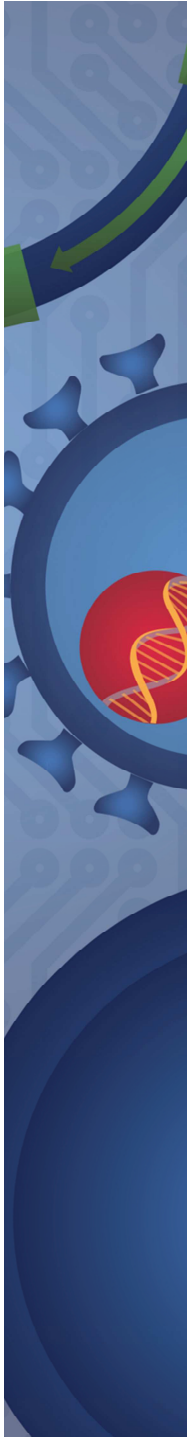


SITC 2014

Enhancing the IQ of CAR T Cells

Michael Jensen, MD
Sinegal Endowed Professor of Pediatrics, UWSOM
Director, Ben Towne Center for Childhood Cancer Research



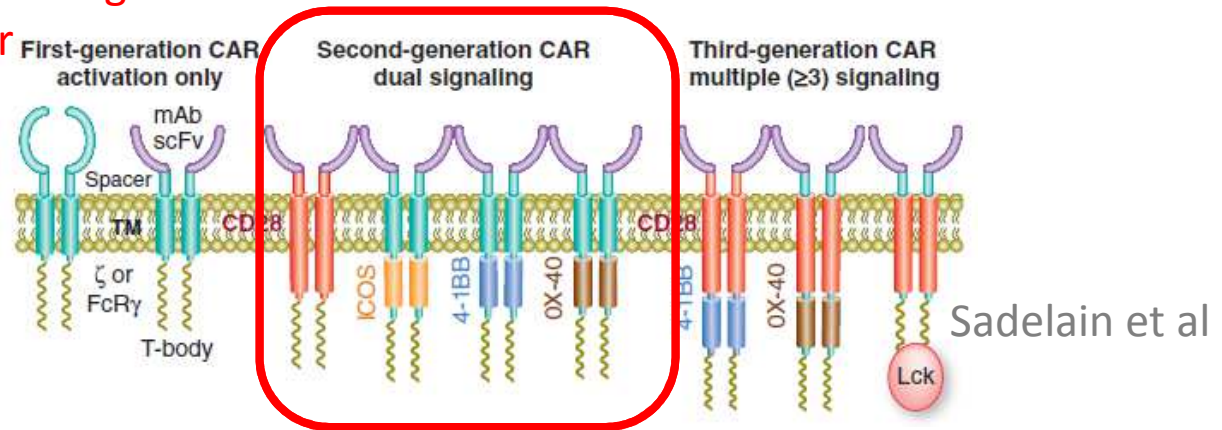


Jensen COI Disclosure:

- scientific co-founder of Juno Therapeutics, Inc. (JTI)
- equity holder in JTI
- inventor of IP licensed to JTI
- SAB/consultant to JTI

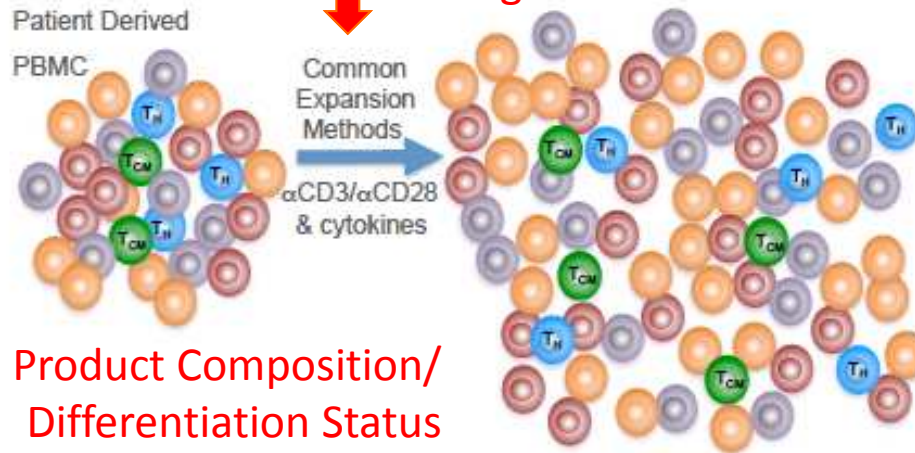
CAR T Cell Therapy Version 1.1- Empiric Designs, Trial and Error, Luck

Limited Engineering
Of Optimized Binding
Domain/Spacer
Tuning

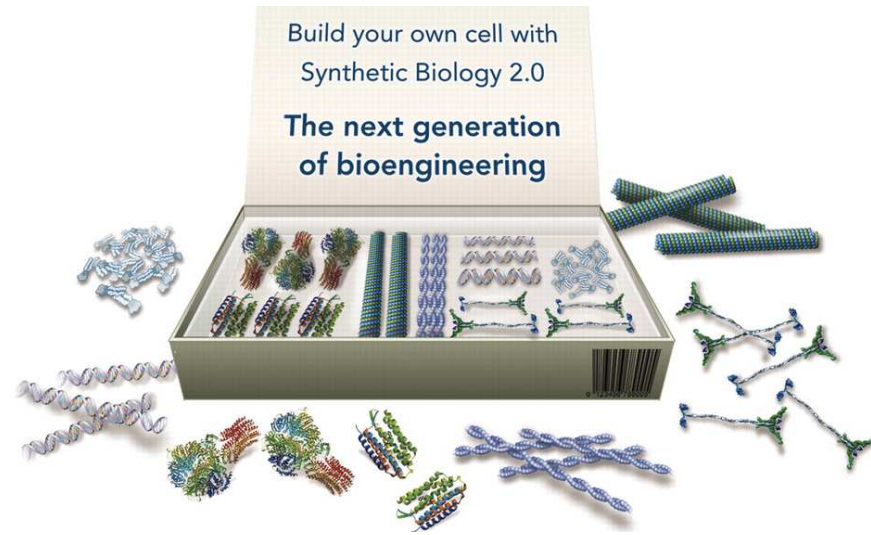


Vector Transduction

Variable Efficiency, Expression Levels,
Unregulated Constitutive Promoter



Variable Cell Product Composition/
Differentiation Status



Synthetic Biology:

-The re-design of existing, natural biological systems for useful purposes.

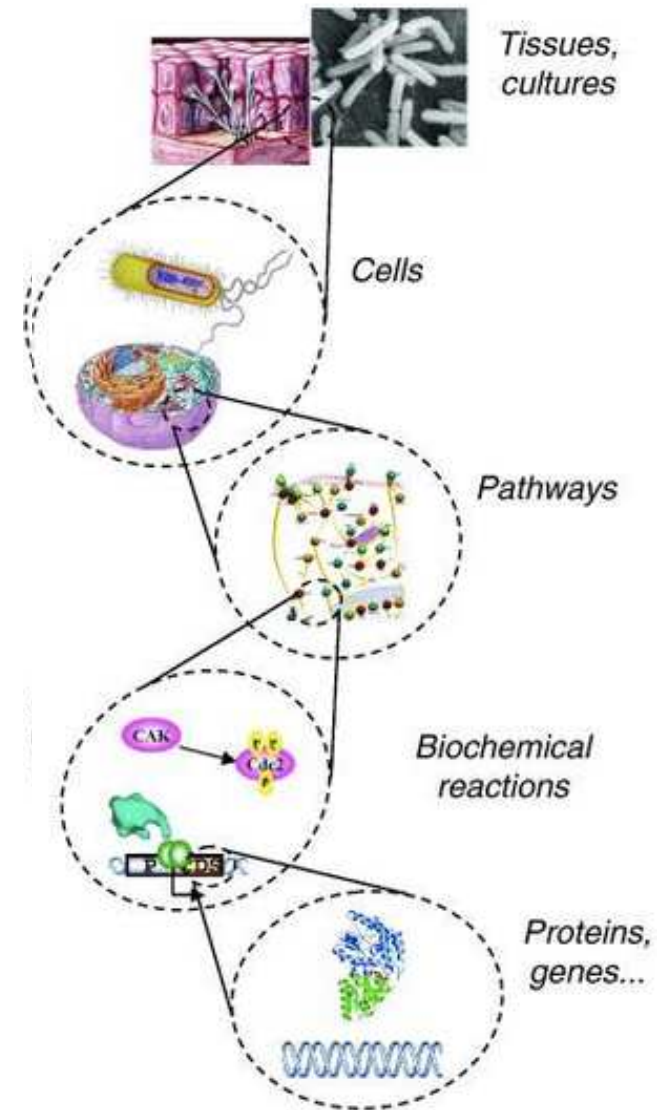
or

-The design and construction of new orthogonal biological parts, devices, and systems. SyntheticBiology.org

Synthetic Biology's (Genetic) Engineering Approach-

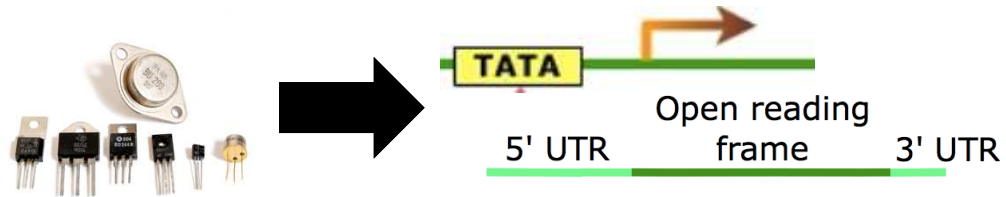
Designing new molecular parts, device modules, circuits, and networks:

- modeling the designed systems & predicting their properties
- making & testing the designs
- updating our understanding from the model/test agreement

