

**Beyond HER2 CAR T cells:
consider how far you have
fallen**



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Disclosure

Research agreement with **Cellmedica** ... NKT cells

Consult for **Adaptimmune** ... tgTCR T cells

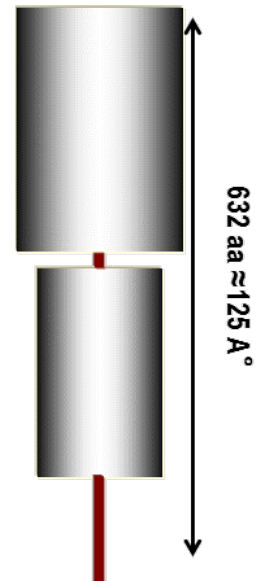
ACEA Bioscience ... pro bono

Concepts

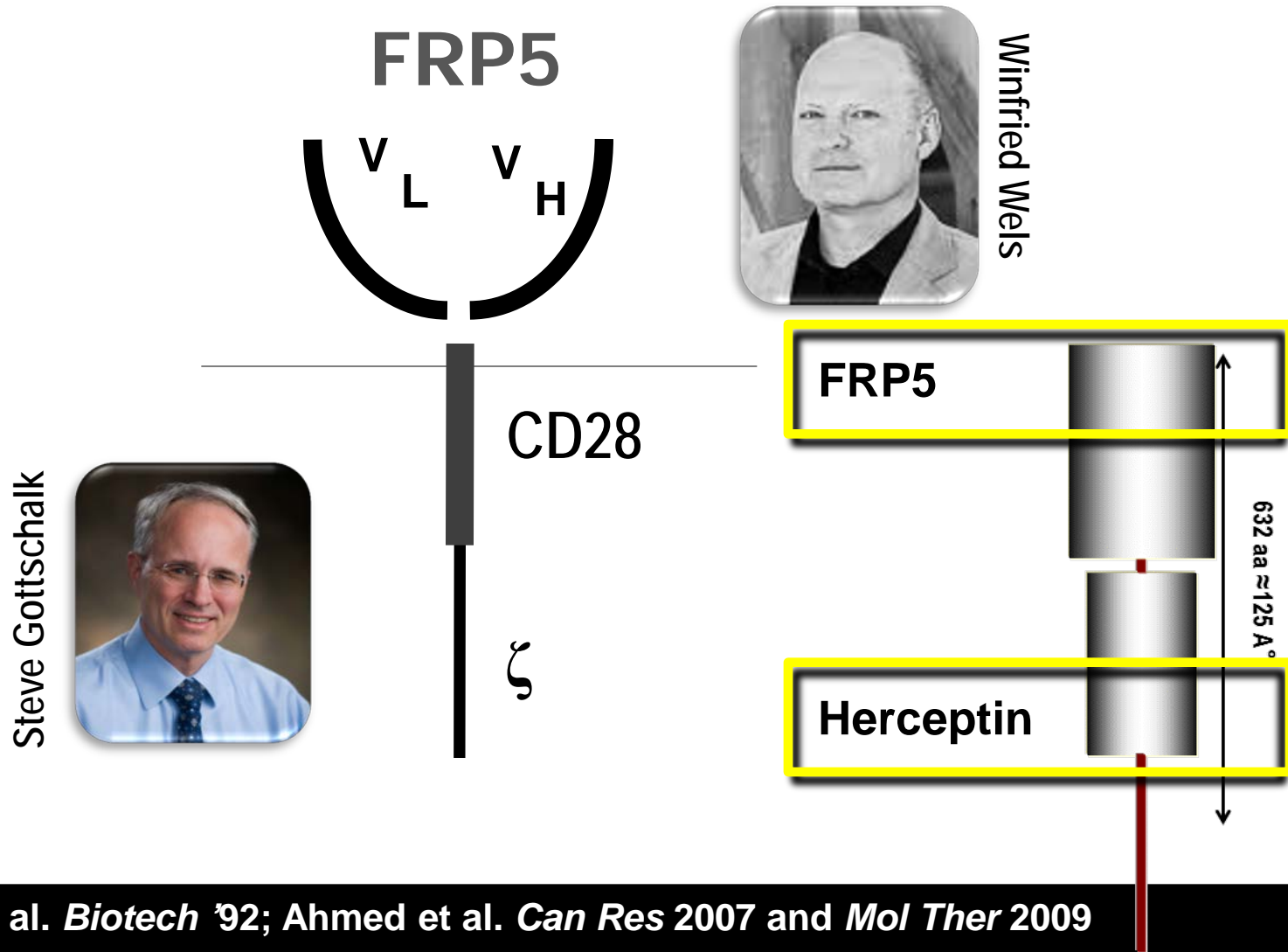
1. Empiric preclinical to clinical development of $HER2$ CAR T-cells
2. Rational development of novel CARs
 1. Target Finding
 2. Exodomain Design
 3. Endodomain Fitting
3. Realistic in preclinical testing
4. Pragmatic pipeline of CAR development

Human Epidermal growth factor Receptor 2

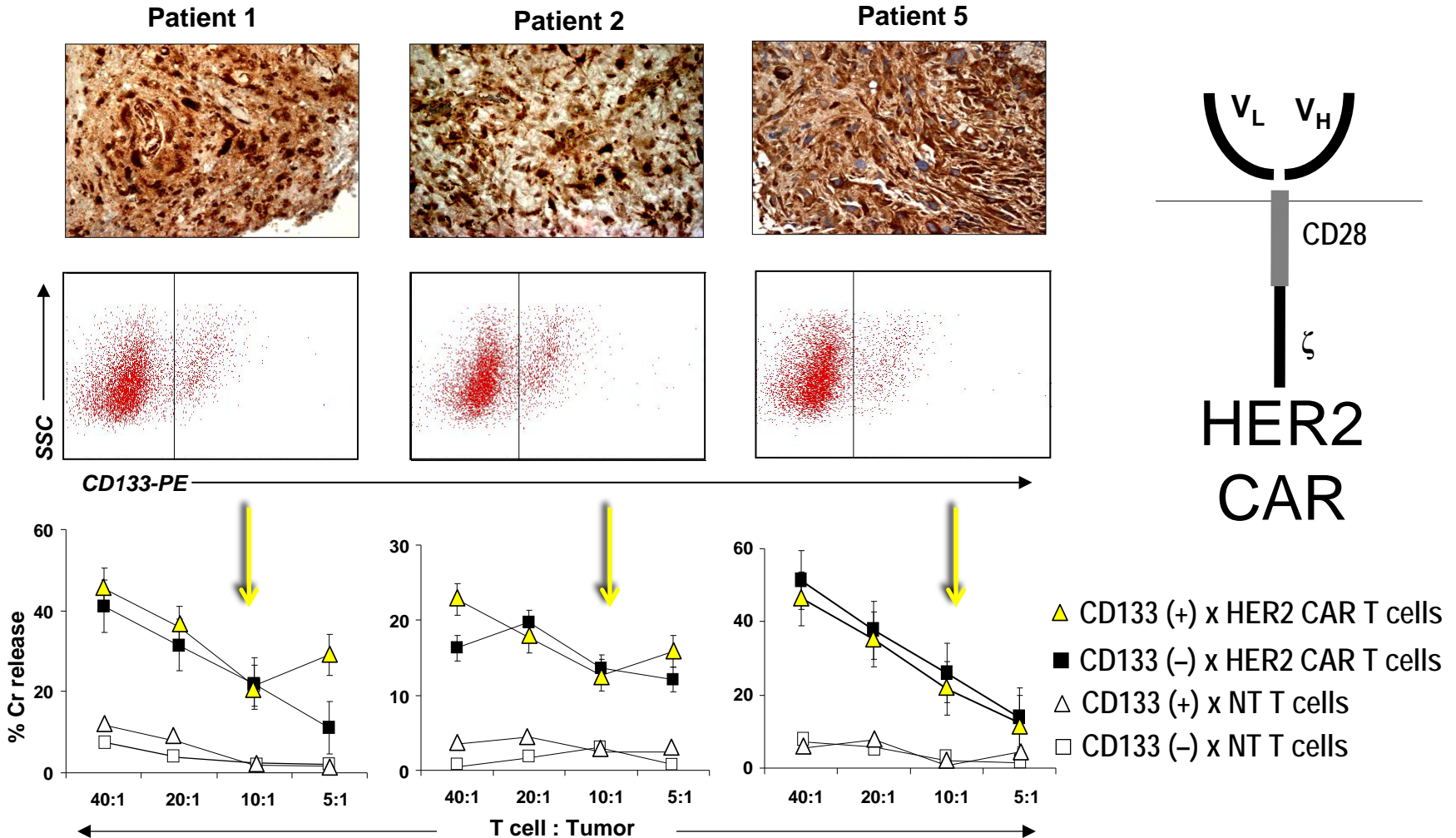
- Multiple malignancies
 - Amplified: breast and ovarian
 - Non-amplified: brain, melanoma & sarcomas
- Aggressive tumor cell behavior
- ↑ in stem cells of GBM & sarcoma
- Associated with ↓ outcome
- Protein ↓↓↓ postnatally



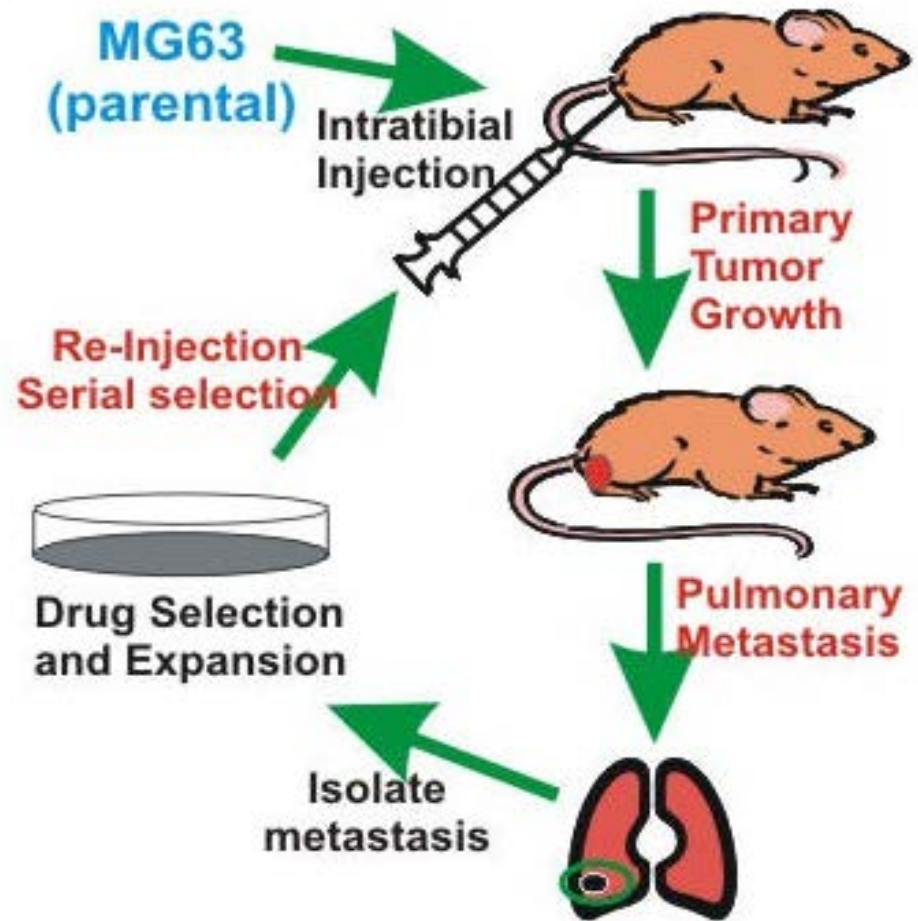
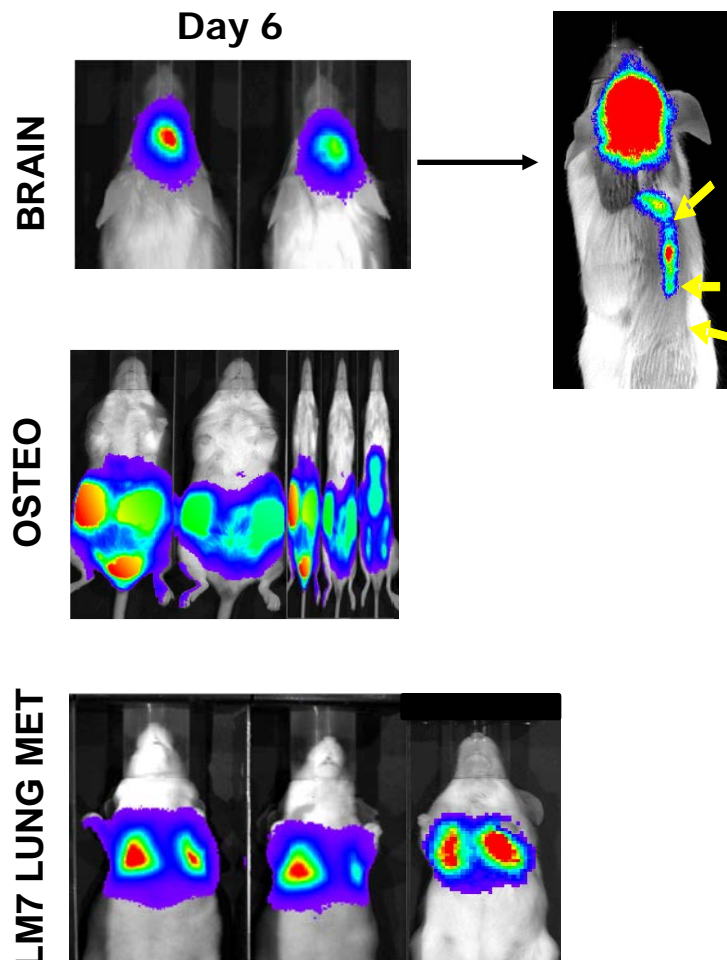
The HER2 CAR



Autologous PDC's



Autologous PDX's





Vita Brawley

HER2 CAR T cells

NT
T cells

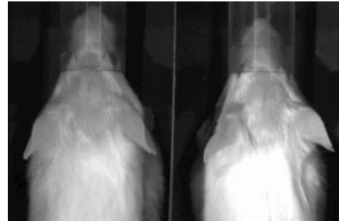
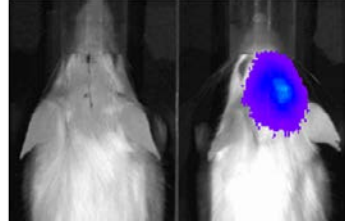
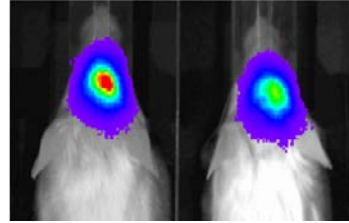
day 6

day 9

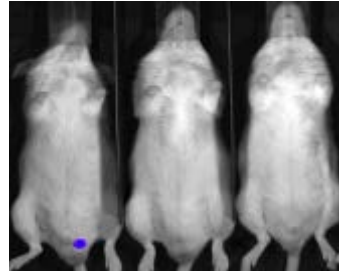
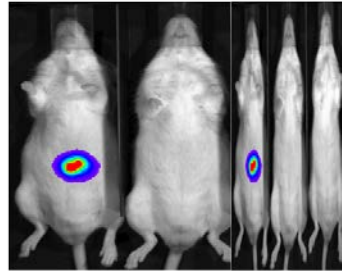
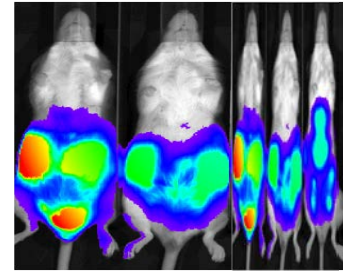
day 15

