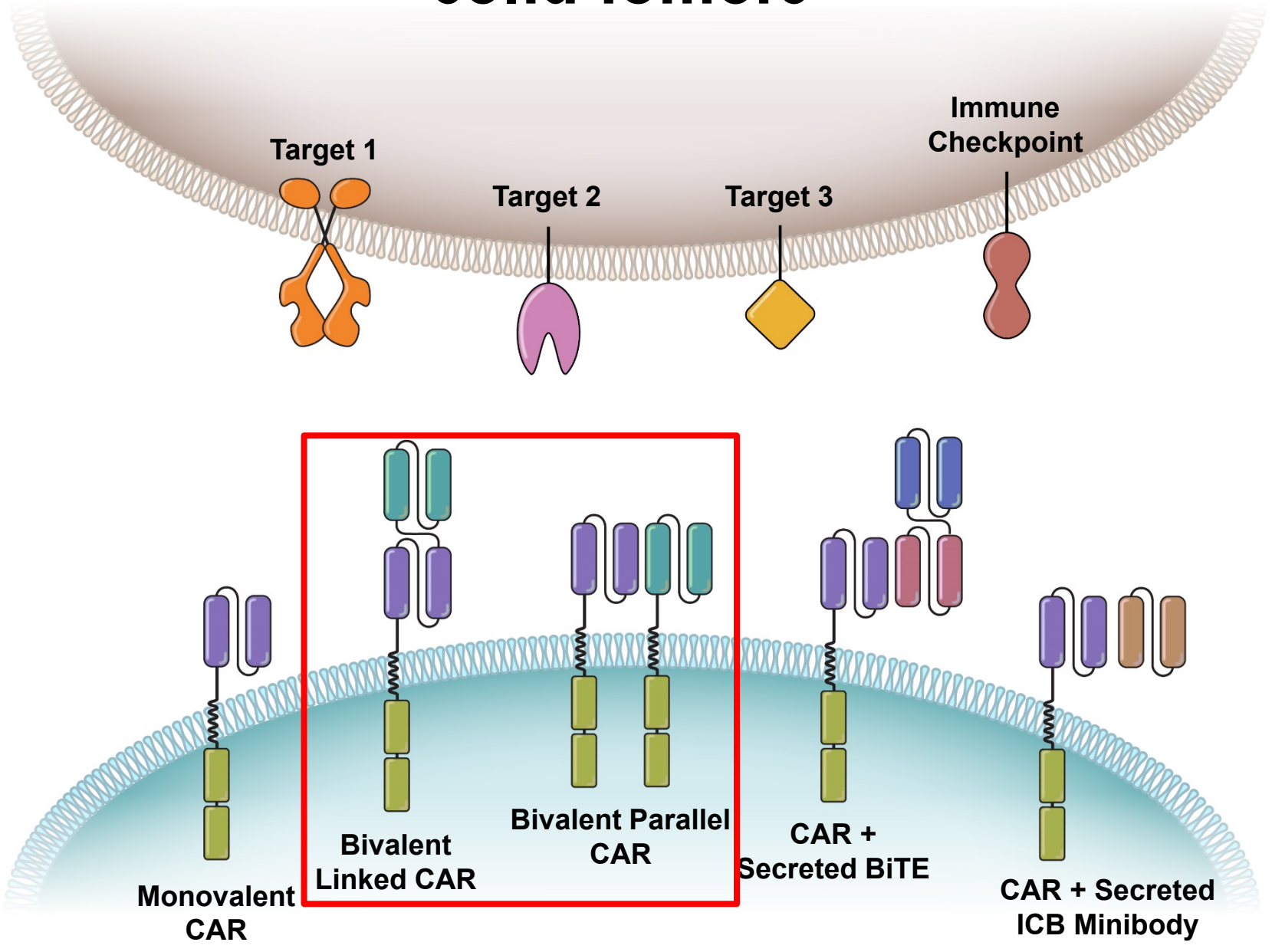
Thokala et al, "High Affinity Chimeric Antigen Receptor with Cross-Reactive scFv to Clinically Relevant EGFR Oncogenic Isoforms." BioRxiv 2020.

IL13RA2 SPECIFIC CAR T CELLS INHIBIT TUMOR GROWTH OF NSG MICE WITH ORTHOTOPIC D270 GBM.