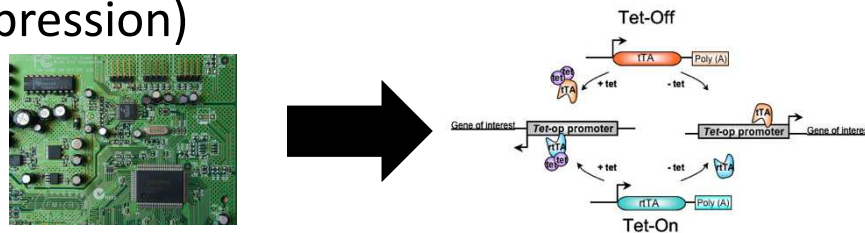


Vocabulary of Synthetic Biologists-

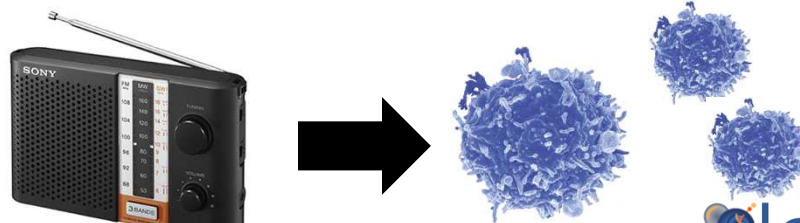
PARTS- DNA sequences encoding some component of the genetic machinery. (e.g. promoter, cDNA, riboswitch, transcription regulator, IRES, etc)



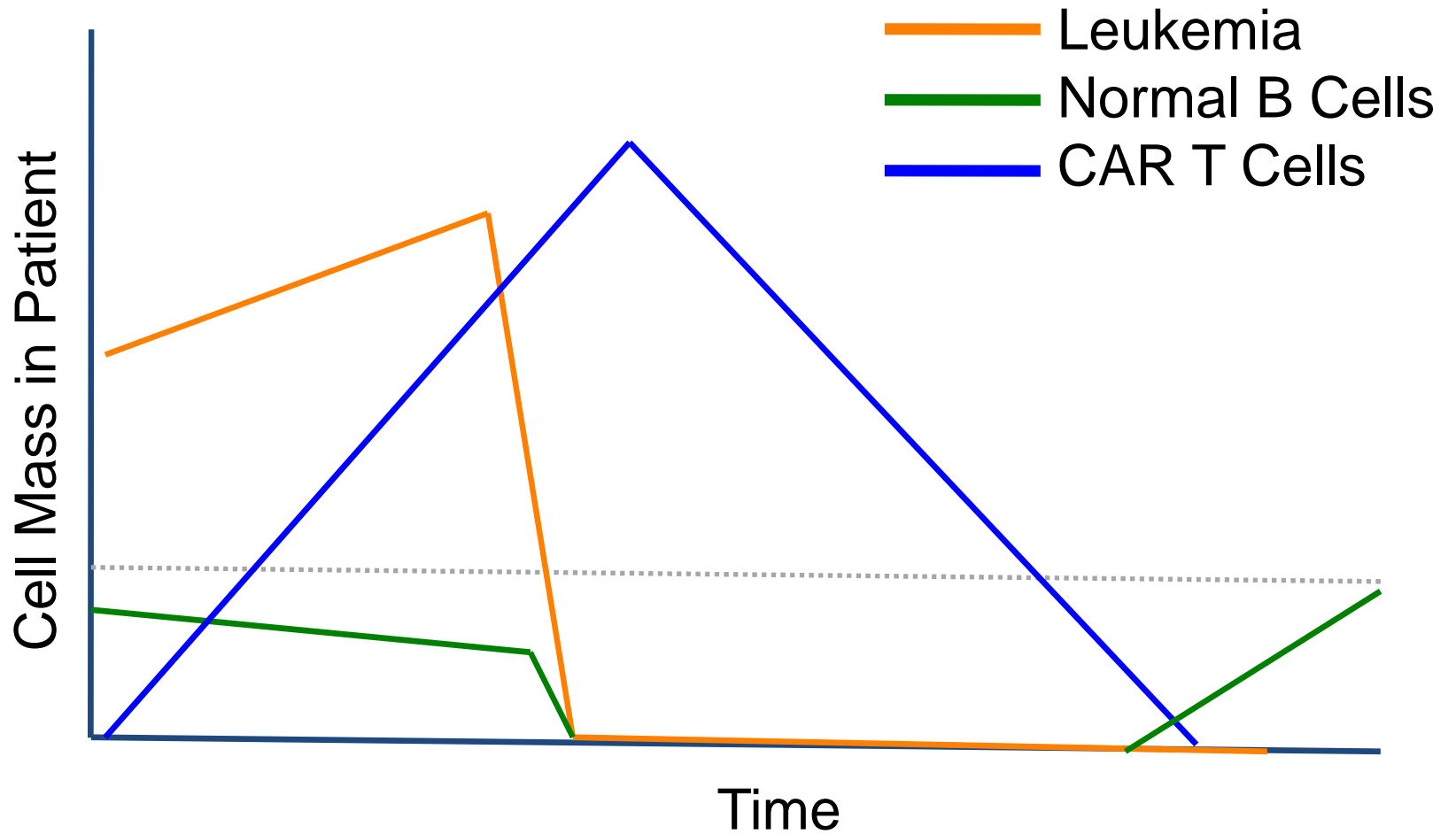
DEVICES- A group of parts that work together to perform a specific function. (exp. small molecule regulated promoter for controlled transgene expression)

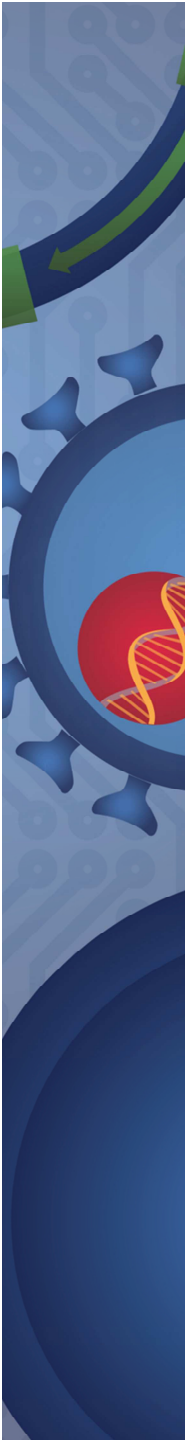


CHASSIS- Organism (host) containing the device (s)

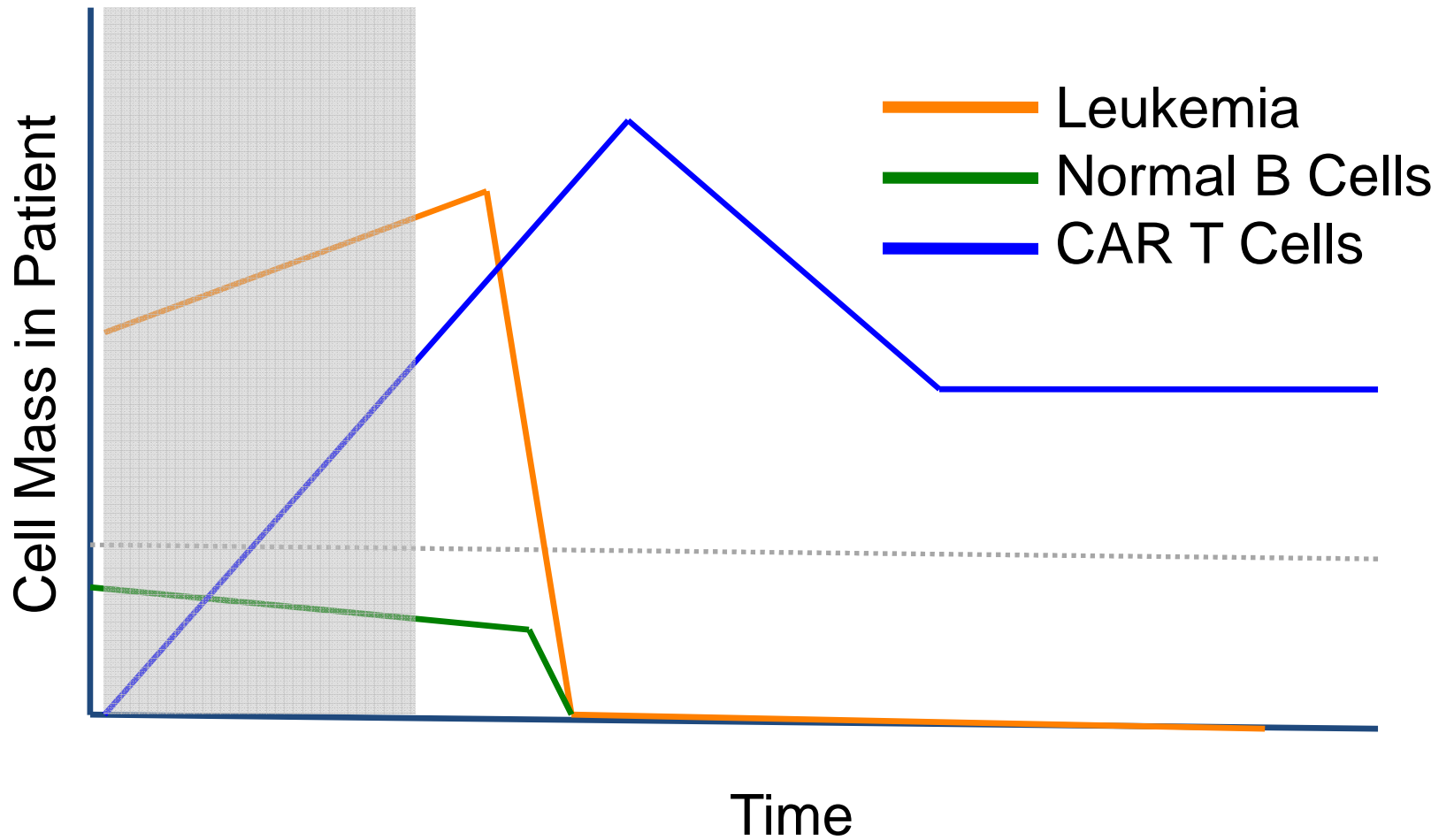


Current State: Polyclonal T Cells/ Constitutively Expressed CAR/No Suicide Mechanism





T Cells Fail to Engraft to
Therapeutic Levels /
Engrafted T Cells Fail
To Retain Anti-tumor
Functional Outputs