HER2.CD28.ζ T cells

MB/GBM/EPN



OSTEO



SARC MET

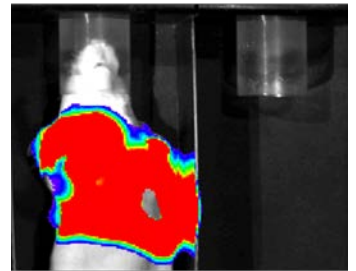
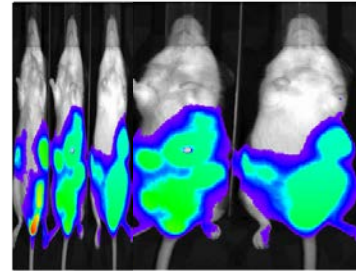
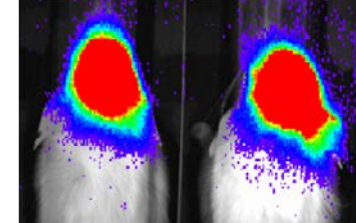
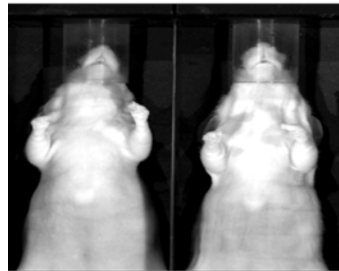
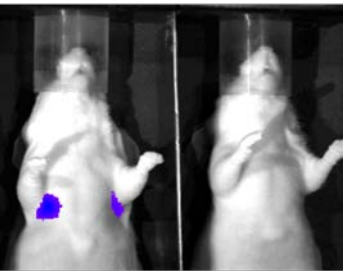
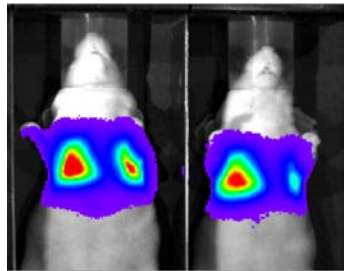
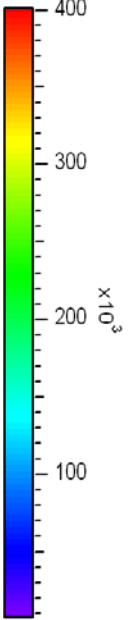


Image
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Max = 1.1131e+06
p/sec/cm²/sr



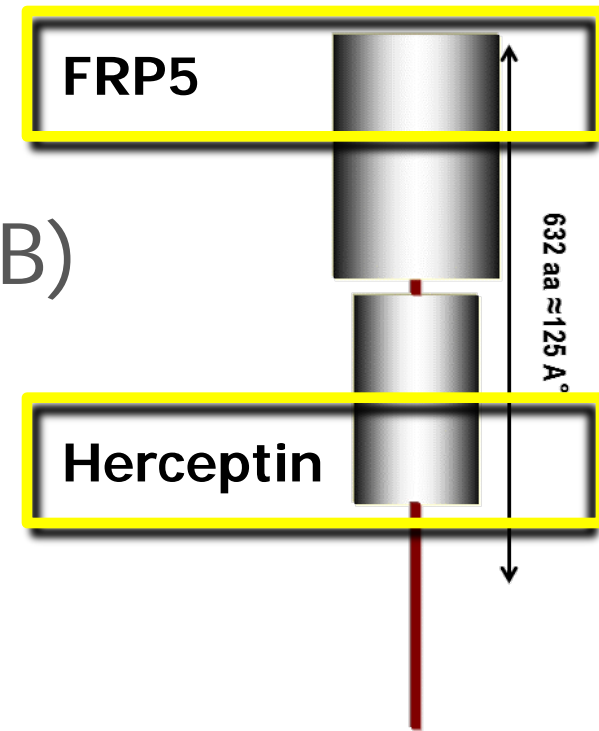
Color Bar
Min = 9180.8
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Fatal Adverse Event

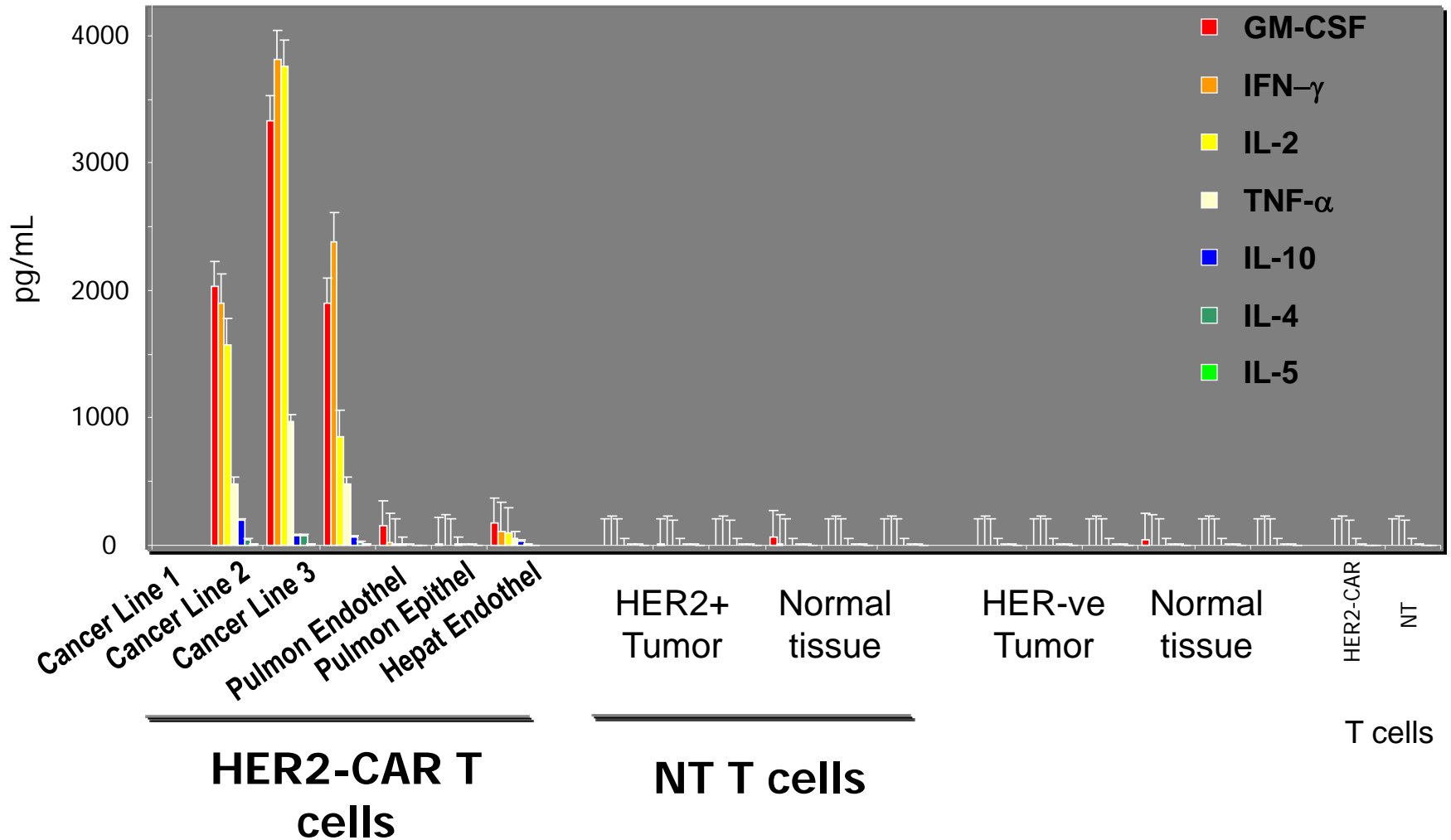
- **Patient:** 39 yr; F; colon cancer metastatic to the lungs and liver
- **Lymphodepletion:** CTX and FLUD
- **Autologous CAR T cells:**
 - CAR based on Trastuzumab (Herceptin®)
 - CD28+4-1BB+CD3.ζ
 - DOSE: 1×10^{10}
- **Adverse Event:** severe dyspnea;
pulmonary edema; respiratory failure;
died day+5
- **Cytokine storm**

A different risk profile

- **DOSE:** 1,000 fold less 1×10^8
- **Trial design:** mCRM escalation
- **Exodomain:** FRP5
- **CAR:** CD28+CD3. ζ (no 41BB)
- **No lympho-depletion**



Specific Cytokine Release



HER2 CAR Trials: *evolution*

2008

2009

2010

2011

2012

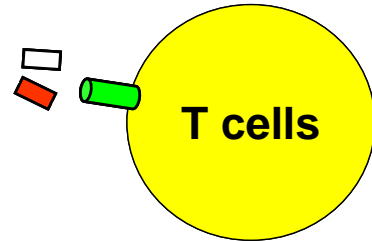
2013

2014

2015

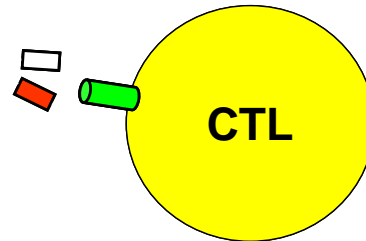
2016

HER2 specific



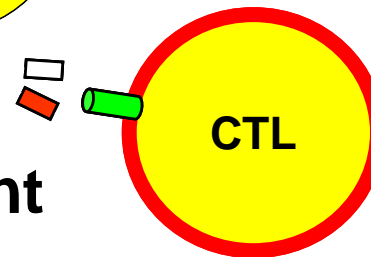
HEROS

+ durable



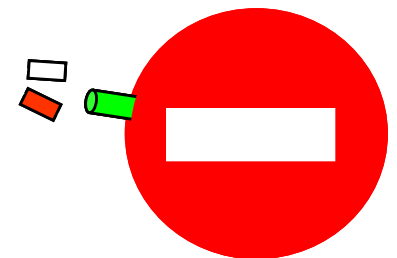
HERT-GBM

+ tumor resistant

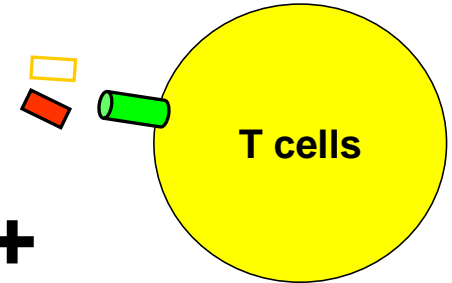


HER-TGFβ

+ stoppable ...



HEROS: *complete*



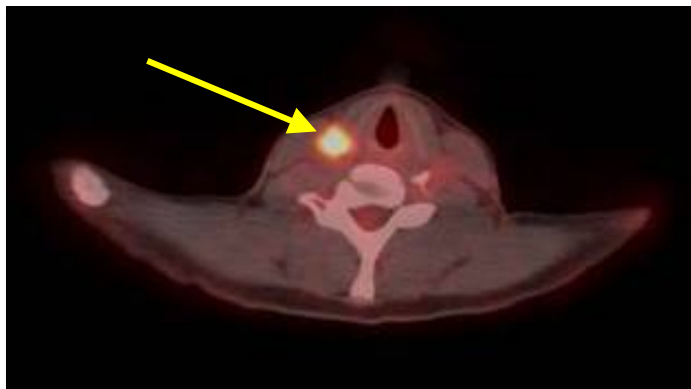
43/55 (~80%) tumors HER2+

Generated 28 cell lines; infused 19

No SAE

Clinically

2 PR; 4 SD (142->600+ days); 13 PD



PREINFUSION

DL6
⇒



POSTINFUSION

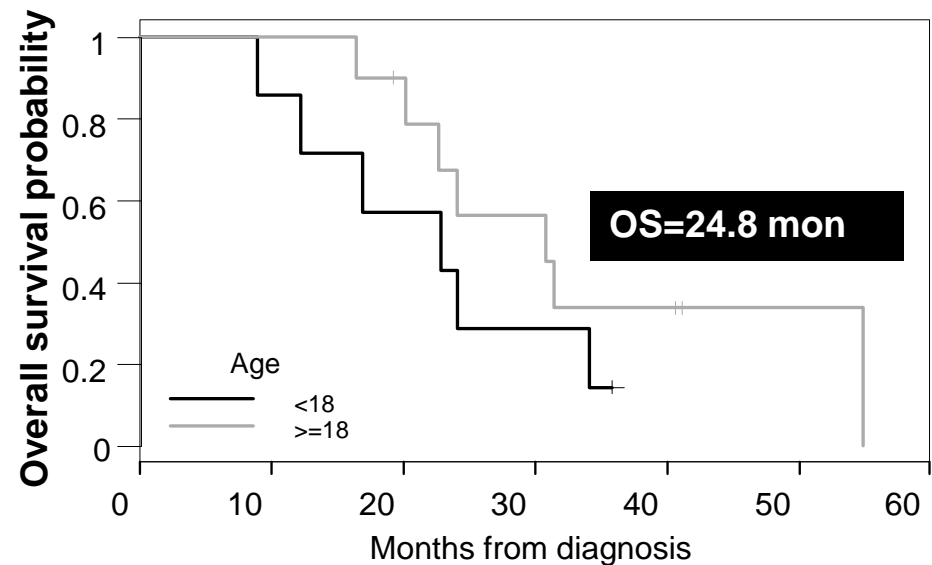
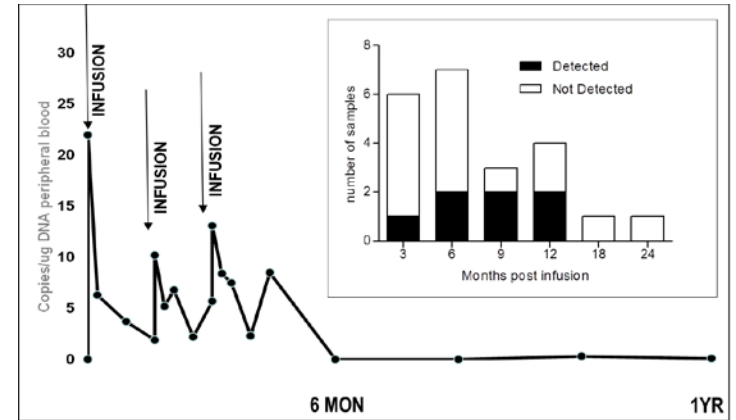
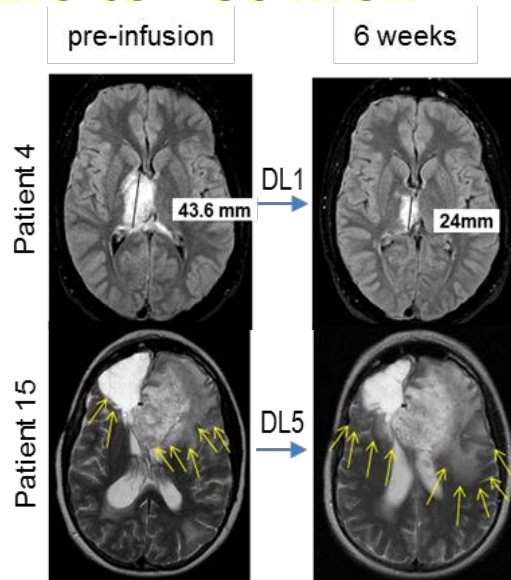
HERT-GBM 17 patients treated