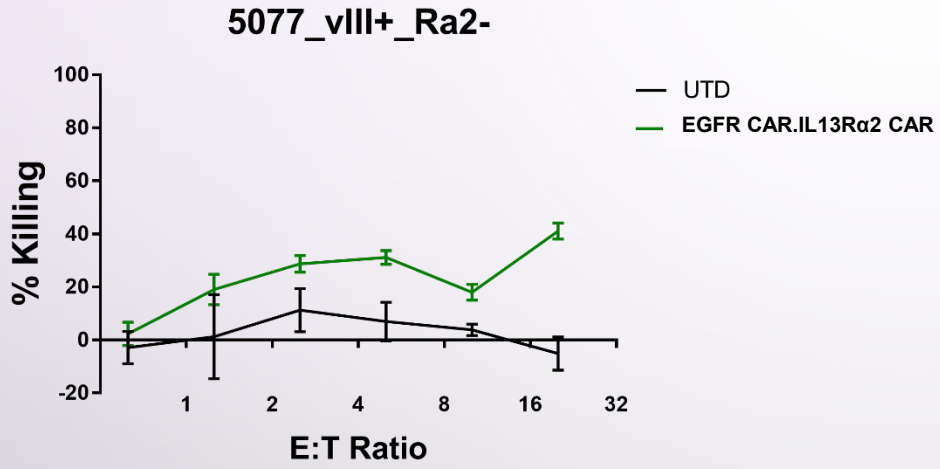


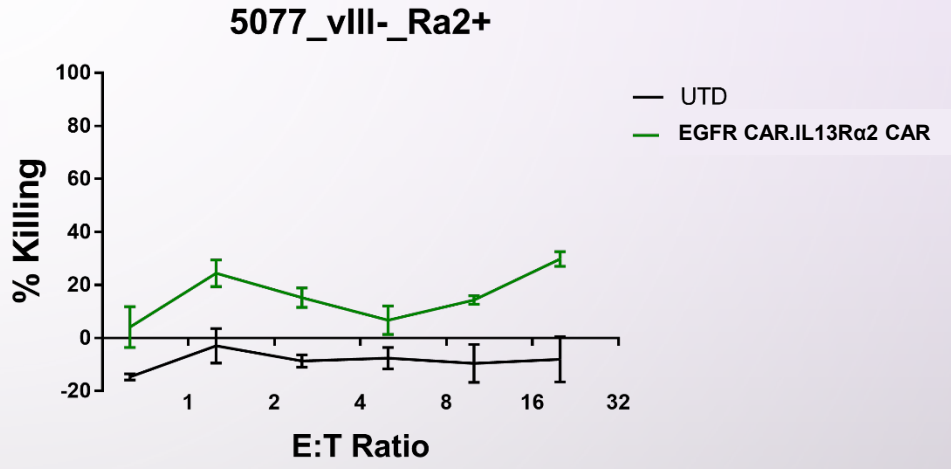
Yin Y, et al. Mol Ther Oncolytics. 2018

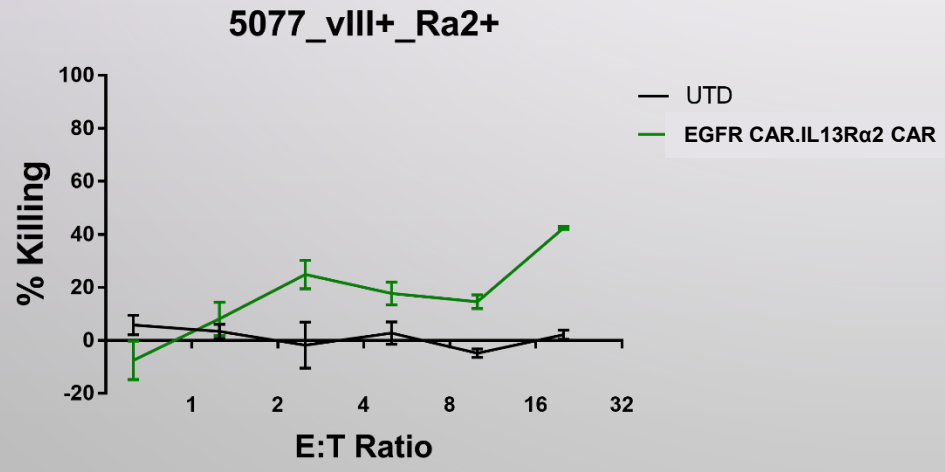
Evolving strategies for next generation T cells for GBM and solid tumors