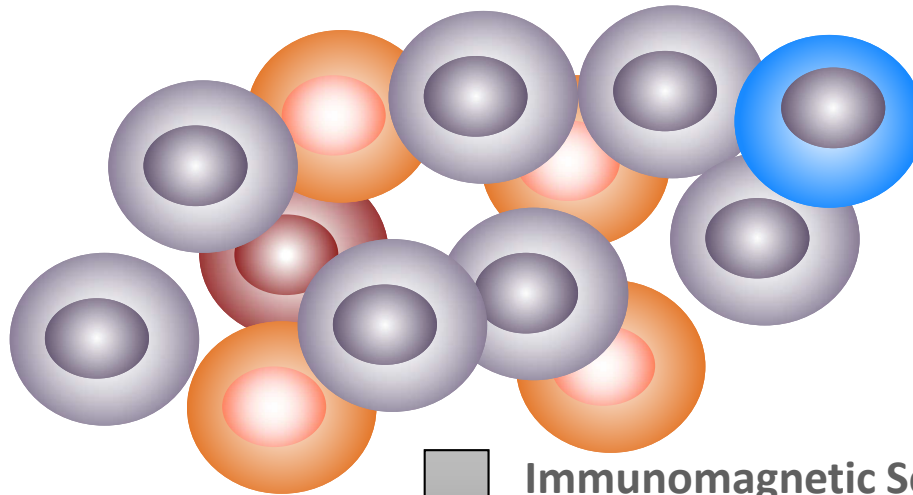


SynBio- Chassis Refinement

I. FORMULATING CAR T CELL
PRODUCTS OF DEFINED COMPOSITION
FOR IMPROVED REPRODUCIBLE
ENGRAFTMENT, EFFICACY, AND SAFETY

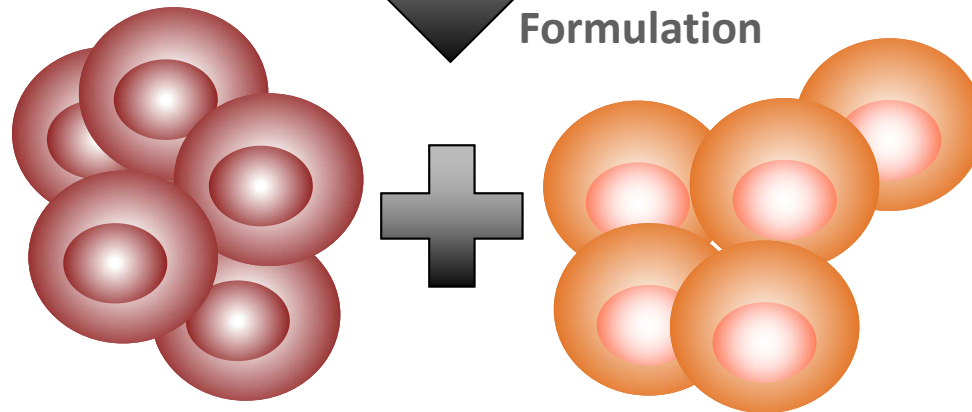
PLAT-02 Defined Product Composition:

PBMC



Immunomagnetic Selection
Lentivirus Transduction
Expansion
Formulation

**FORMULATED
CAR T CELL
PRODUCT**



100% CAR⁺CD8⁺

100% CAR⁺CD4⁺

1

:

1

MANUFACTURING CAR T CELL PRODUCTS OF DEFINED COMPOSITION:

LEUKAPHERESIS/PBMC ISOLATION

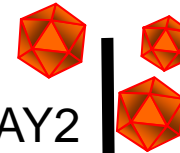


DAY 1
ACTIVATION OF T CELLS
(ANTI-CD3/CD28 BEADS)



DAY 2
LENTIVIRUS
TRANSDUCTION

EXPANSION IN
CYTOKINES



Mid-Process
Bead Removal/
EGFRt Positive
Selection

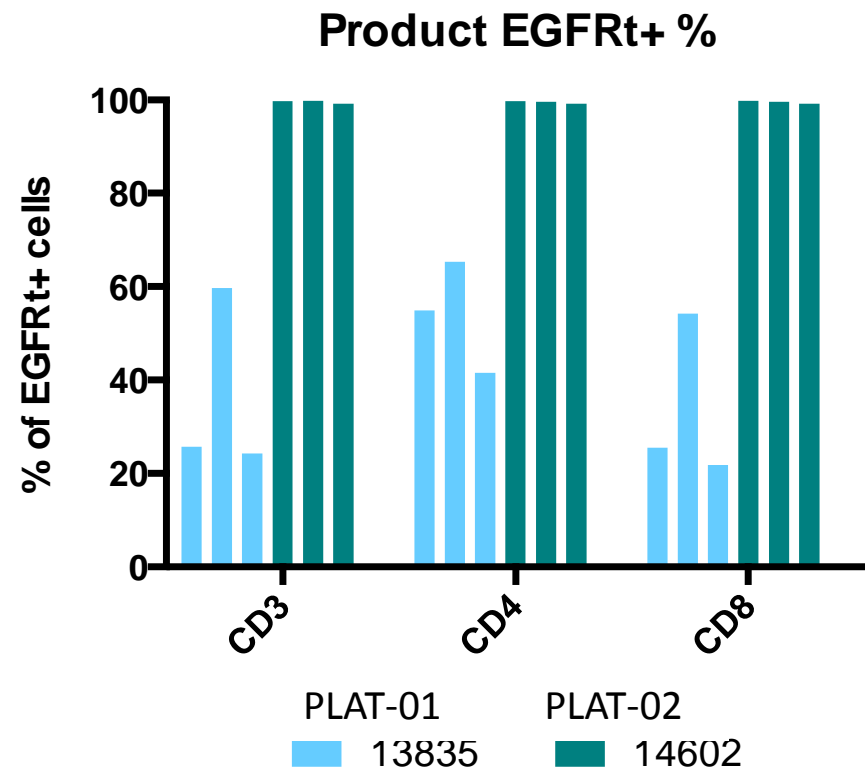
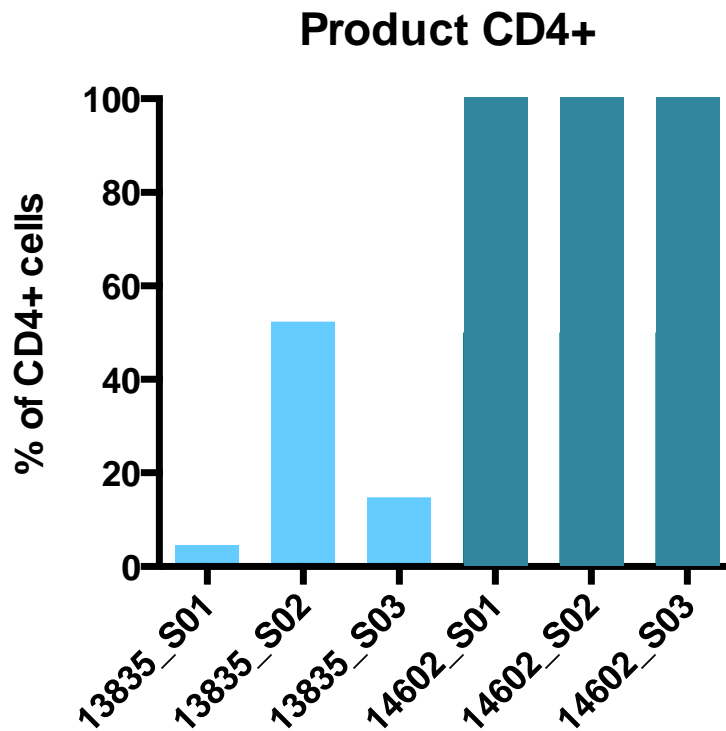


DAY 1
PURIFICATION OF
CD4 and CD8 SUBSETS

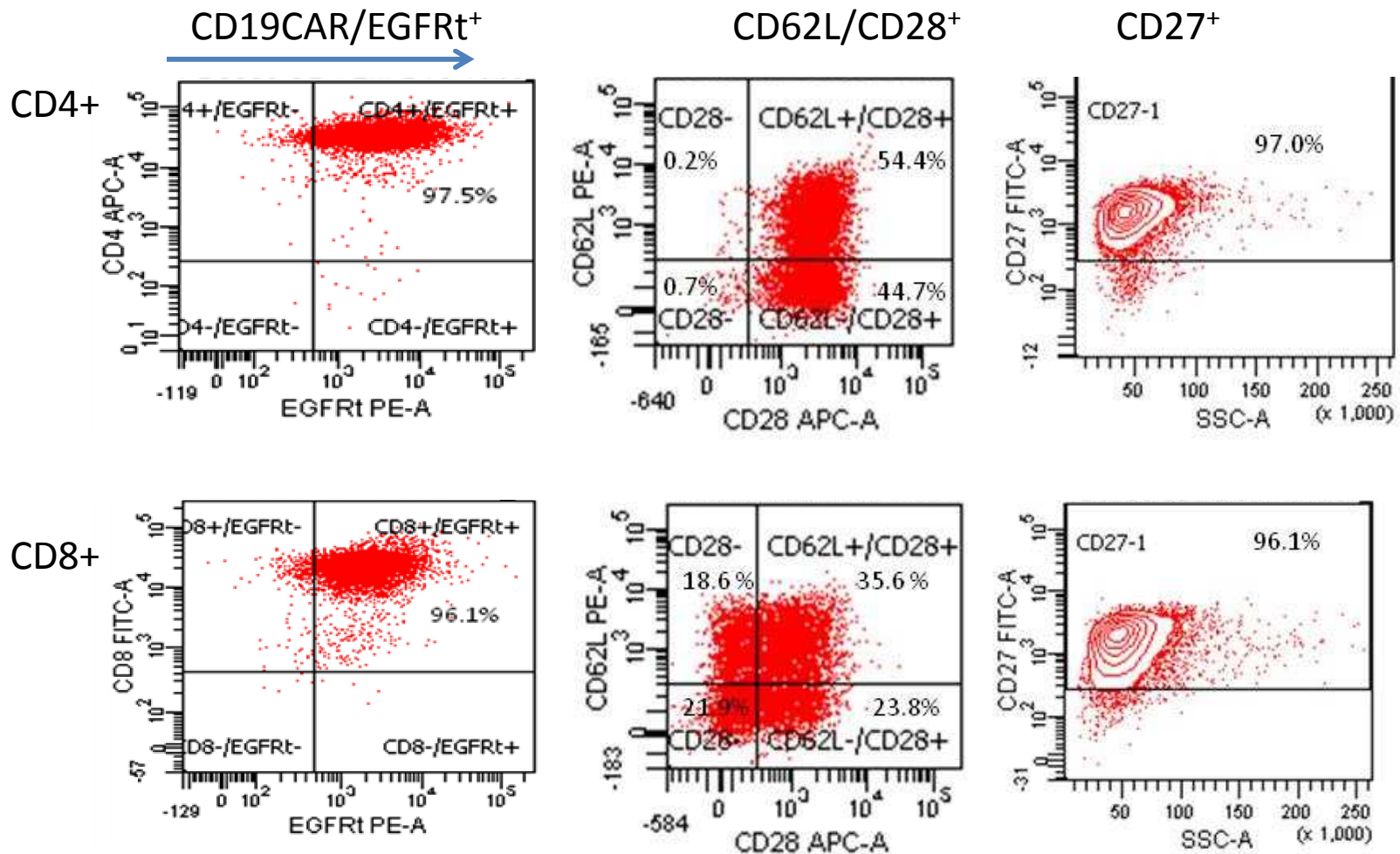


DAY 14-21 CRYOPRESERVATION

Defined Composition CAR T Cell Product Uniformity Compared to Unformulated Products