Progressive GBM
No SAE & No CRS
Attrition 40%

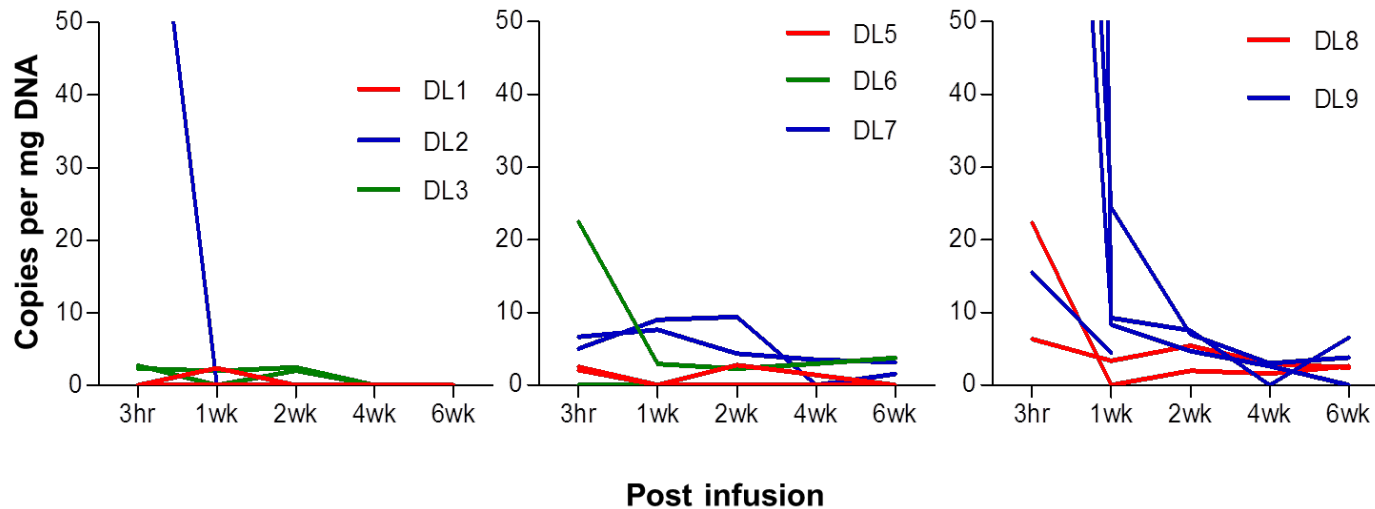
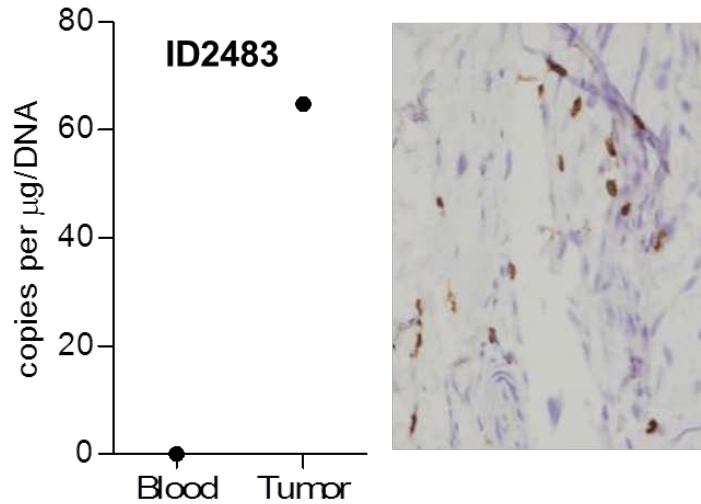
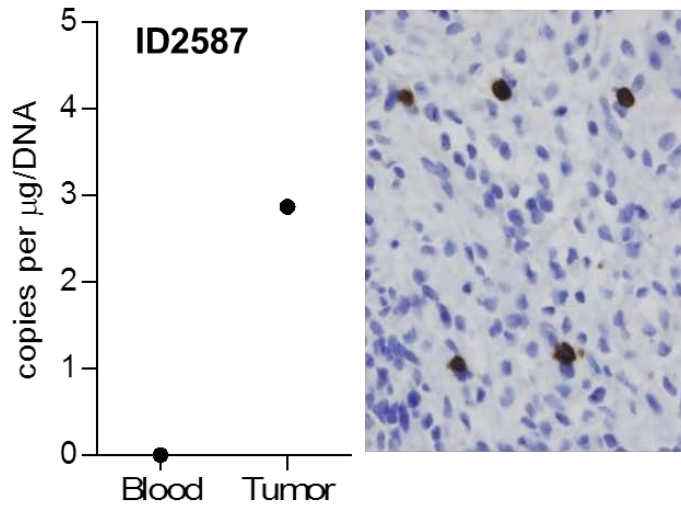
7 SD 2.5 to >30 mon

1 PR

8 PD



T cells home but don't expand



HEROS 2.0

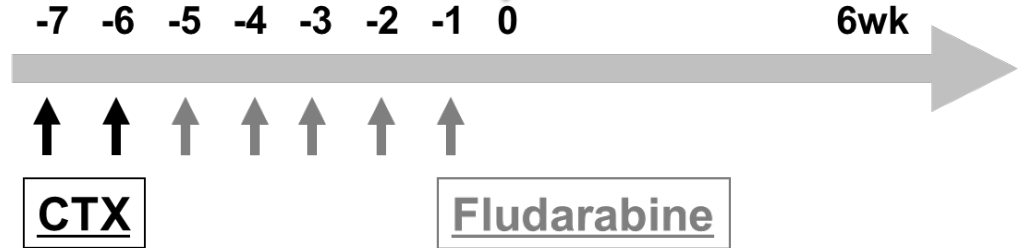
Auto HER2 T cells

$1 \times 10^8 / m^2$



Re-evaluation

6wk

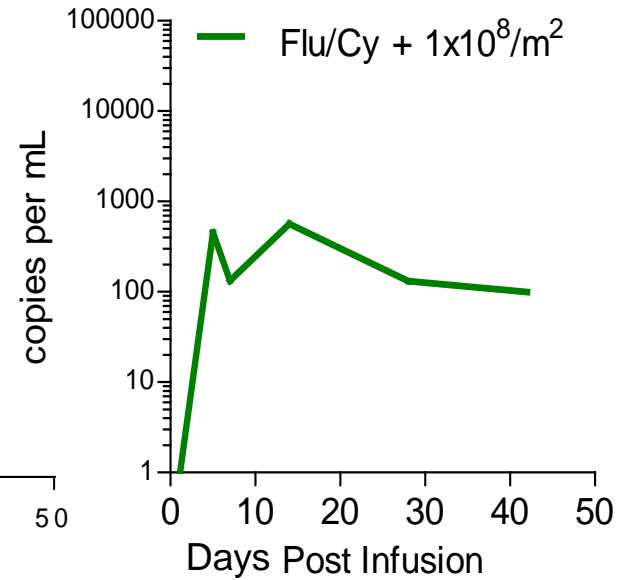
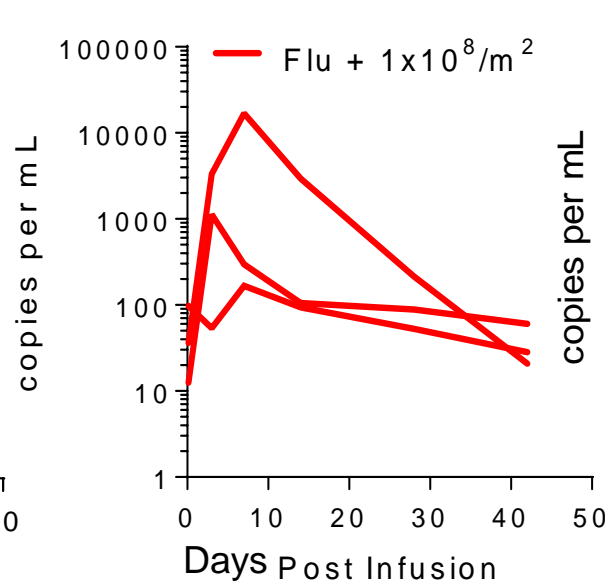
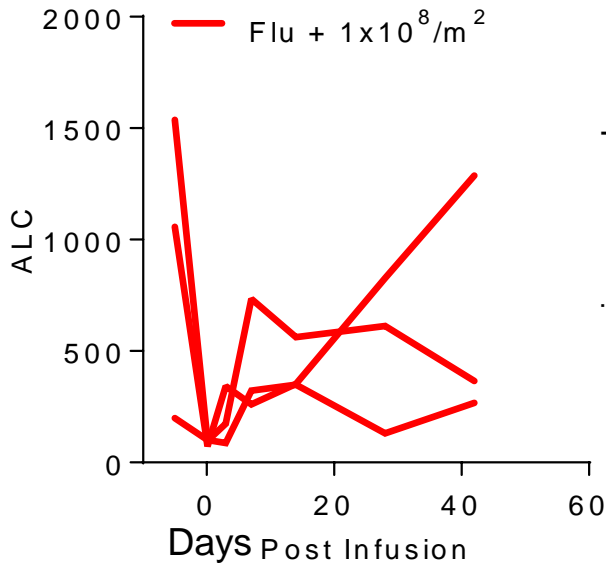


Flu only (3 pts)

Flu + CTX (2 pts)

Lymphodepletion

CART cell Expansion



HEROS 2.0 is currently recruiting

HEROS 2.0

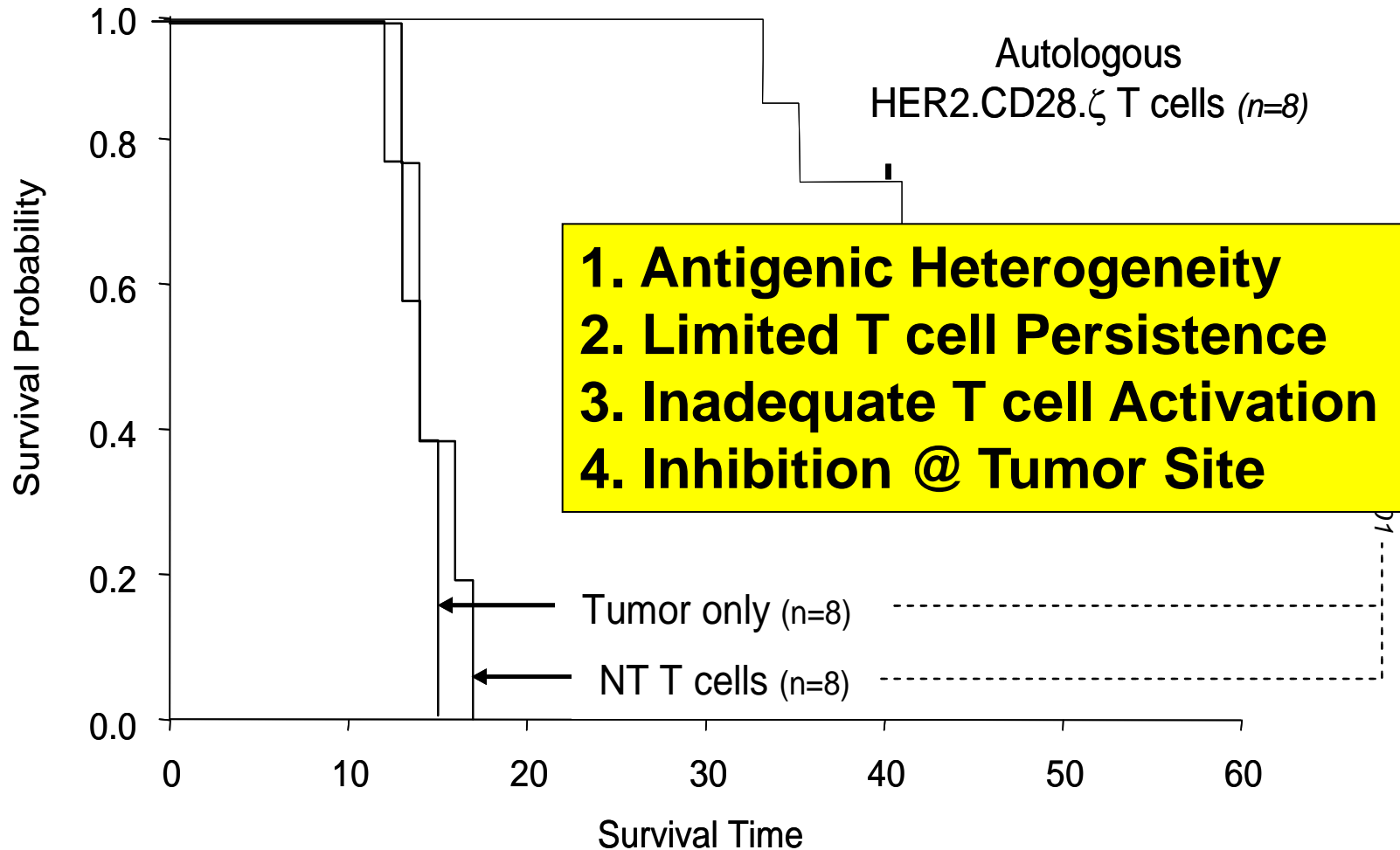
Toxicity:

- **3 patients:** CRS grade 1 (fever + CRP 8.9)
- **1 patient:** CRS grade 2 (fever + CRP 7.2)
 - hypotension (low dose dopamine for ~ 36 hours)
 - transient oxygen requirement

Responses (5 patients):

- **2 patient:** PD
- **2 patients:** SD 8-12 months
- **1 patient:** CR

Why do tumors relapse?