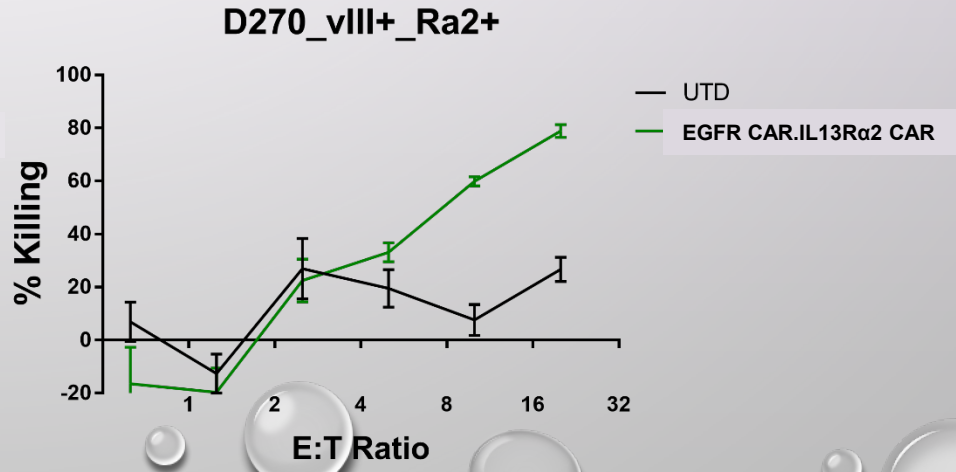


BIVALENT CAR KILLS SINGLE- OR DOUBLE-TARGET EXPRESSING TUMORS *IN VITRO* TO A COMPARABLE DEGREE

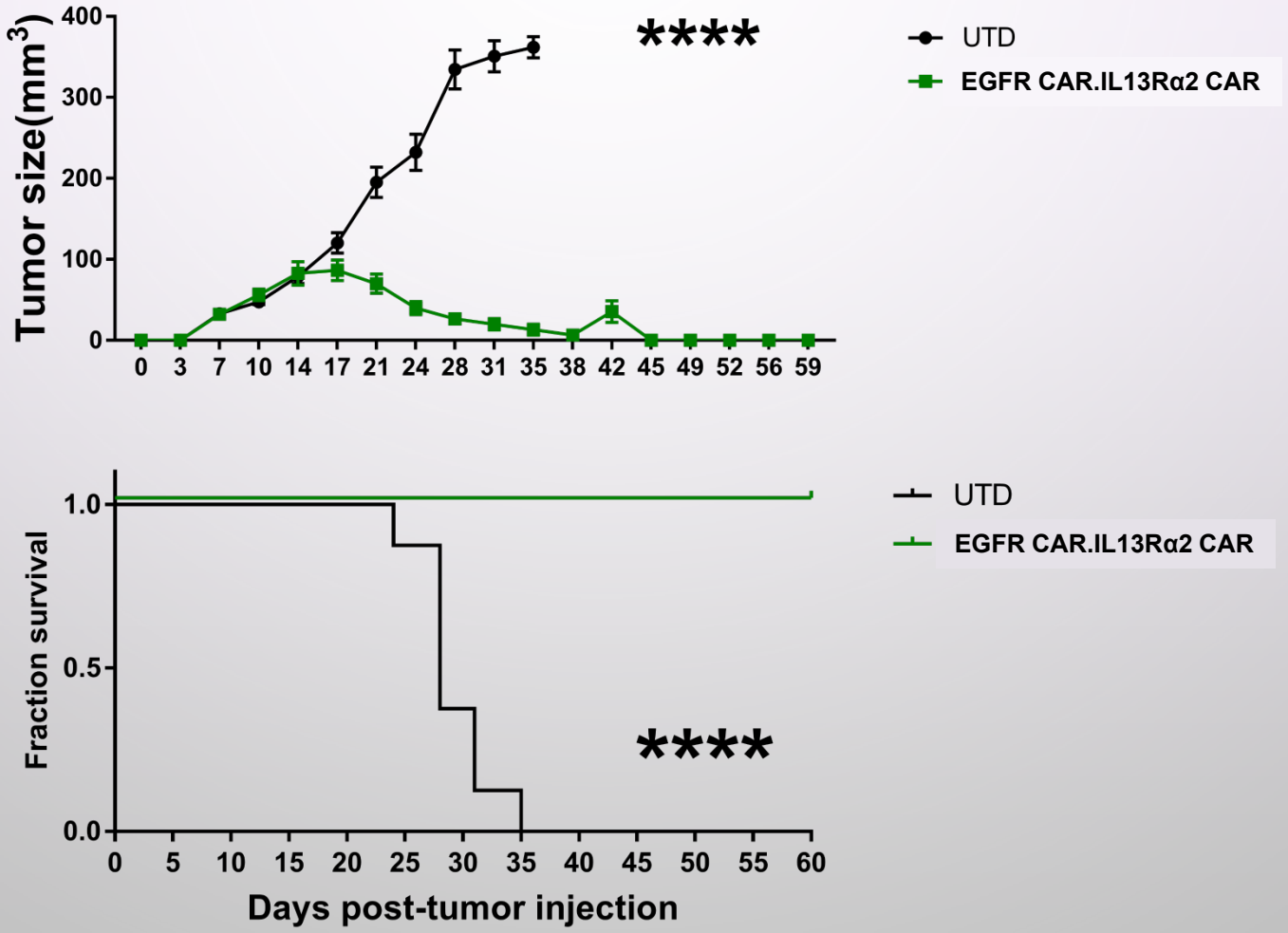








BIVALENT CAR KILLS ESTABLISHED SINGLE- OR DOUBLE-TARGET EXPRESSING D270 TUMORS *IN VIVO*



PENN CELLULAR IMMUNOTHERAPY FOR GBM: ISSUES

1. Multivalent CAR IND preclinical data package being assembled (Q4, 2021)
2. *De novo* or recurrent GBM? Different approaches for each disease?
3. Valency of CAR T cells? How many antigens should be targeted?
4. Role of checkpoint blockade in combinations to modulate GBM
5. Additional combinations: BiTEs and ICBs *in situ*
6. T cell function in *de novo* and recurrent patients? Allogeneic CAR T cells?