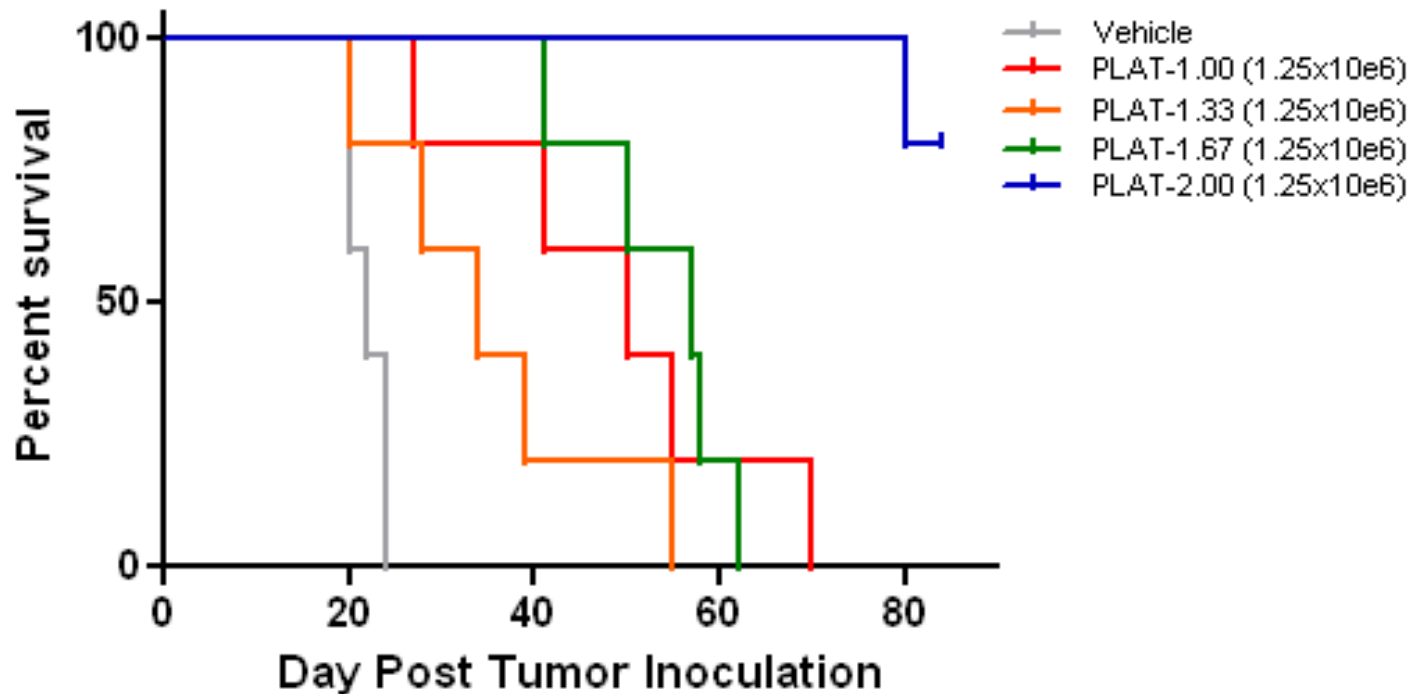


Phenotype of Expanded Defined Composition CD19CAR T Cell Products At Time of Cryopreservation (Day 11-18):



Superior In Vivo Anti-tumor Activity of Defined Composition CD19CAR T Cell Products (1:1 CD4/CD8 Cell Dose, 100% CAR+)

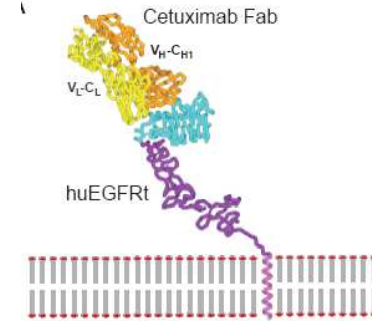
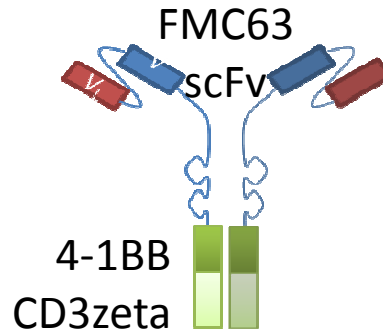
(Compared to Undefined or Single Parameter Selected Products)



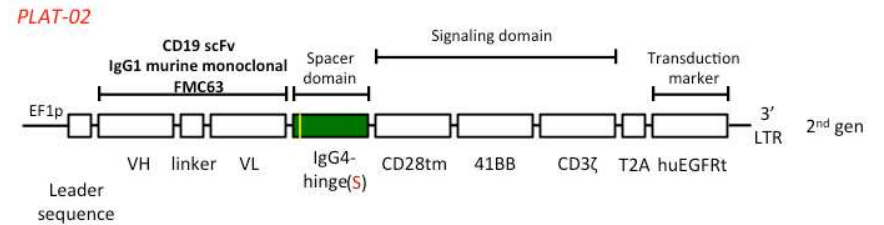
CURRENT STATE: Version 2.0 (SCRI PLAT-02)

Parts List-

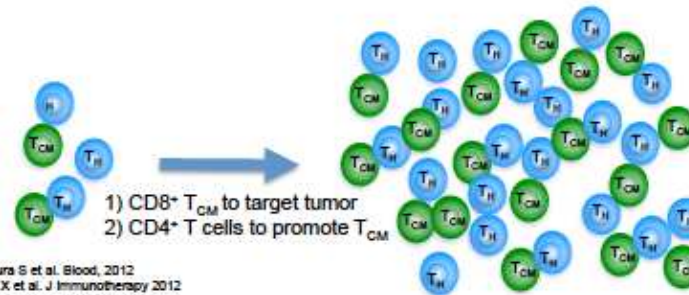
EF1 α -promoter
T2A Linker



Devices- G3 SIN Lenti



Chassis- Defined combinations of T cell subsets



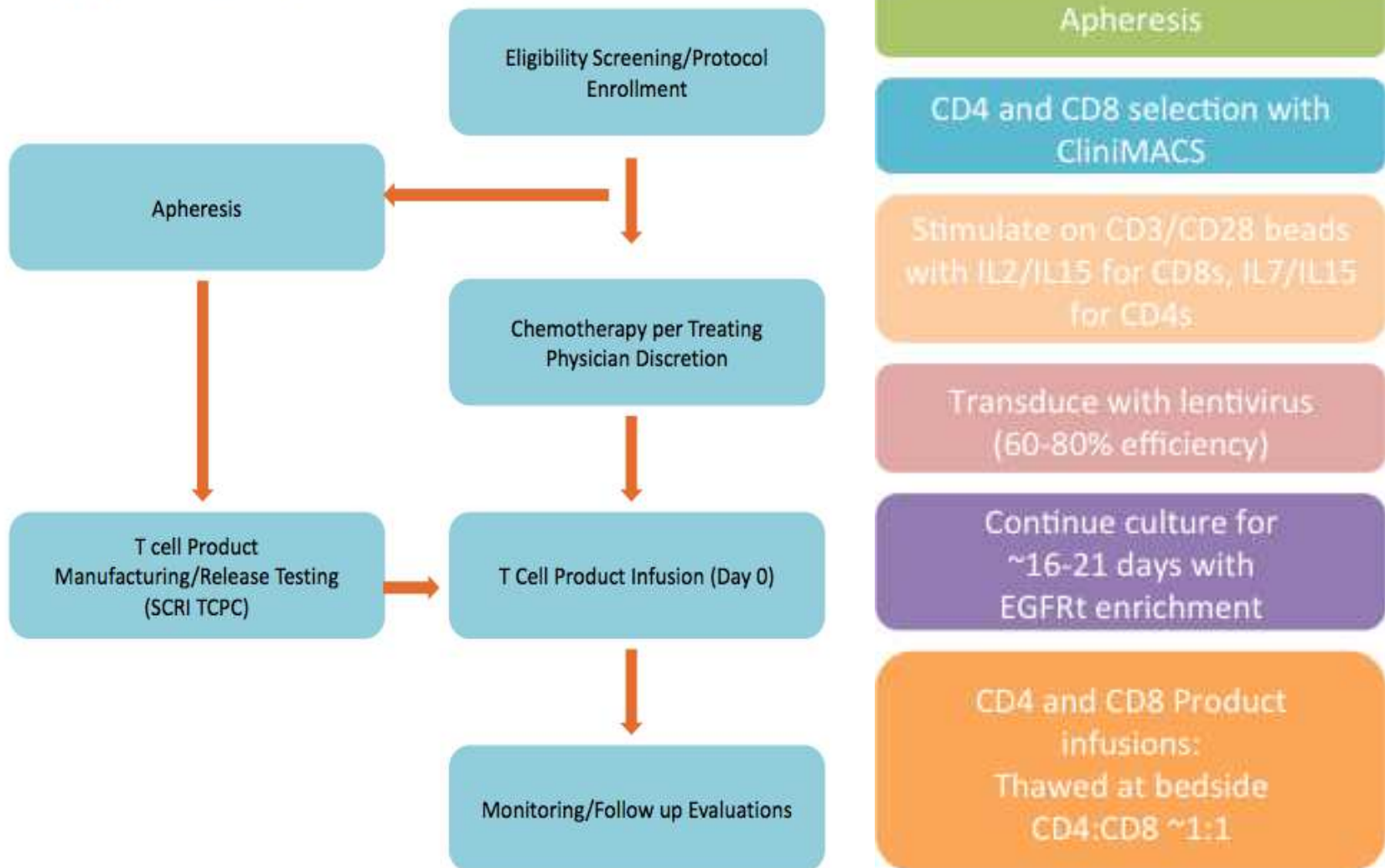
Terakura S et al. Blood, 2012
Wang X et al. J Immunotherapy 2012
Stemberger C et al. PLoS One, 2012