The Great Escape



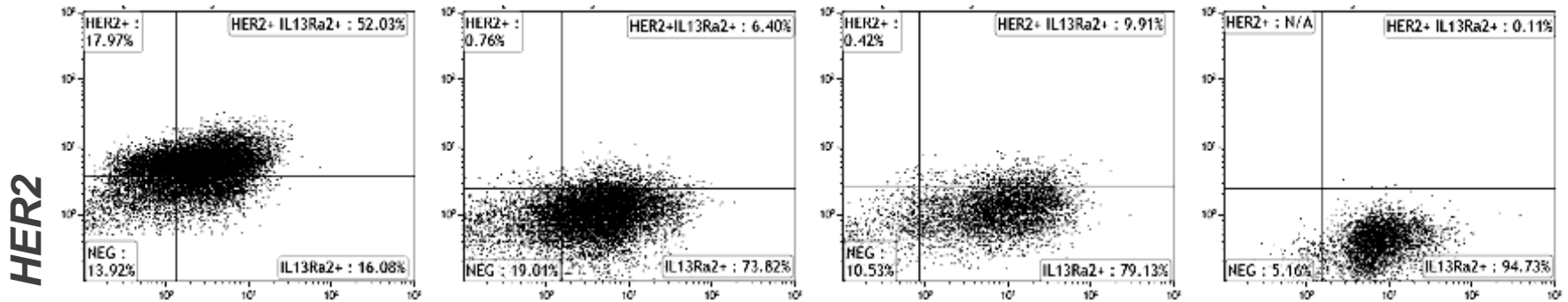
Meena Hegde

Day 1

3

7

14

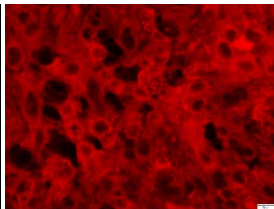
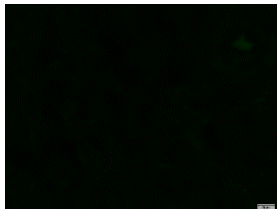
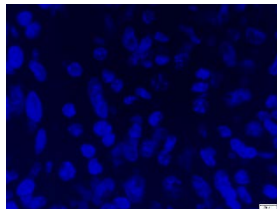
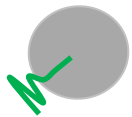


IL13R α 2

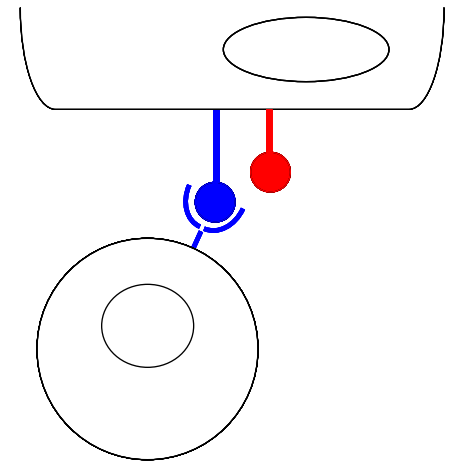
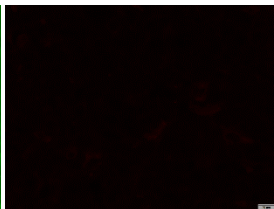
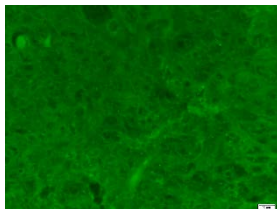
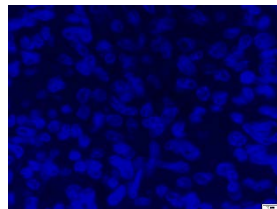
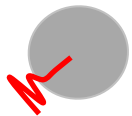
HER2

IL13R α 2

HER2 CAR

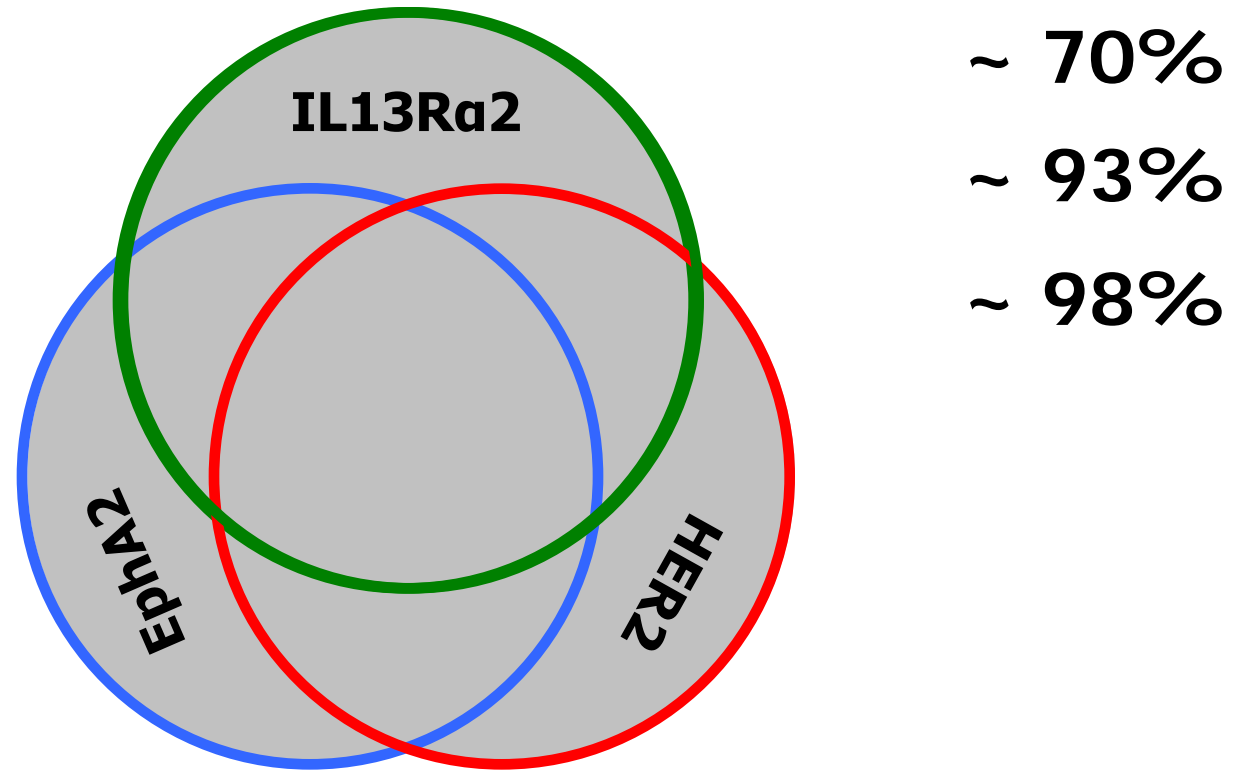


IL13R α 2 CAR



HER2 CAR T cell

GBM: *intratumoral heterogeneity*

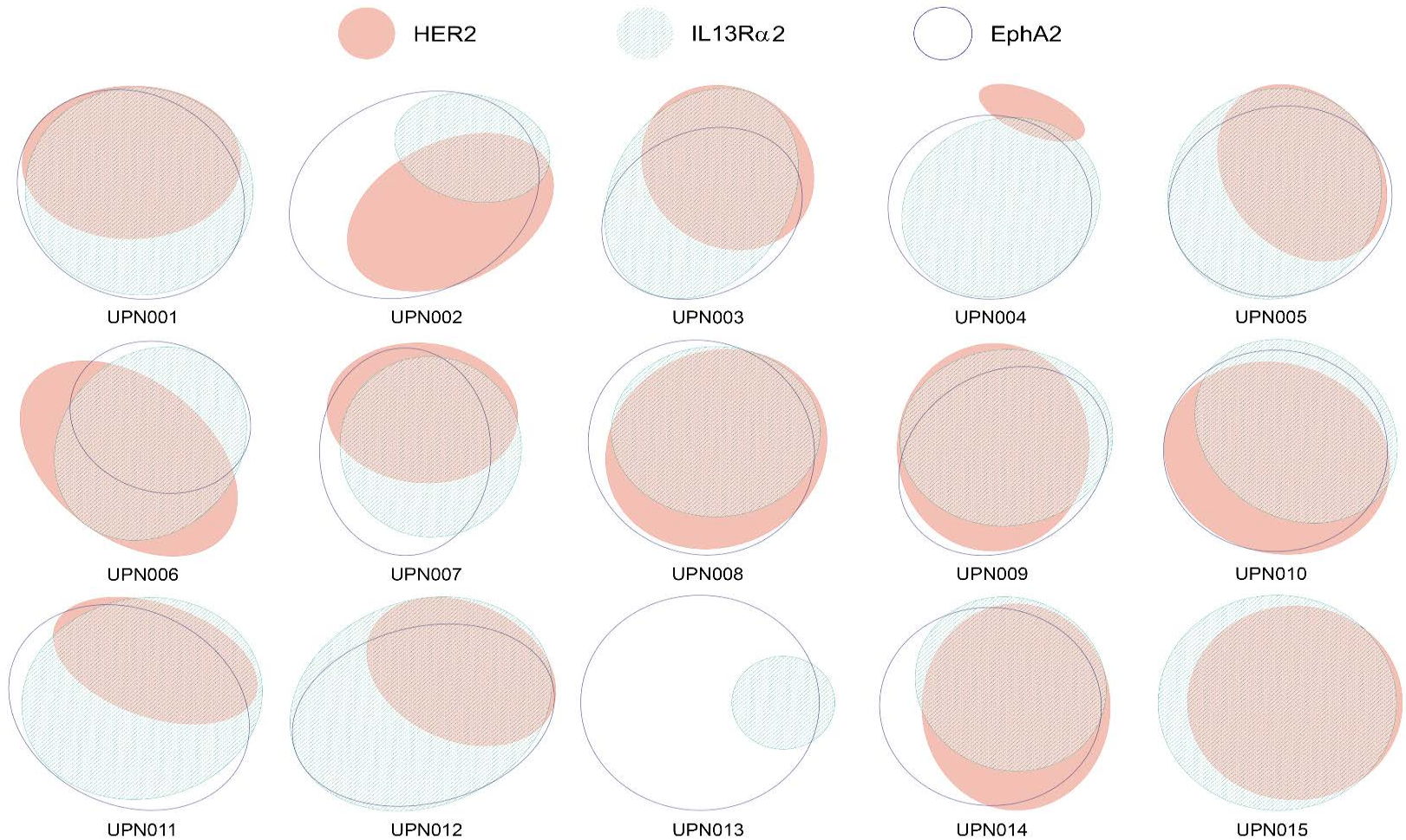


20 GBM (surgical excision)

Flowcytometry on $\geq 100,000$ cells

IFC on 5+ cuts; 6+ hpf; 200+ cells/hpf

GBM: *intertumoral heterogeneity*



15 SAMPLES; MULTIPLE PIECES POOLED; 100,000-200,000 FLOW EVENTS

Hypothesis



Possible advantages:

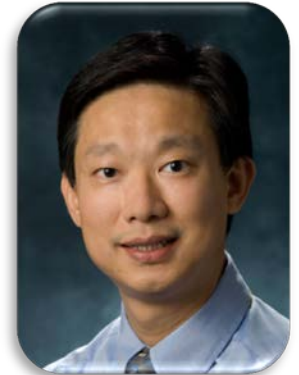
Circumvent tumor escape

- Heterogenous expression of target
- Down regulation of target
- Antigen loss variants

Improve T cell activation



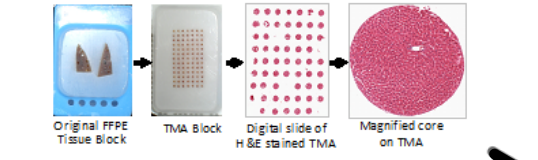
Tumor Profile



Chris Man

ES	MB	OS
ROR ₁	ROR ₂	SFRP ₁
MUC ₁	PDPN	ROR ₂
STEAP ₁	FCER ₂	PDPN
SFRP ₁	SFRP ₁	NCAM ₁
NCAM ₁	ROR ₁	MUC ₁₆
L1CAM	NCAM ₁	L1CAM
IL ₁₁ RA	KDR	KDR
FOLH ₁	IL ₁₃ RA ₂	IL ₁₃ RA ₂
CLDN ₁	CLDN ₁	EPHA ₂
CD ₂₄₈	BCHE	CSPG ₄
BCHE	ALK	CD ₂₄₈
ALK	ADAM ₁₂	CA ₉
ADAM ₁₂		BCHE
		ANTXR ₁
		ADAM ₁₂

250 OS SETS



+



MINIMUM
UNIVERSAL
PROFILE

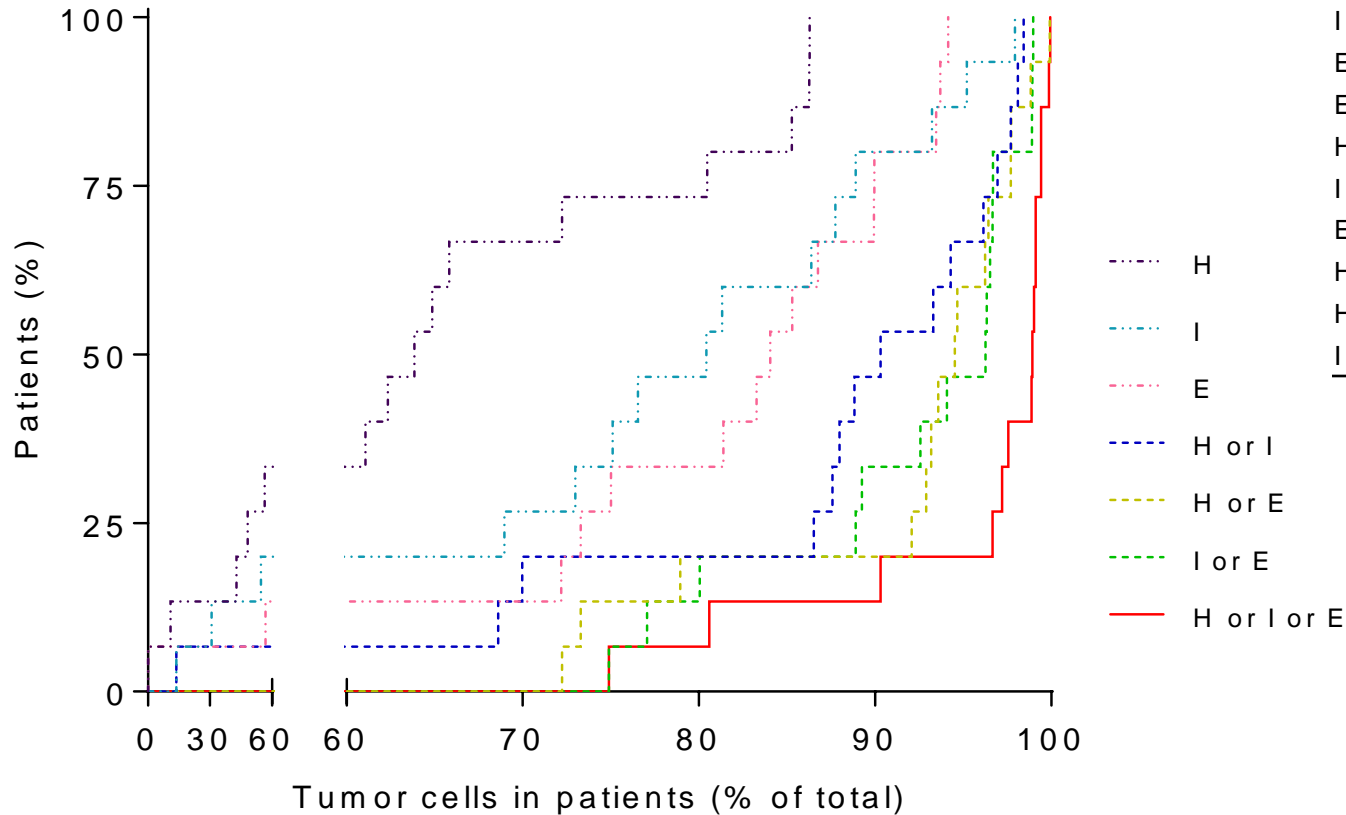


Kevin B

Tending to 100%

Routine

Analysis	P Value
H vs. H or I	<.0001
H vs. H or E	0.0001
I vs. H or I	0.0001
I vs. I or E	0.0001
E vs. H or E	0.0001
E vs. I or E	<.0001
H vs. H or I or E	<.0001
I vs. H or I or E	<.0001
E vs. H or I or E	<.0001
H or I vs. H or I or E	0.0001
H or E vs. H or I or E	<.0001
I or E vs. H or I or E	0.0001