7. When to give CAR T cells-> how much residual target available? Neo-adjuvant trial design?
8. CNS delivery: systemic and/or loco-regional?

PENN GBM TCE (>20 PIS, 10 DEPARTMENTS)

- **DONALD O'ROURKE, MD-NEUROSURGERY, CCI**
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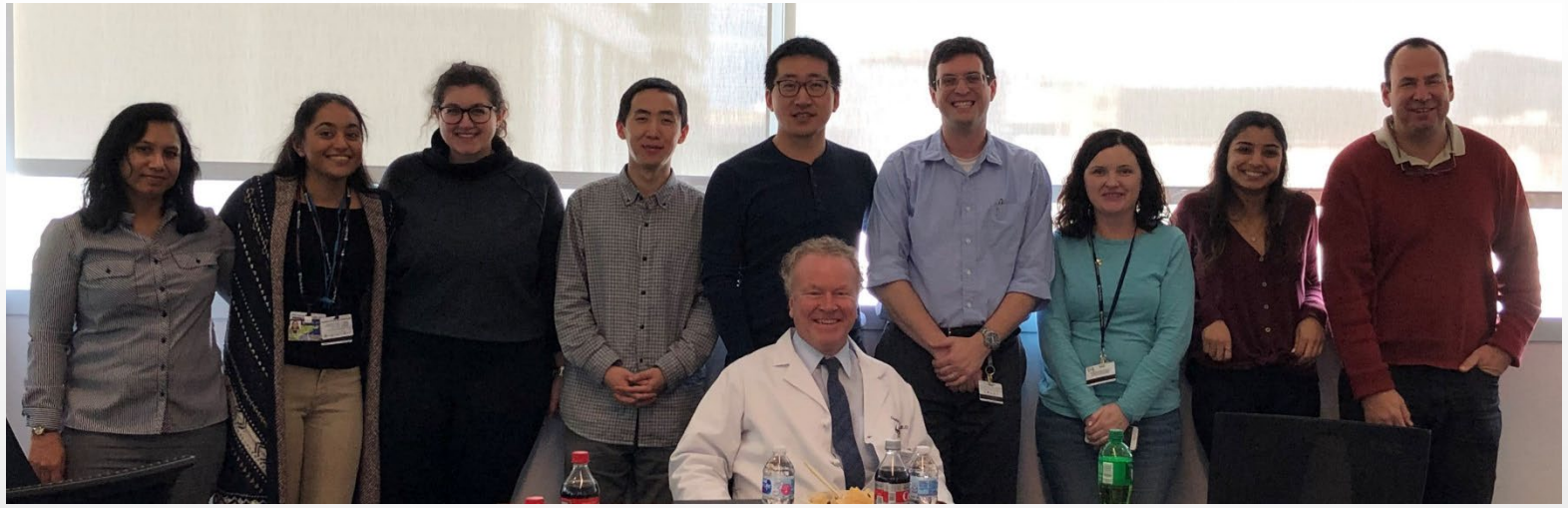
THE O'ROURKE GBM LAB



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  - JESSE RODRIGUEZ
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  - VICKY ZHANG
  - JOE DURGIN
  - THILAN TUDOR
  - OLIVER TANG
  - MADELINE MANDELL
  - TOBENNA IBEABUCHI
  - JACKIE CHEN

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  - PENN CENTER FOR PRECISION MEDICINE