Pediatric Leukemia CD19CAR Adoptive Therapy Trials

Site	Defined Cells	Vector	scFv	ECD Spacer	Co-stim	Selection/ Suicide
MSKCC	No	Retro	SJ251	CD28partial	CD28	No/No
CHOP	No	Lenti	FMC63	CD8hinge	4-1BB	No/No
NCI	No	Retro	FMC63		CD28	No/No
Baylor	EBV	Retro	FMC63	Full IgG1	CD28	No/No
SCRI	CD4:CD8	Lenti	FMC63	IgG4hinge	4-1BB	Yes/Yes

PLAT-02: A Phase 1/2 Trial of Defined Composition CD19CAR T Cell Adoptive Therapy For Refractory Relapsed and Post-HSCT Recurrent Pediatric ALL

EXPERIMENTAL DESIGN SCHEMA



PLAT-02: Post alloHSCT Patient Profile

Cohort	Patient ID	Age (y)	Relapse#	Amount of disease in BM at enrollment by MPF	Current progress in study/off study
1A	14602-S01	21	2	90	In long term follow up
1A	14602-S02	22	2	0.4	In long term follow up
1A	14602-S03	21	2	90	In long term follow up
1A	14602-S04	11	2	0.04	In long term follow up
1A	14602-S05	19	2	2	In long term follow up
1A	14602-S06	4	2	54	D+42
1B	14602-S07	1	2	69	D+21
1B	14602-S08	23	1	98.6	D+7
1B	14602-S09	6	2	30	Pre T cell infusion
TBD	14602-S10	17	2	1.94	Pre T cell infusion
TBD	14602-S11	15	2	23	Pre T cell infusion
TBD	14602-S12	12	3	0.04	Pre T cell infusion

Update 10-18-2014: 15 pts enrolled, 11 pts infused

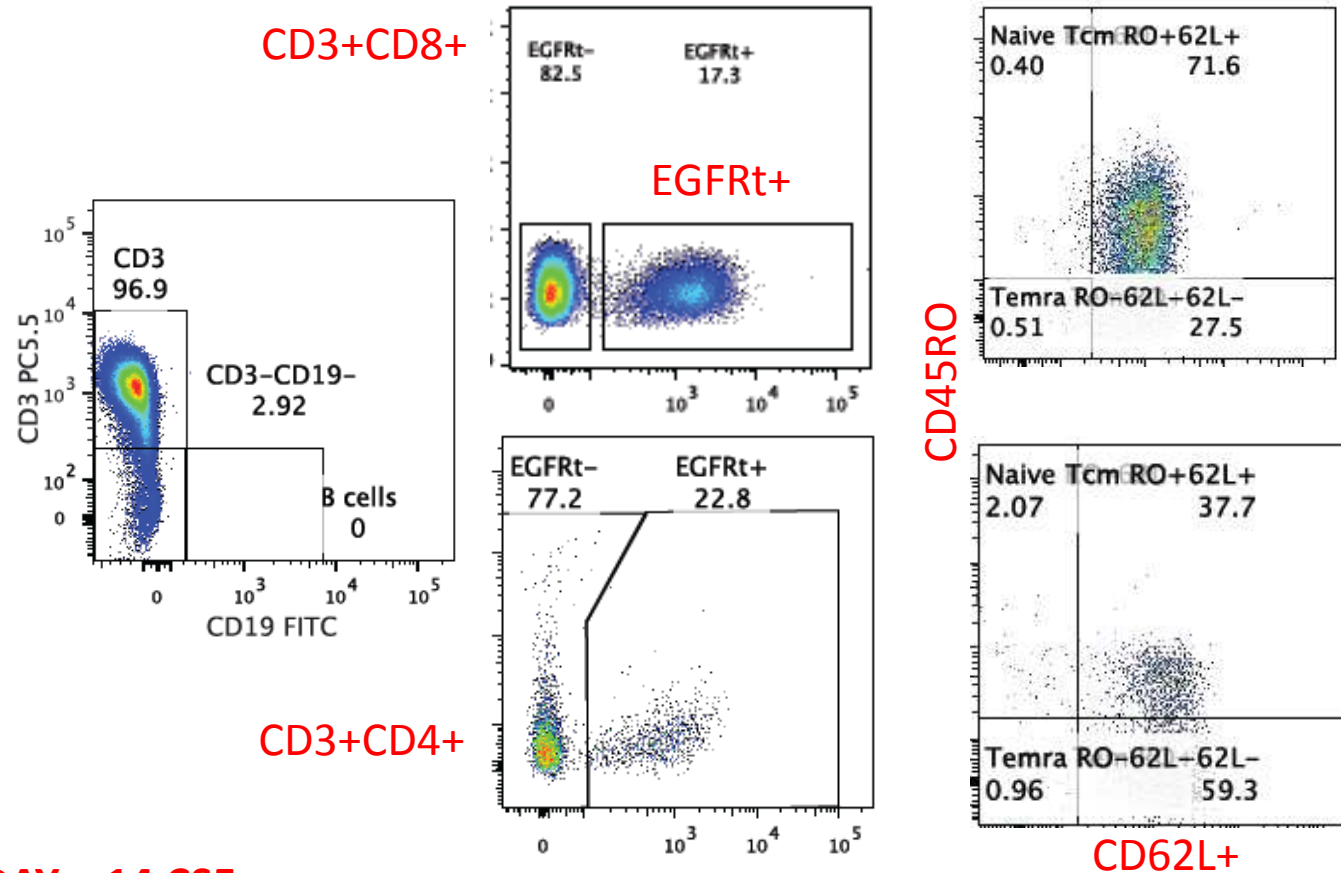
S09 and S10 Infused

S09 PR after first dose, received second dose, no response.

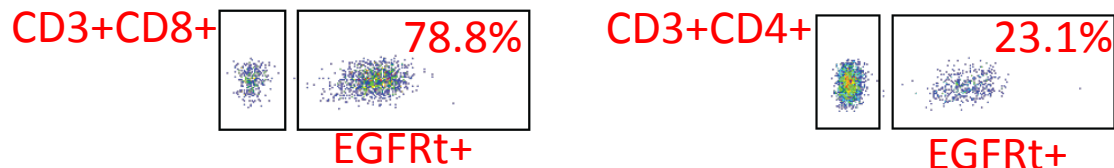
S10/S11 MRD- CR

PLAT-02: Post-alloHSCT ALL Relapse/Pt Derived Donor Origin T Cells
CD4/CD8 1:1 AntiCD19CAR(4-1BBzeta)-EGFRt
 Dose 250,000 cells/kg of CD4 product and CD8 product

Peripheral Blood Day +14:



DAY + 14 CSF:



PLAT-02: Day +7 Tumor Burden vs Response

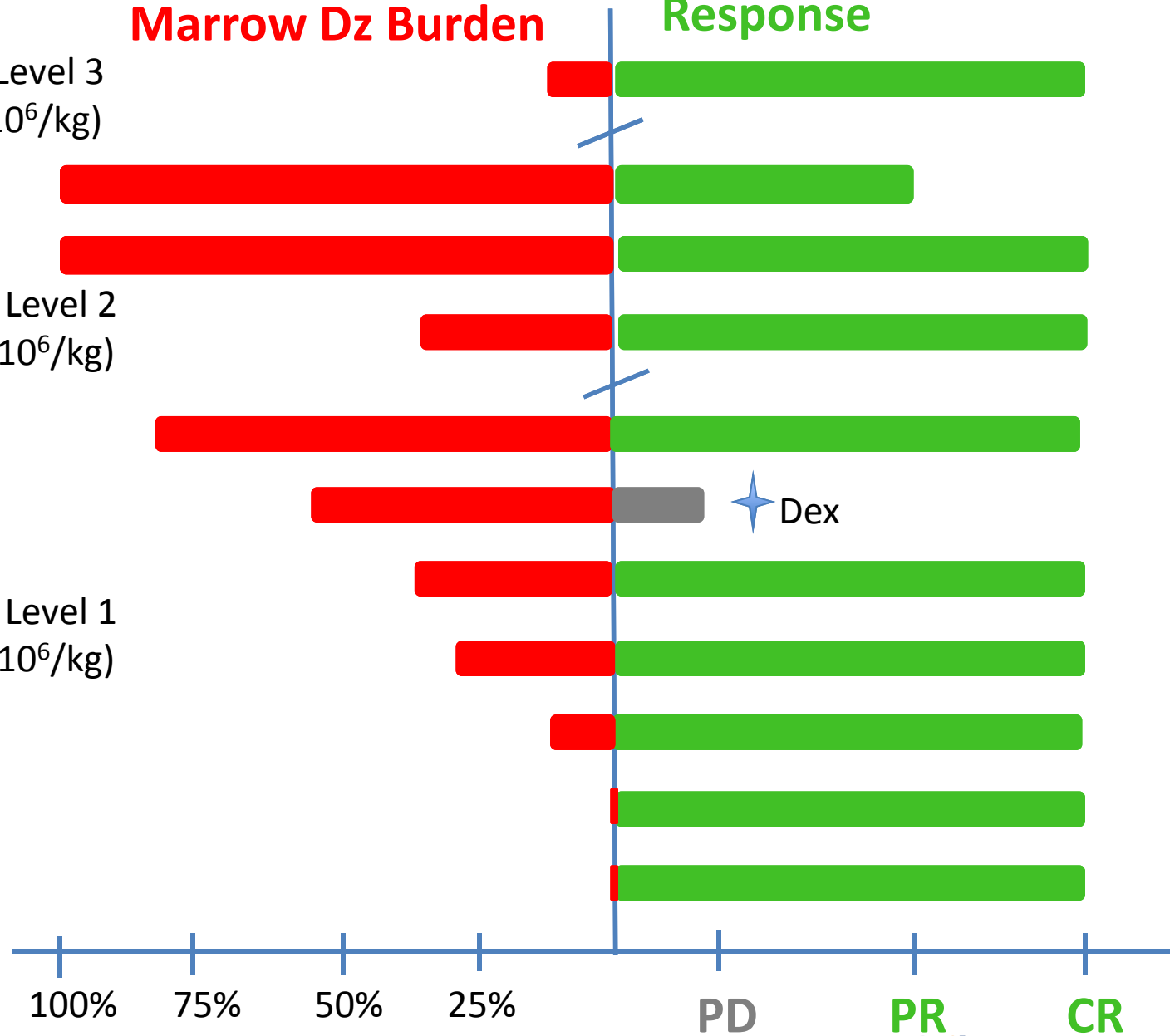
Marrow Dz Burden

Response

Dose Level 3
($5.0 \times 10^6/\text{kg}$)