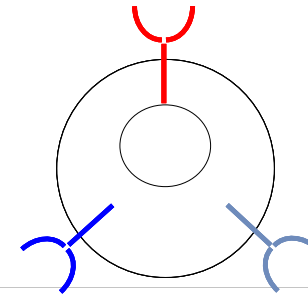




5' UTR

Kevin B

UCAR T cells



U.CAR

Insert 4.6 kb

2000

4000

6000

Accl

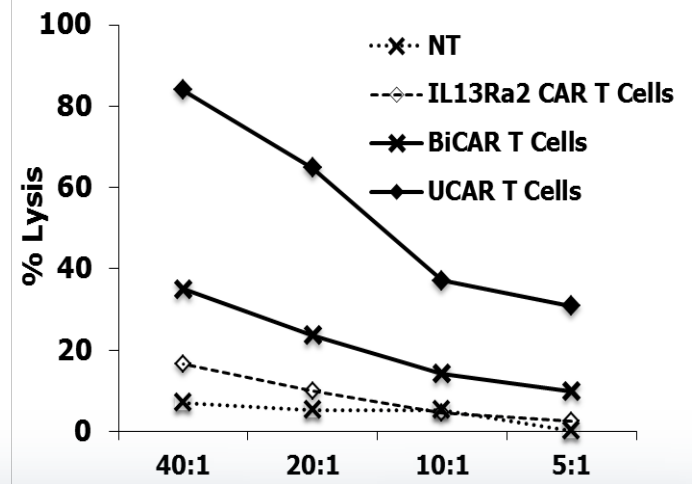
HER2

IL13R α 2

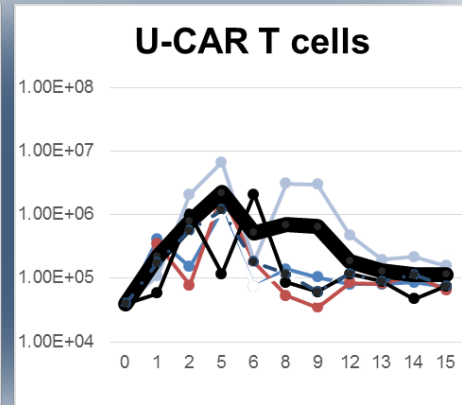
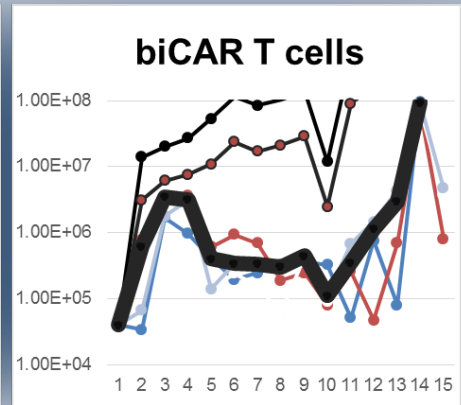
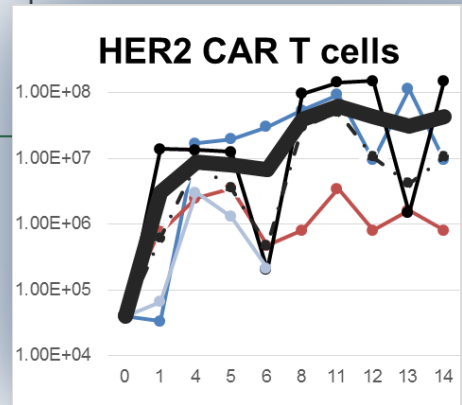
EphA2

Autologous GBM PDXs 1:20

Patient 001 Glioma



Glioblastoma
Ependymoma
Medulloblastoma

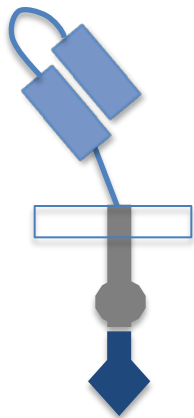




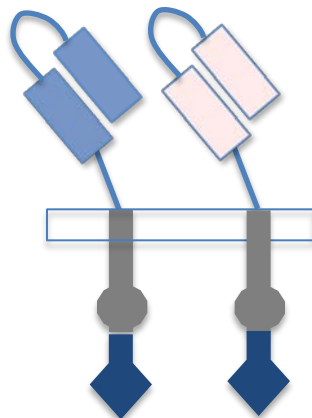
Smart CARs



The Boolean Logic in Action

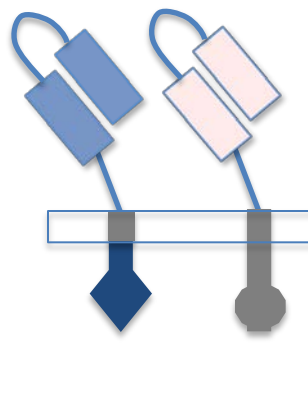


CAR T cell



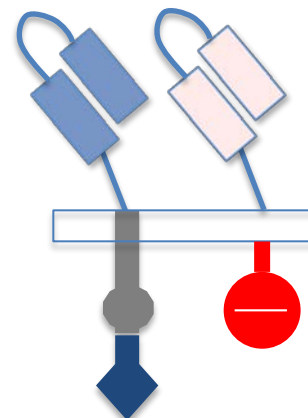
Multi-CAR
T cell

OR



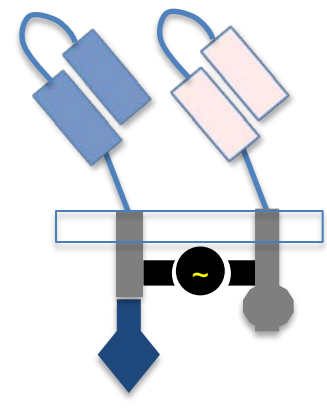
Conditional
CAR T cell

AND



Gated-CAR
T cell
(iCAR)

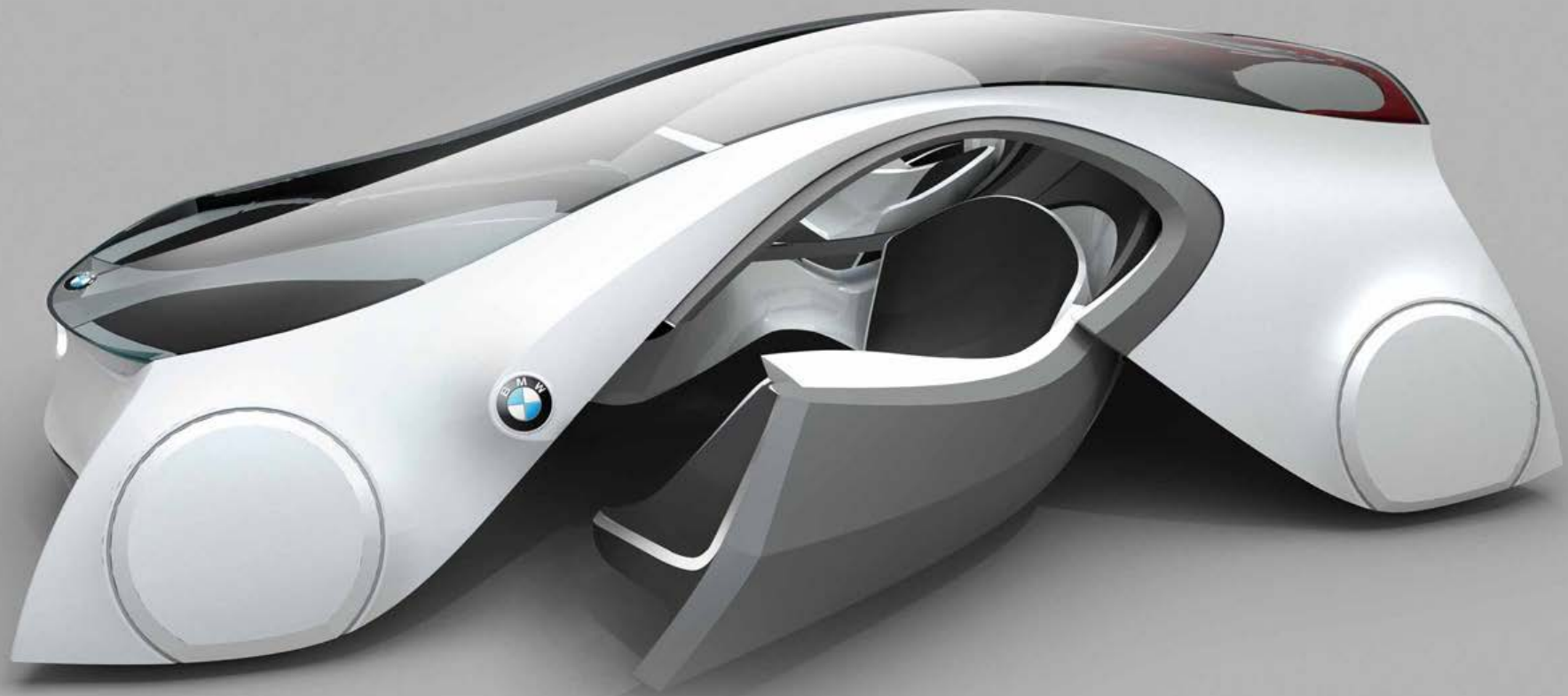
NOT



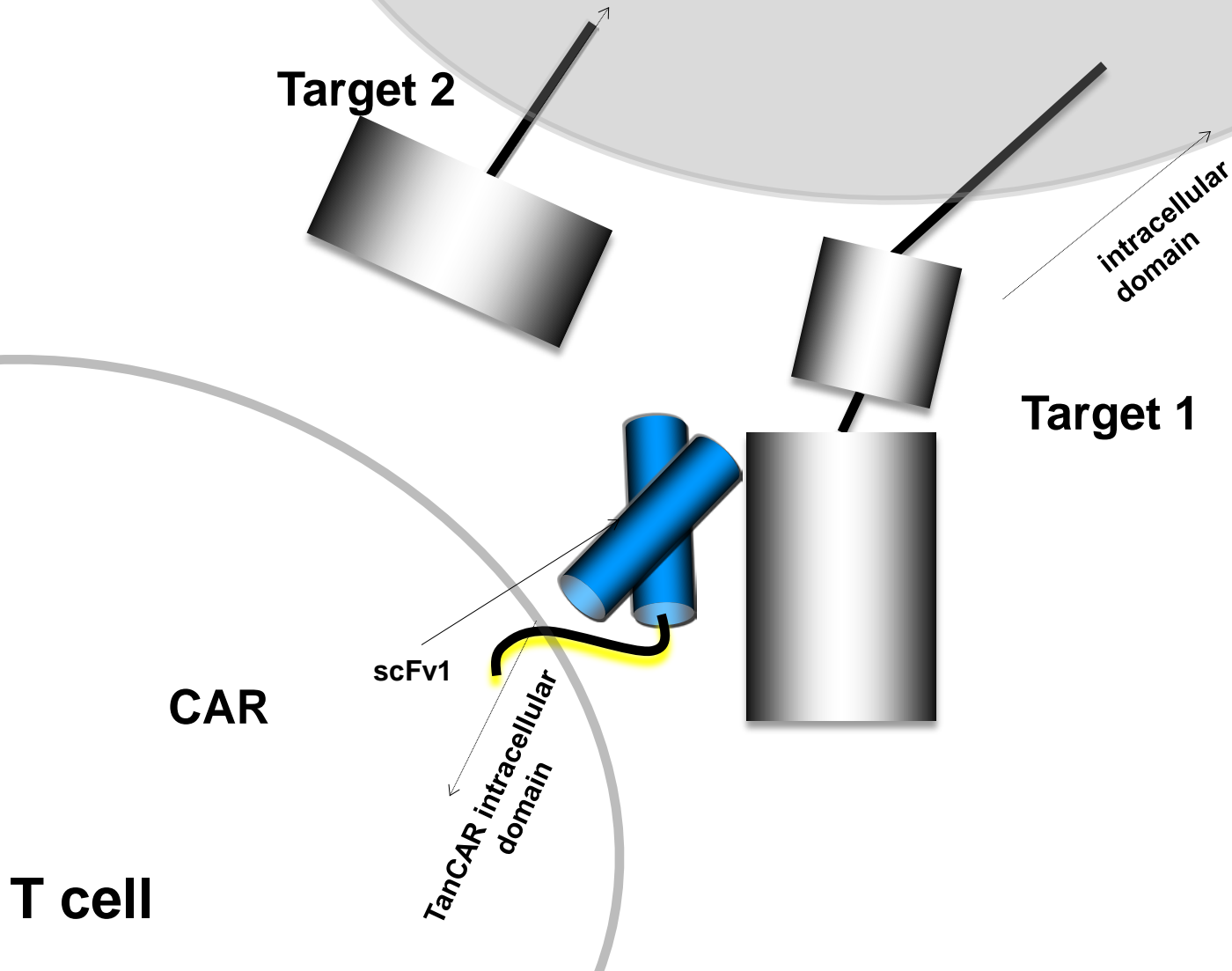
On/Off
CAR T cell
(GoCART)