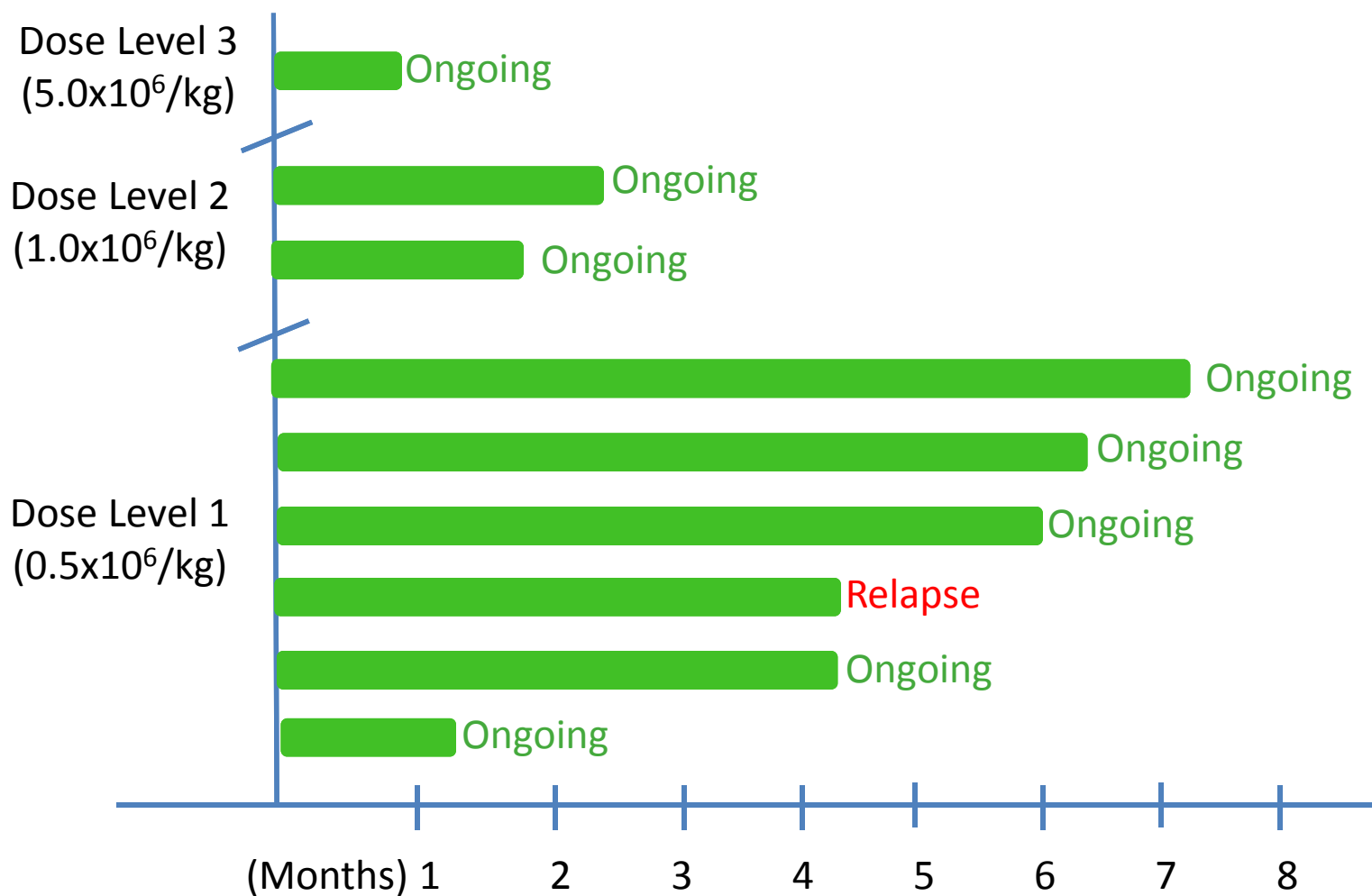
Dose Level 2
($1.0 \times 10^6/\text{kg}$)

Dose Level 1
($0.5 \times 10^6/\text{kg}$)



PLAT-02: Remission Duration (Intent to Treat/Post-HSCT Relapse)

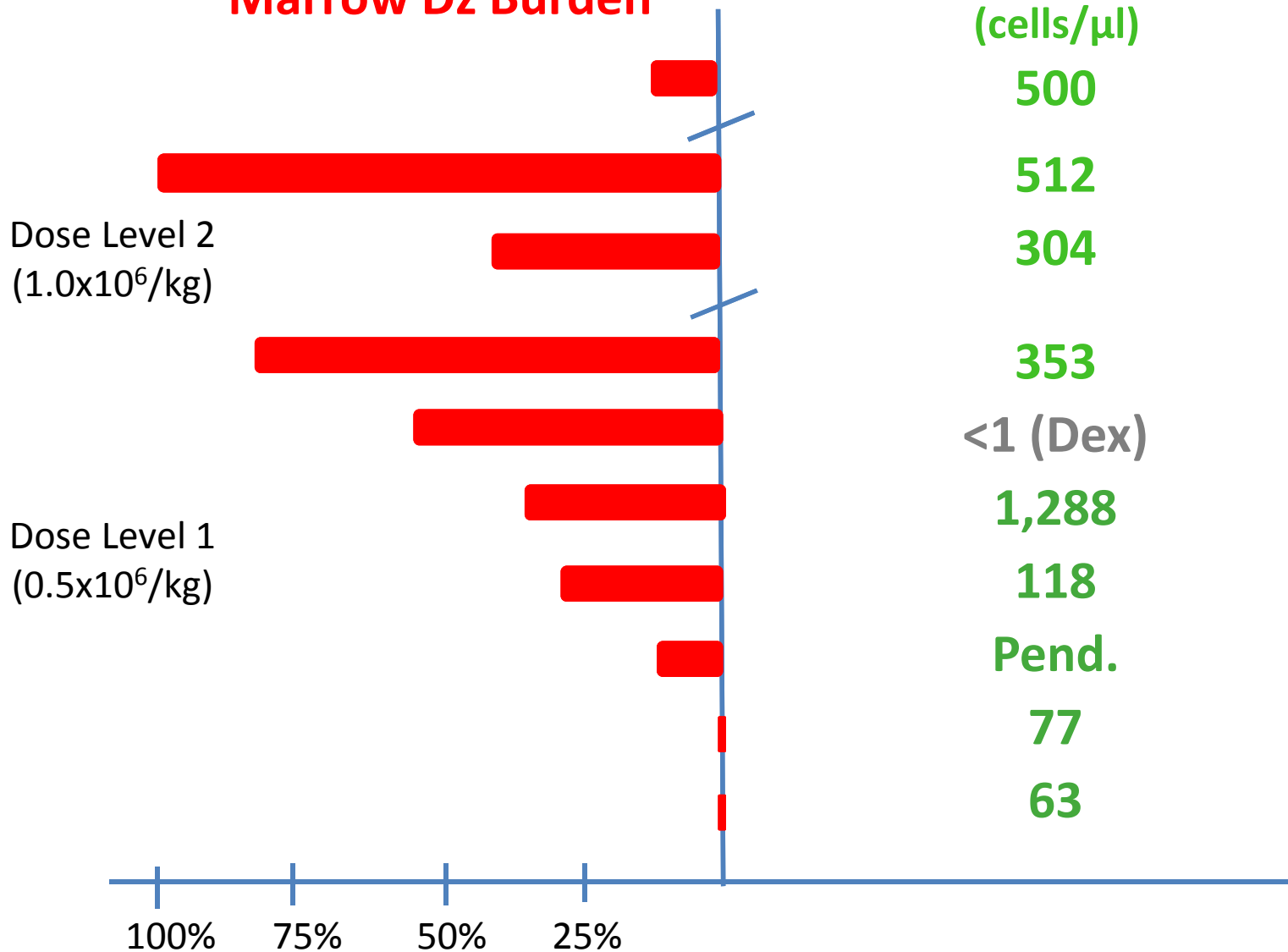
Complete Response (MRD-neg by MPF) →



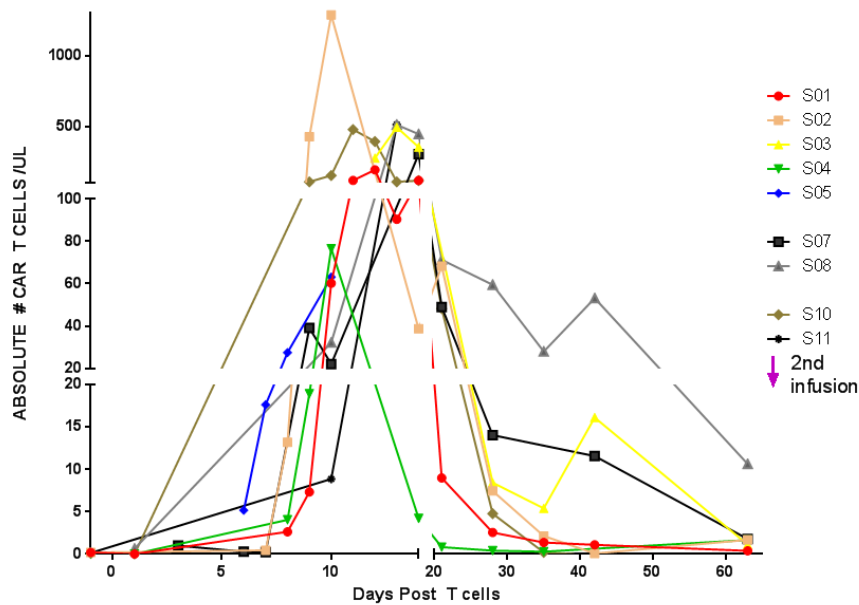
PLAT-02: Day +7 Tumor Burden vs Engraftment