PRN

Concept CARs



in silico Design: the virtual world



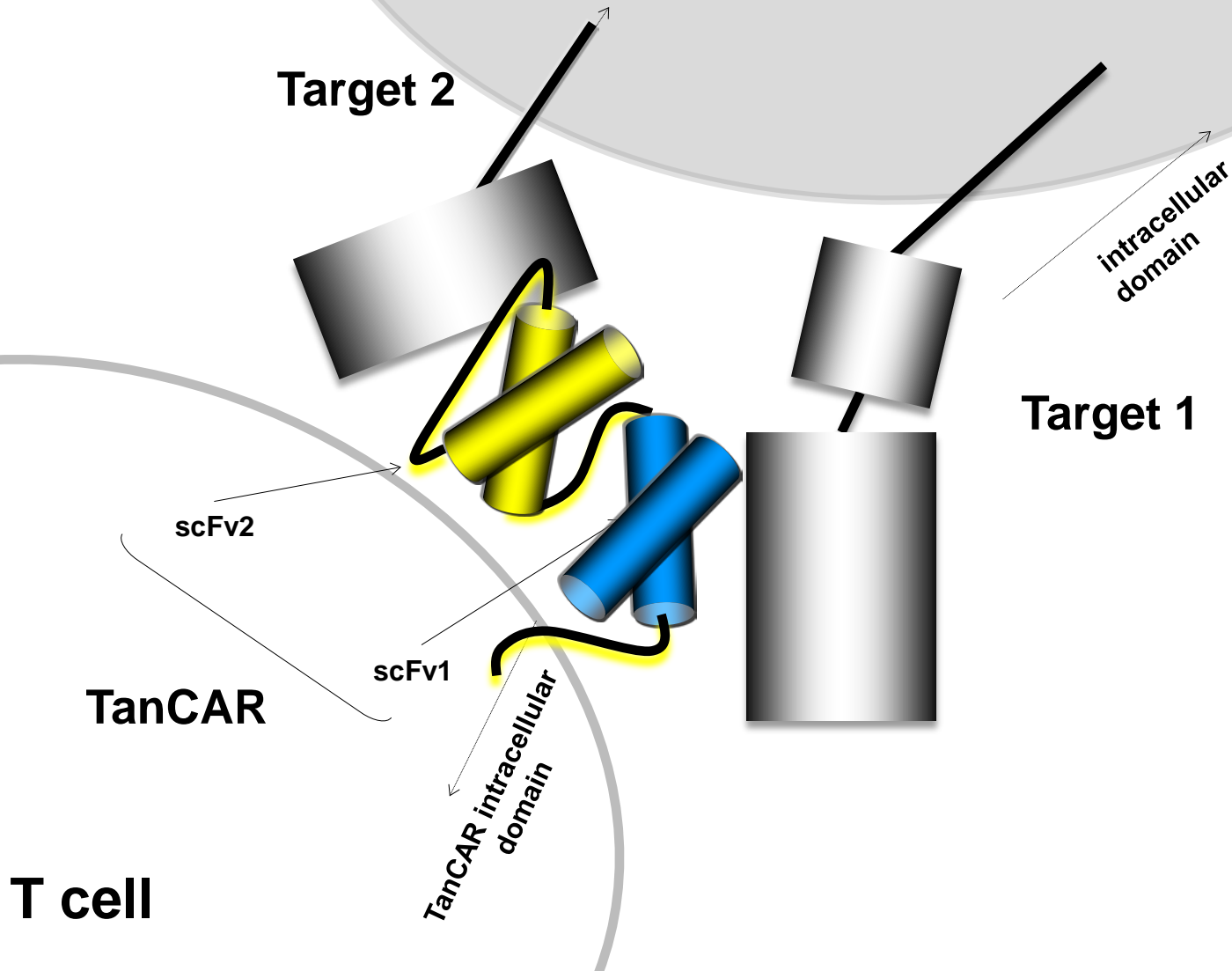
Zakaria Grada



Meena Hegde



in silico Design: the virtual world



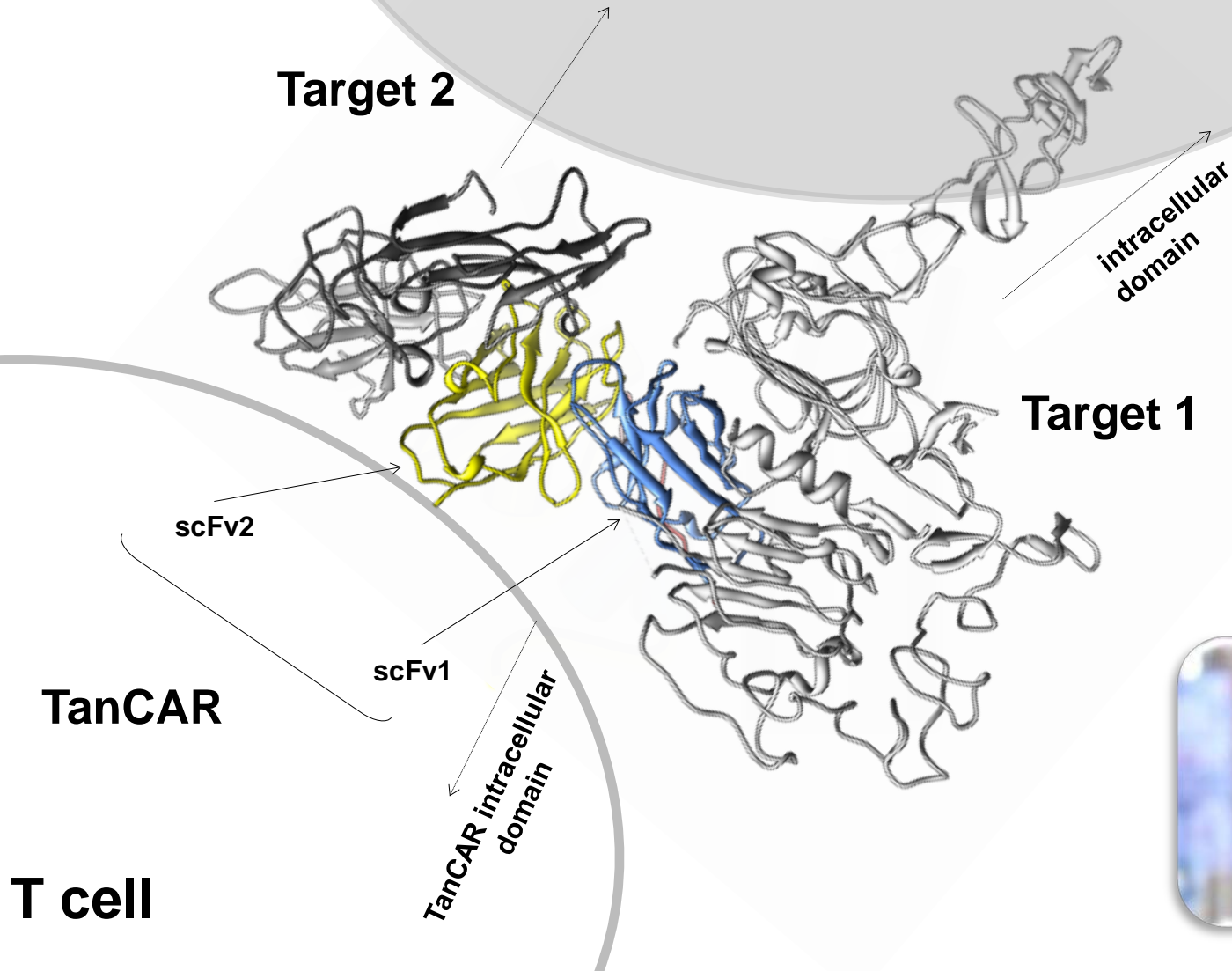
Zakaria Grada



Meena Hegde



in silico Design: the virtual world

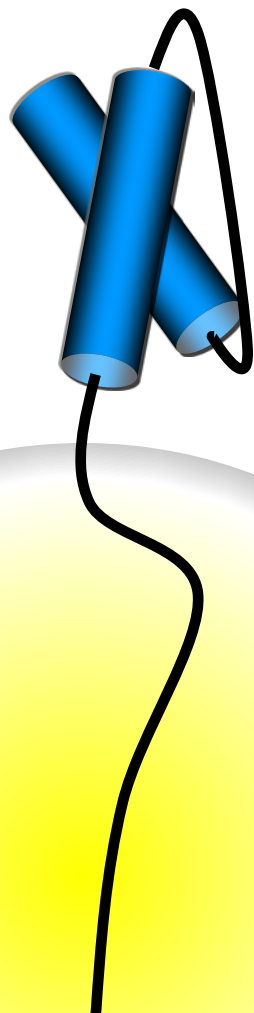


Matthew Baker

Imaging Toolbox: *exodomain*

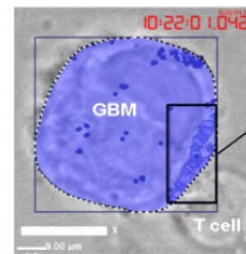


Maini Mukherjee

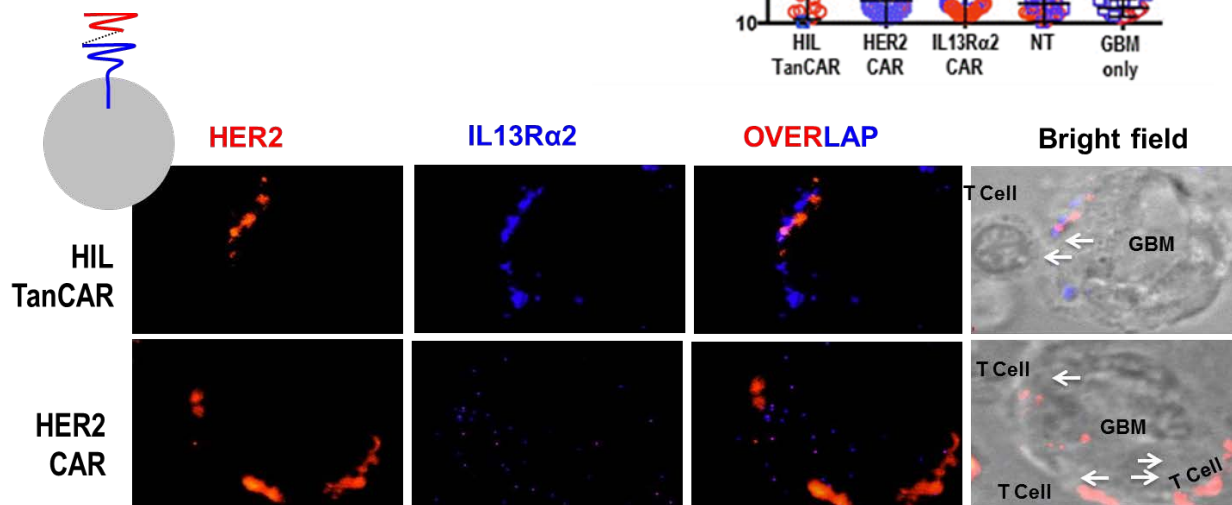
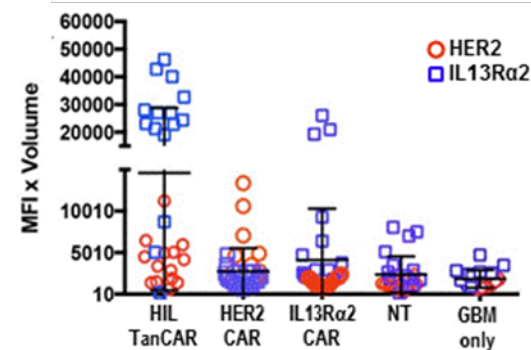


CAR Exodomain

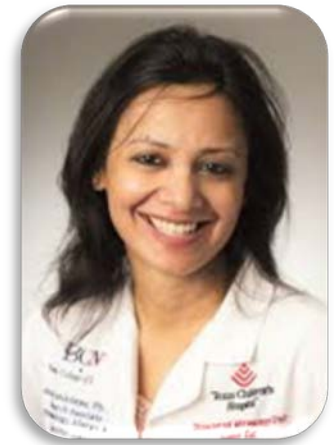
- Ligand Aggregates at IS
- CAR Aggregates at IS
- Synapse Properties
 - F-Actin
 - LFA-1



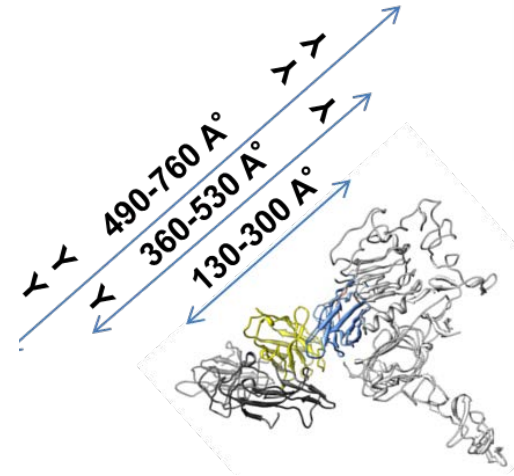
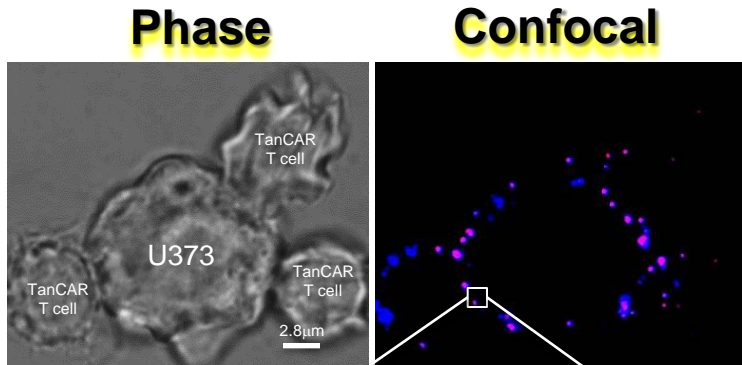
Immune Synapse



bi valency



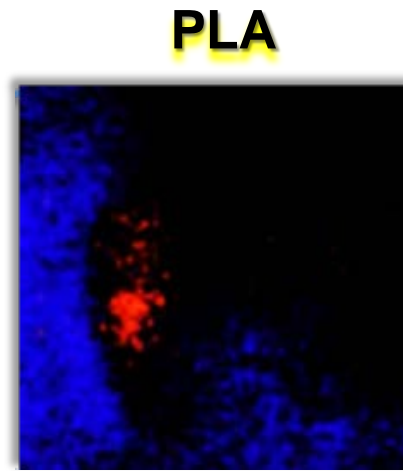
Malini Mukherjee



<3000Å°



<1500Å°

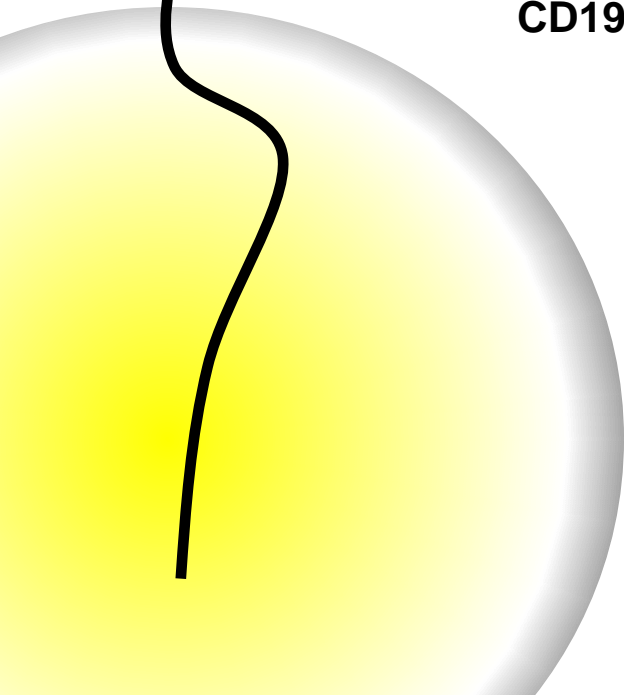
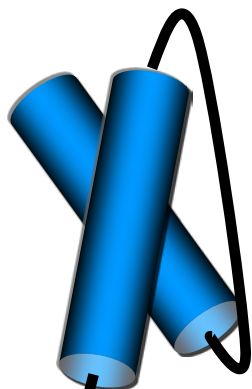


<400Å°

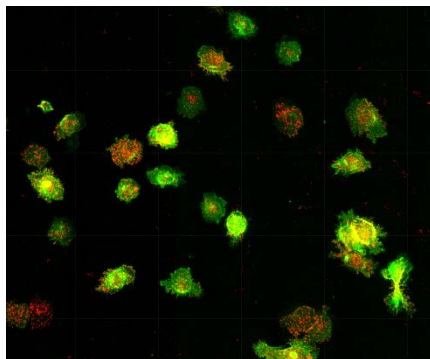


<80Å°

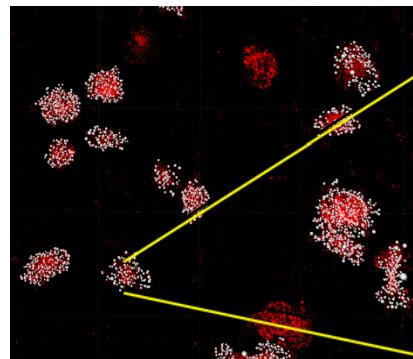
Imaging Toolbox: *endodomain*



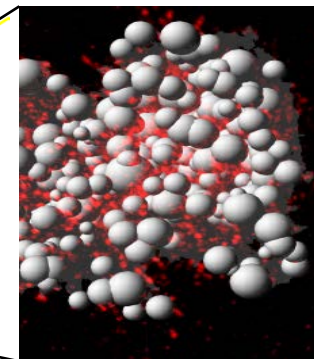
pLck



CD19.28z CAR GFP
T cells



Imaris® Rendition

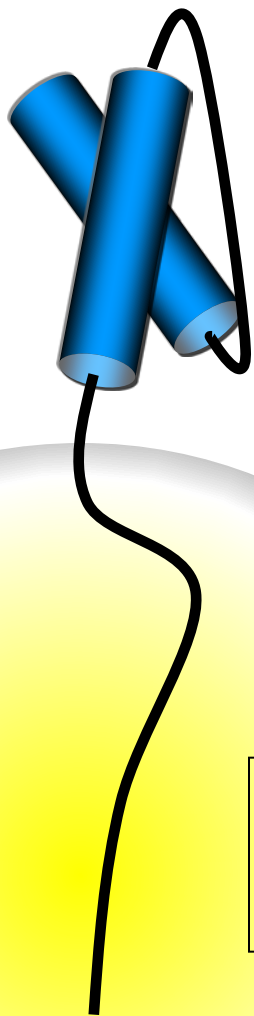


CAR Signaling

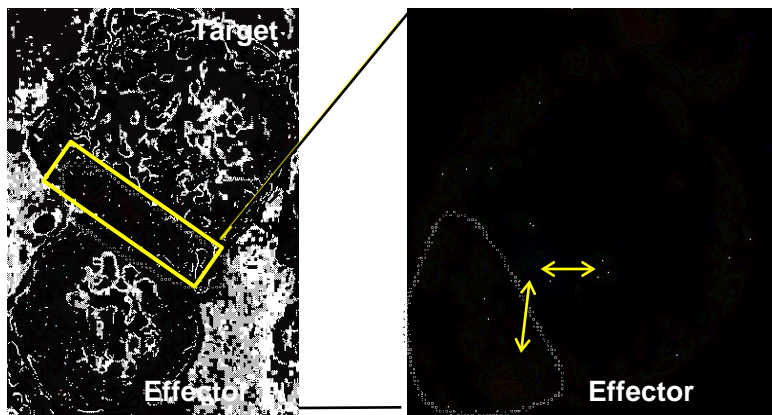
- pLck
- pZap70

CD45
CD4/CD8.

Imaging Toolbox: *killing machinery*

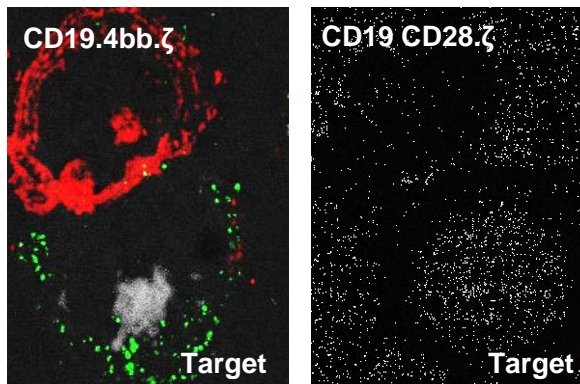


Immunological Synapse

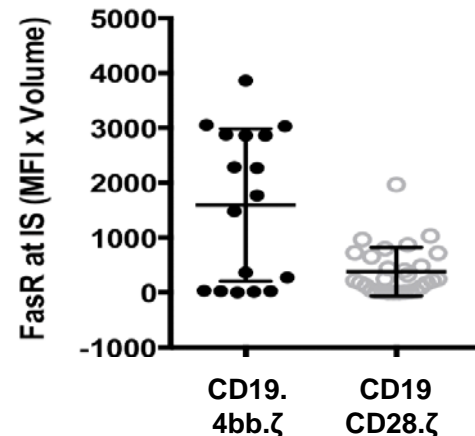


F-Actin, **Perforin**, **Pericentrin**

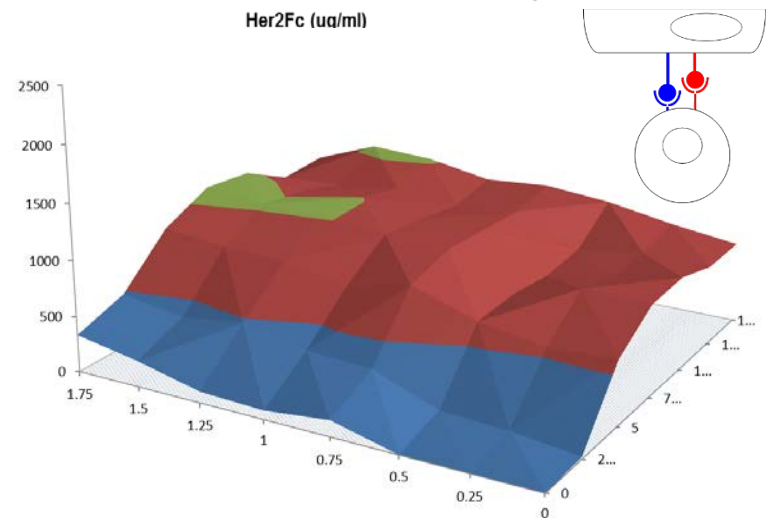
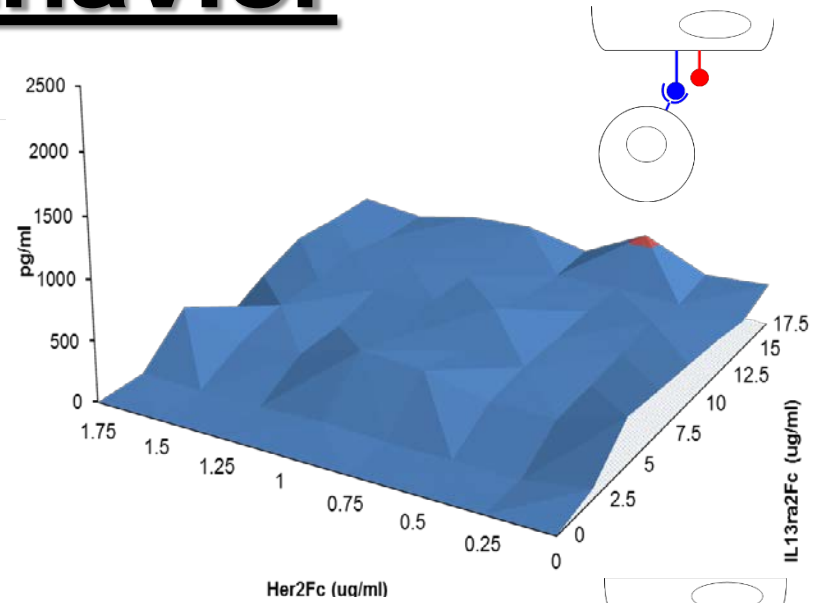
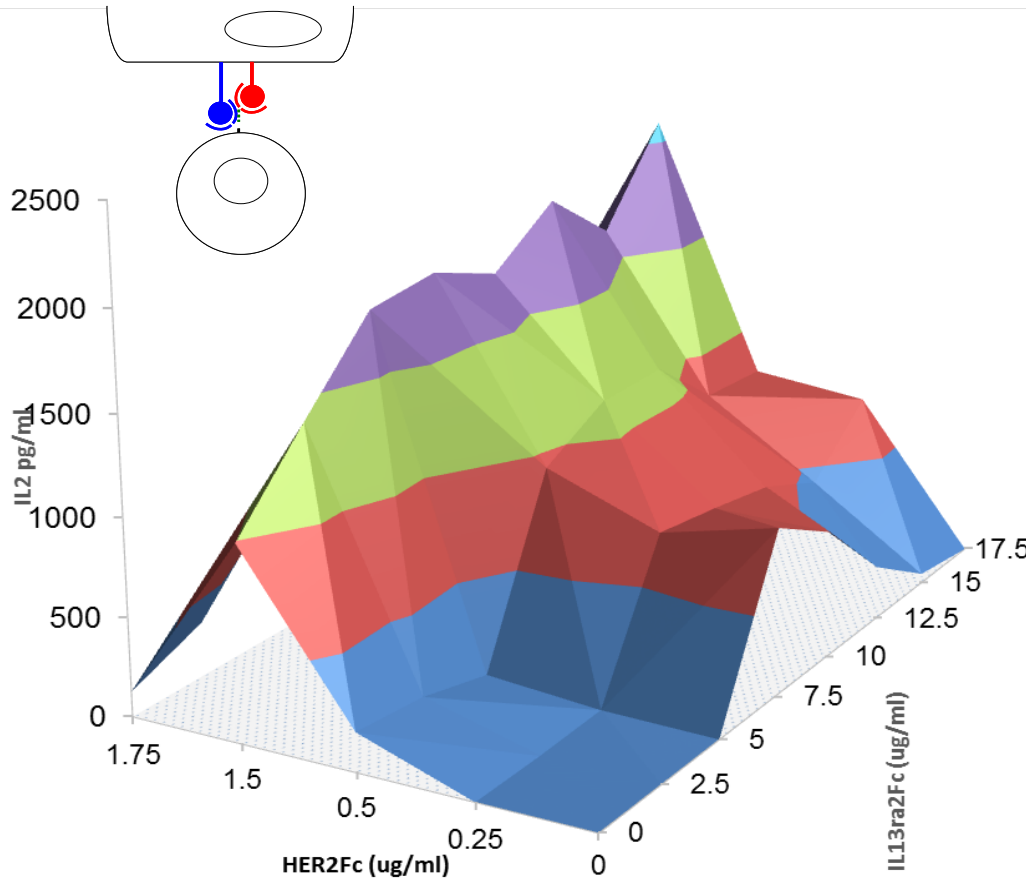
Death Pathway
 - Fas
 - Perforin



Fas receptor **Fas Ligand**



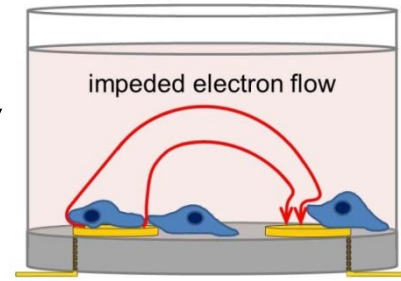
TanCAR behavior



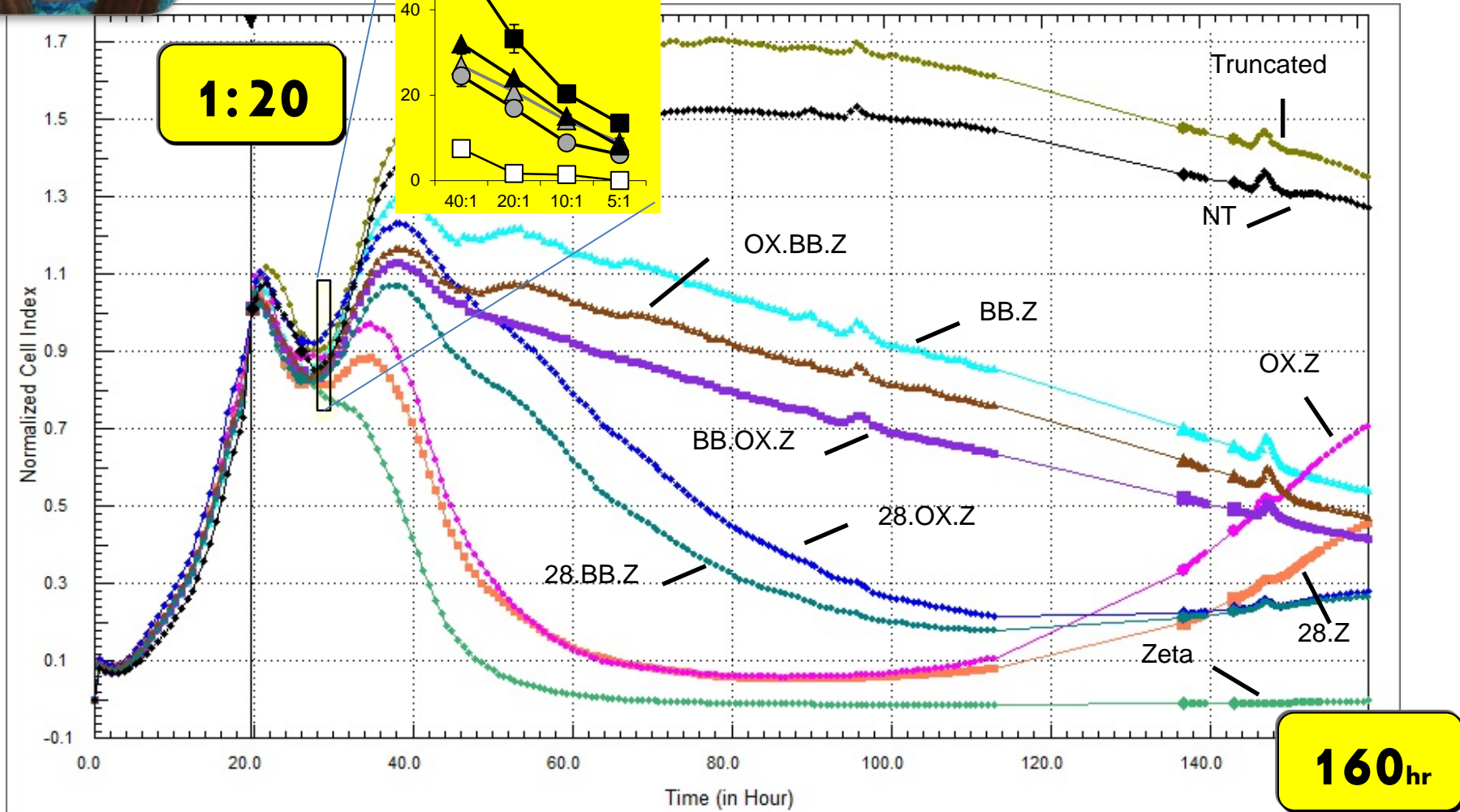


Kristen Fousek

Endodomain "fitting"

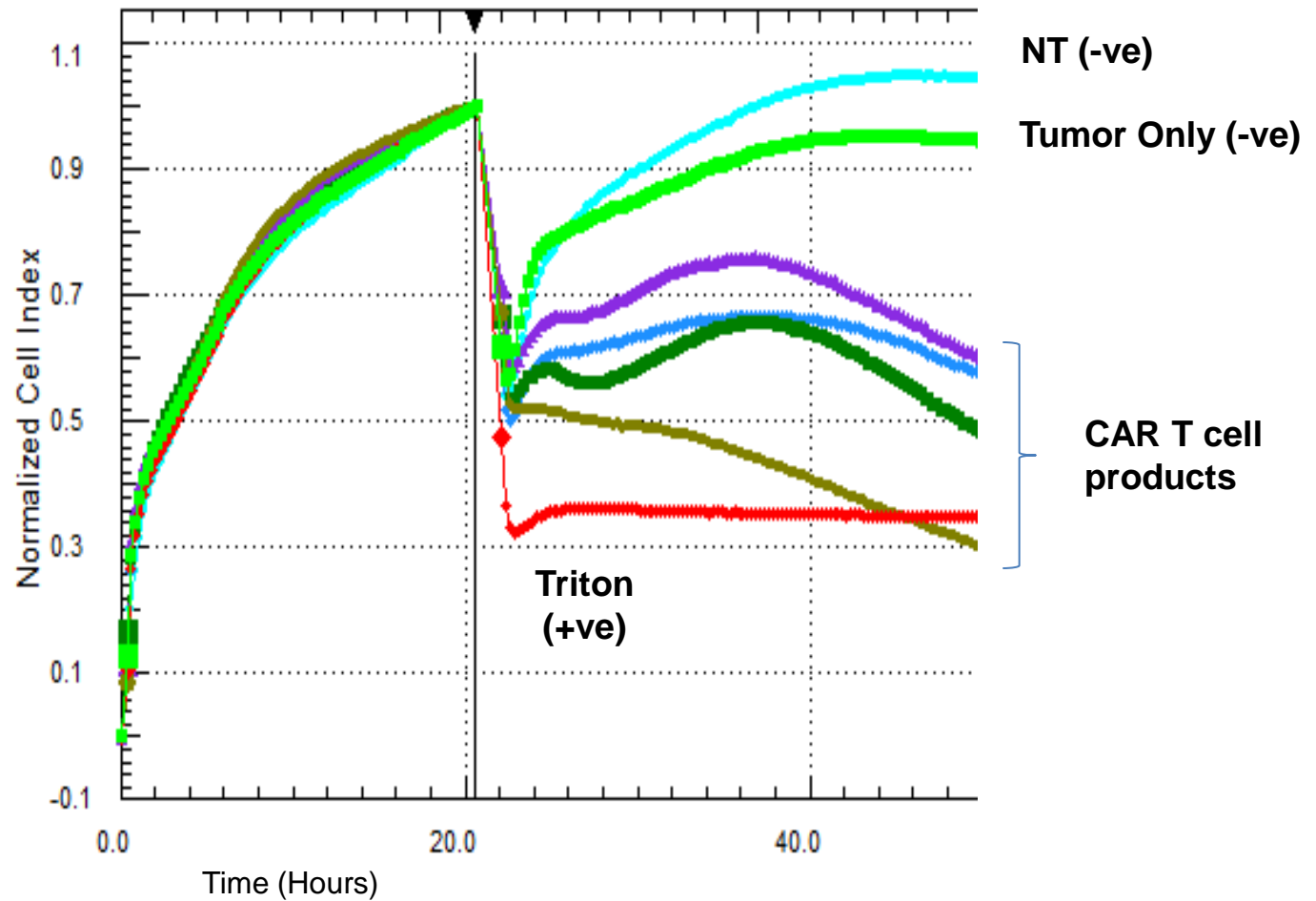


U373-GBM

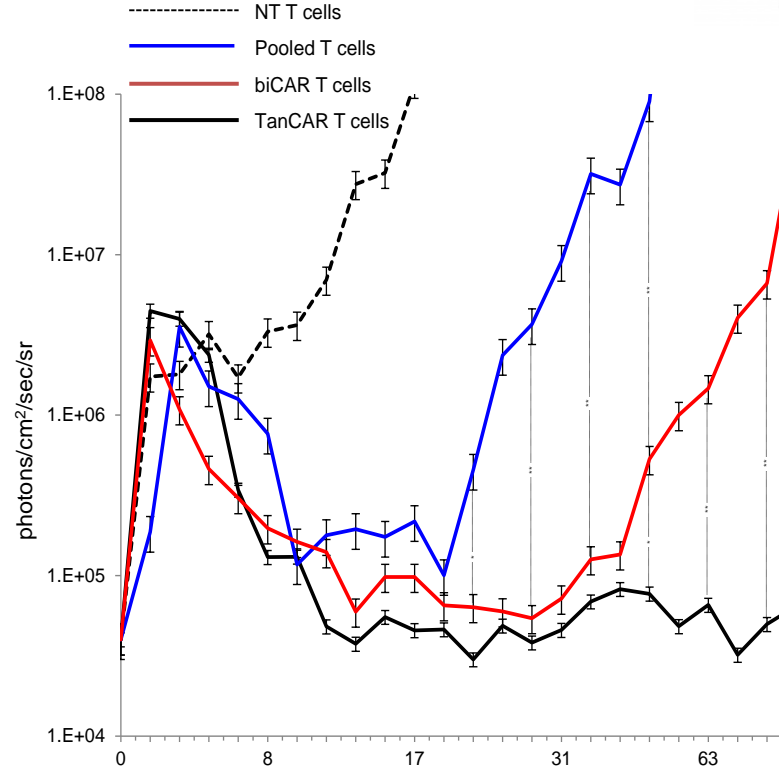
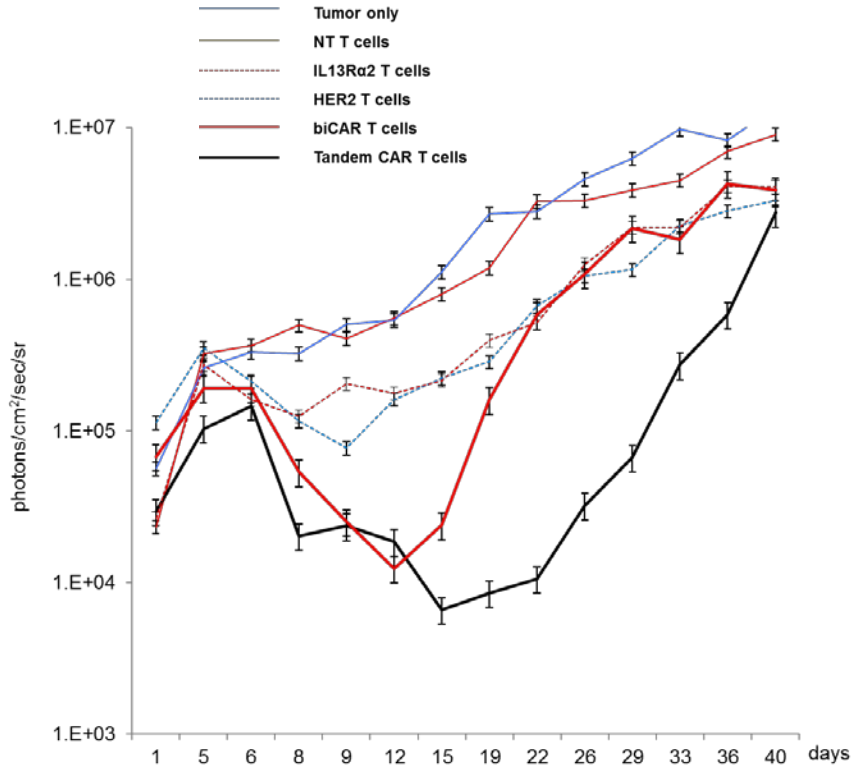


... & now **NON** adherent cells

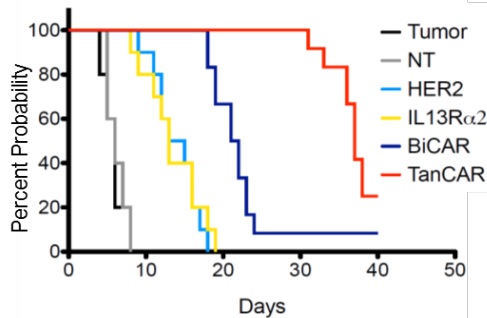
Daudi



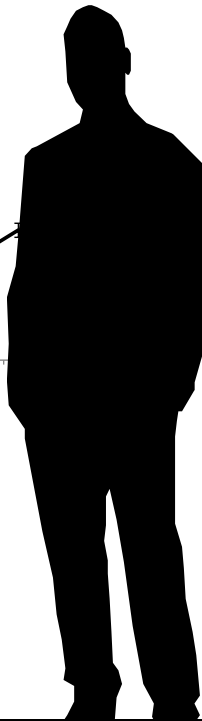
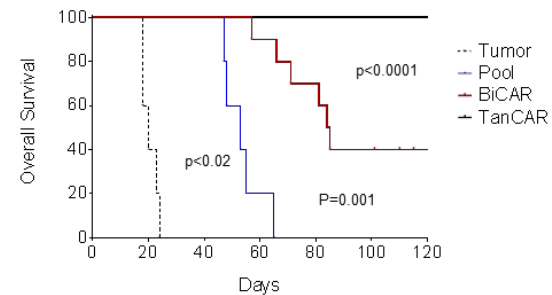
of Surrealism & Realism

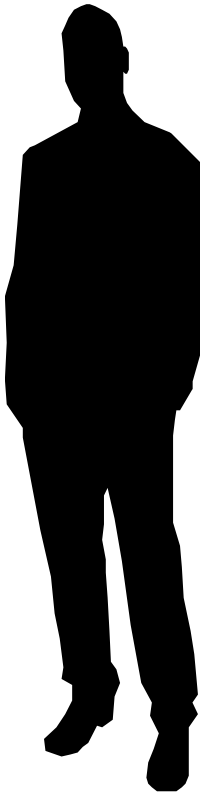


1:30



1:3



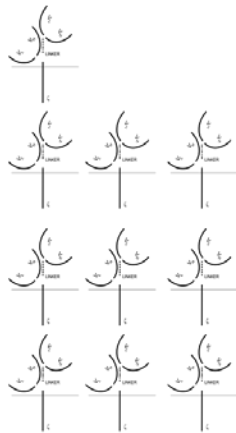


Preclinical CAR Assembly Line

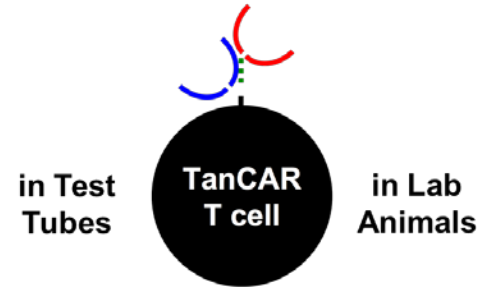
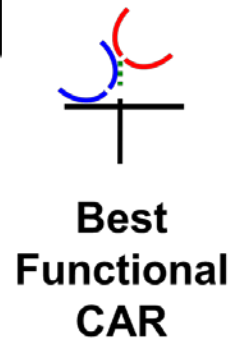
Target(s)



Simulation

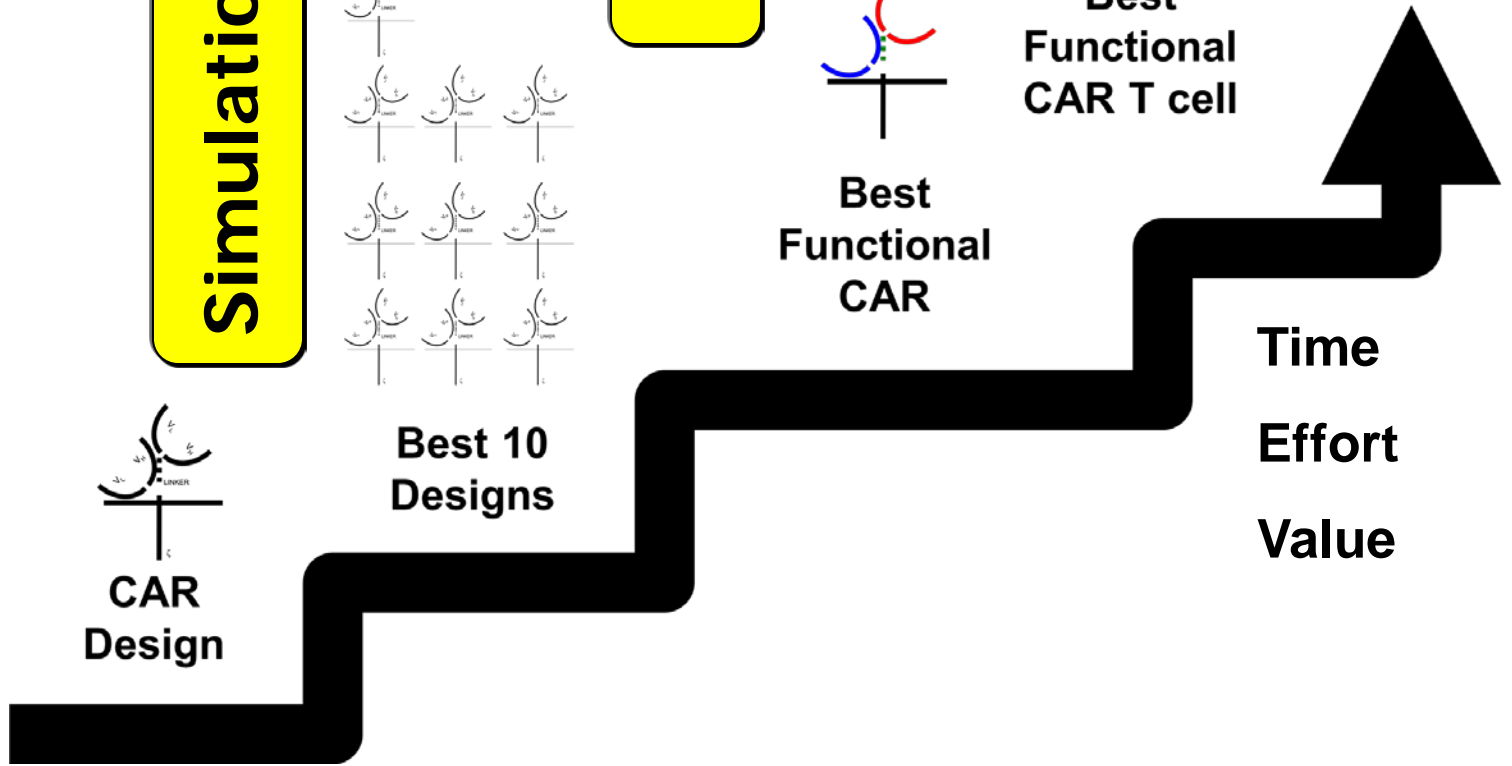


Function



Best Functional CAR T cell

Time
Effort
Value



Look how far you have fallen THEN repent!

Revelation 2:5

1. Empiric preclinical to clinical development of ^{HER2}CAR T-cells
2. Rational development of novel CARs

SILICO > VITRO > VIVO

3. Realistic in preclinical testing
4. Unresolved: modeling toxicity

Acknowledgments

THE PATIENTS

Center for Cell and Gene Therapy
Baylor College of Medicine

Stephen Gottschalk
Helen Heslop
Malcolm Brenner

Baylor College of Medicine
Texas Children's Hospital
The Methodist

MD Anderson Cancer Center-UT (P Anderson,
E Kleinerman)

The Methodist Research Institute (Y. Kew, R.
Grossman, SZ Powell, D Baskin, J Zhang)

Children's Mercy and PACT (Doug Meyers)

The National Cancer Institute (B St Croix)

University of Florida (B Fletcher)

Georg Speyer Haus (J Koch; W Wels)





Tiara Byrd

TEM8 in TNBC