Marrow Dz Burden

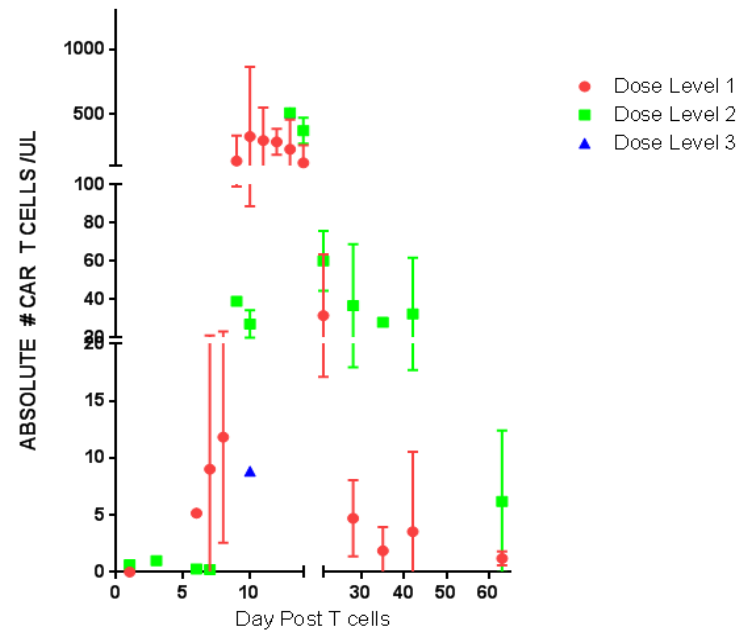
**Peak EGFR^t T Cells in PB
(cells/ μ l)**



PLAT-02: Magnitude and Duration of CAR/EGFRt⁺ T Cell Persistence

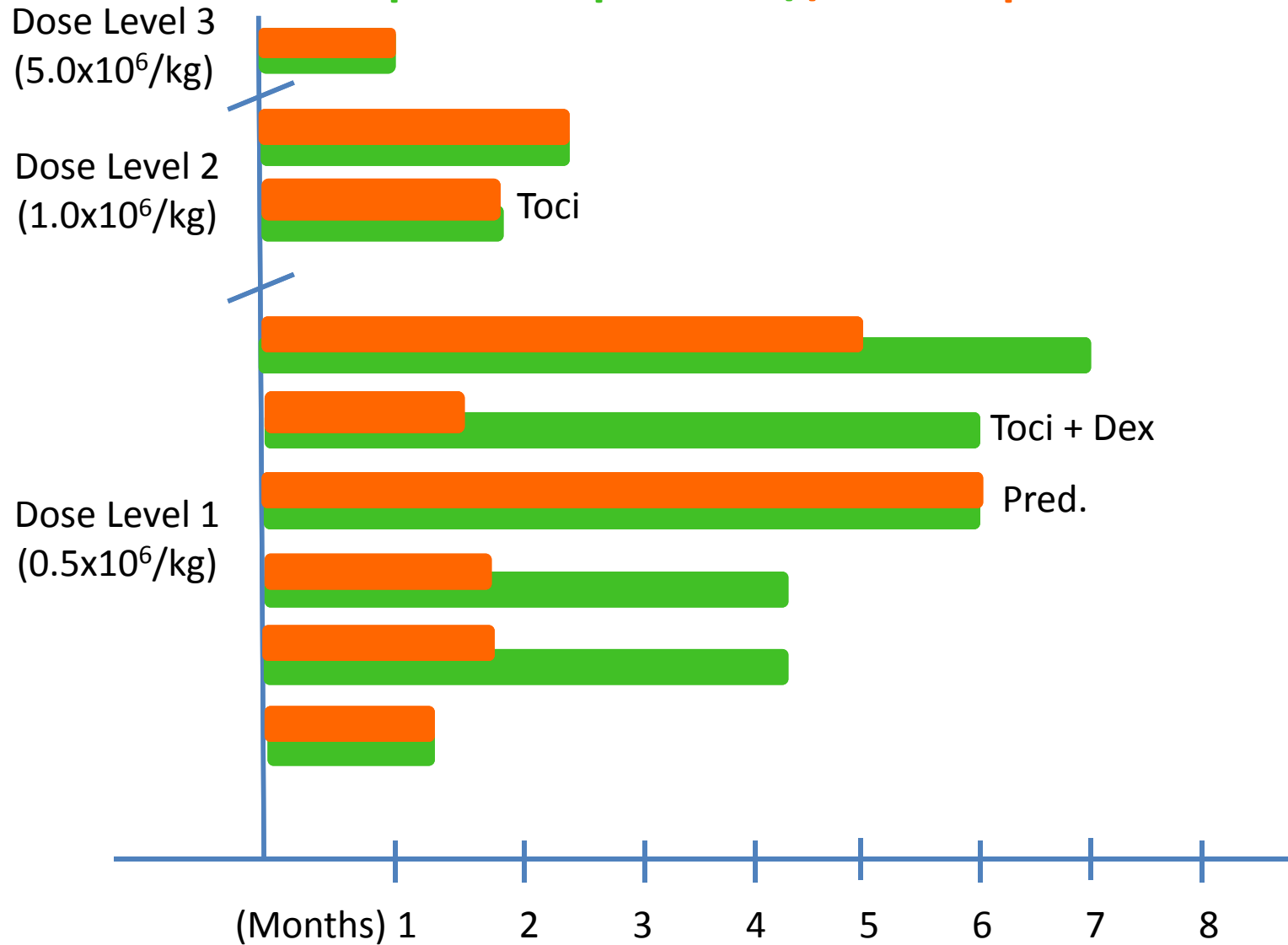


Dose Level ABSOLUTE # CAR T CELLS/UL



PLAT-02: Duration of B Cell Aplasia

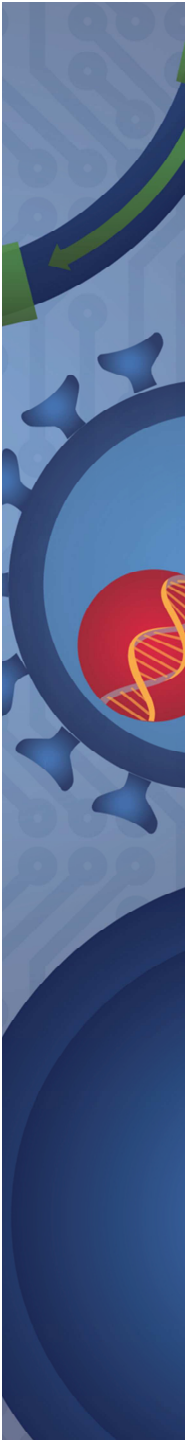
Complete Response → // B Cell Aplasia →



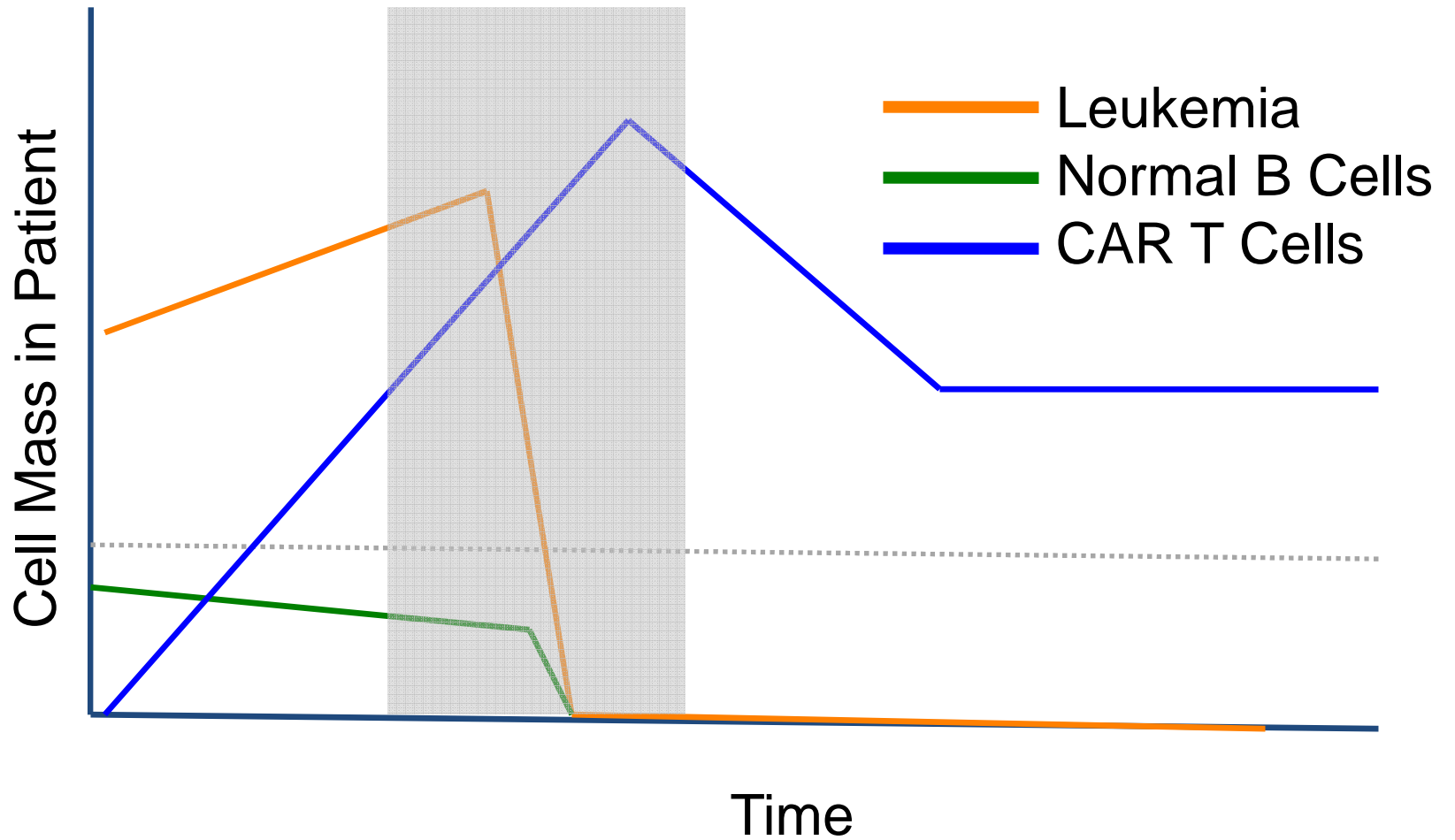


Summary I:

- 1. Feasible (100% based on intent to treat) to manufacture defined composition products.**
- 2. Bioactive against ALL, high peak engraftment, duration of engraftment is heterogeneous.**
- 3. Products have high frequencies of CD62L/CD28/CD27⁺ T Cells.**



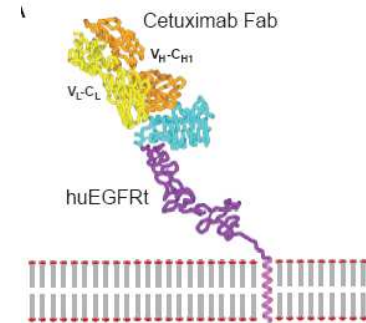
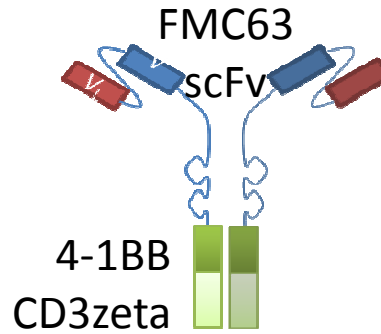
LIFE THREATENING
TOXICITIES ARISING FROM
UNREGULATED CAR T CELL
FUNCTIONAL OUPUTS



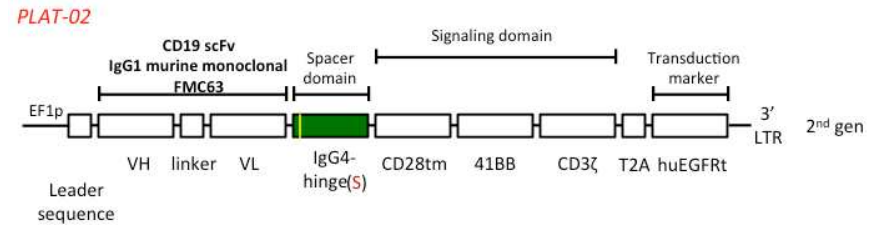
CURRENT STATE: Version 2.0 (JCAR14, 17)

Parts List-

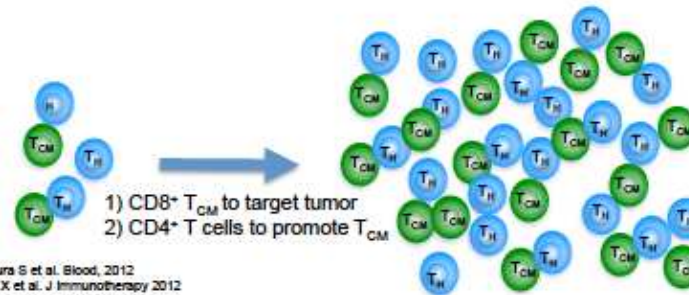
EF1 α -promoter
T2A Linker



Devices- G3 SIN Lenti



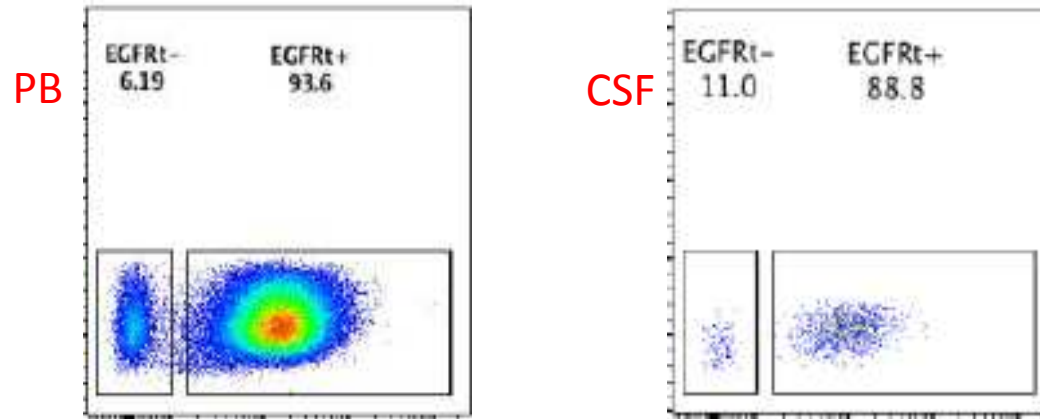
Chassis- Defined combinations of T cell subsets



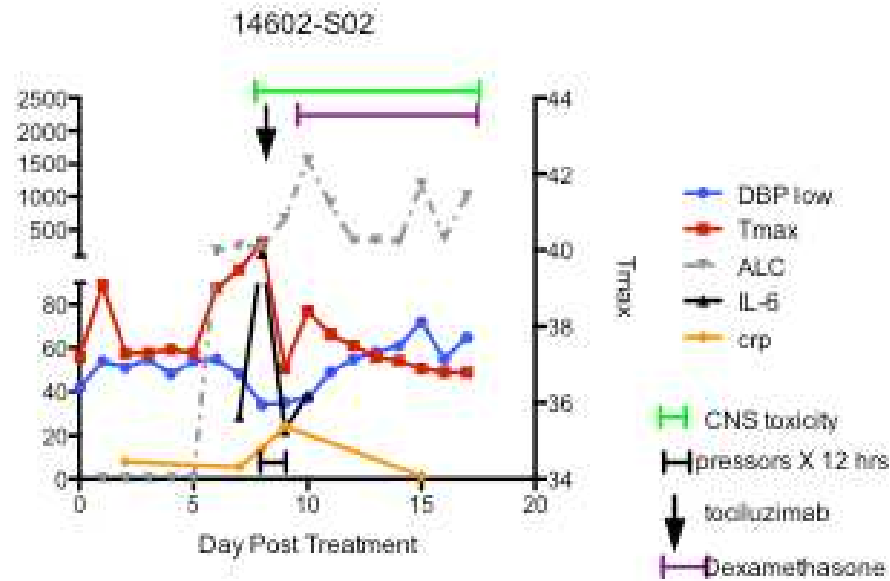
Terakura S et al. Blood, 2012
Wang X et al. J Immunotherapy 2012
Stemberger C et al. PLoS One, 2012

CAR T Cells Are Constitutively "ON"-

S02 CAR T Cell Engraftment-

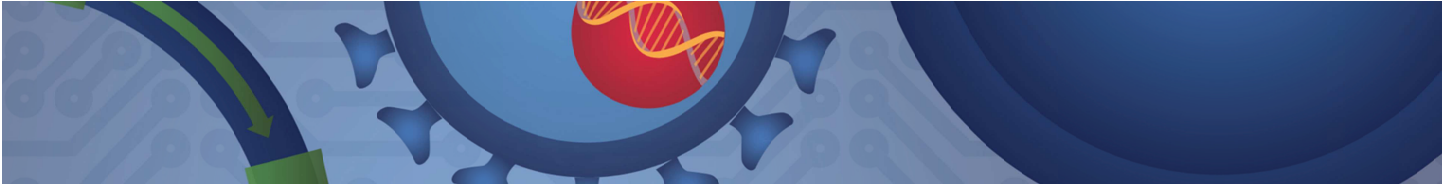


Severe Sx's/Pressors, Toci, Dex





II. CLINICAL CONTROLLED CAR T CELLS THROUGH REGULATED TRANSGENE EXPRESSION



The ideal system for regulating transgene expression in CART cells

- A clinically relevant transgene regulatory system should:
 - Demonstrate selective and specific regulation by ligand
 - Stringent OFF state *
 - High inducibility *
 - Non-immunogenic
 - Regulation by a safe, well tolerated ligand
- While other transcriptional regulatory system exists for transgene regulation, few possess sufficient number of these key attributes to permit clinical application



bioDEVICE ASSEMBLY:
Transgene Expression Rheostats For Regulated
TgX Expression:

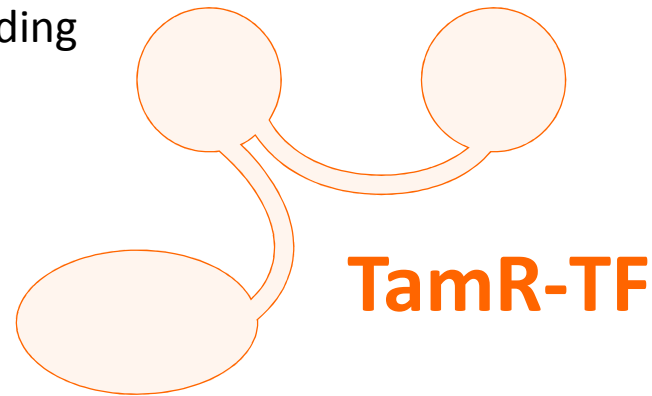


**TamR Transcriptional
Regulatory System**

TamR-Transcription Platform- *Parts I*

Human Estrogen Receptor LBD
Tuned For Tamoxifen Binding

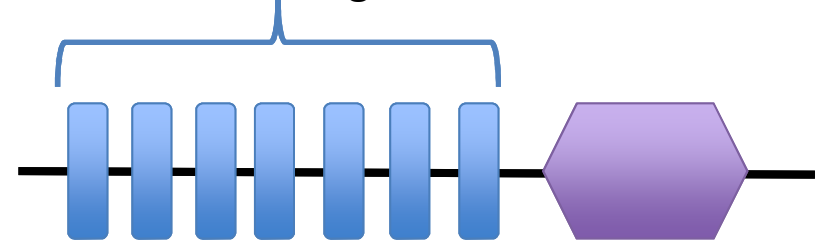
Human RelA
Transactivation Domain



Human Hepatocyte Nuclear Factor-1 α
DNA Binding Domain

**TamR-TF Responsive
Synthetic Promoter**

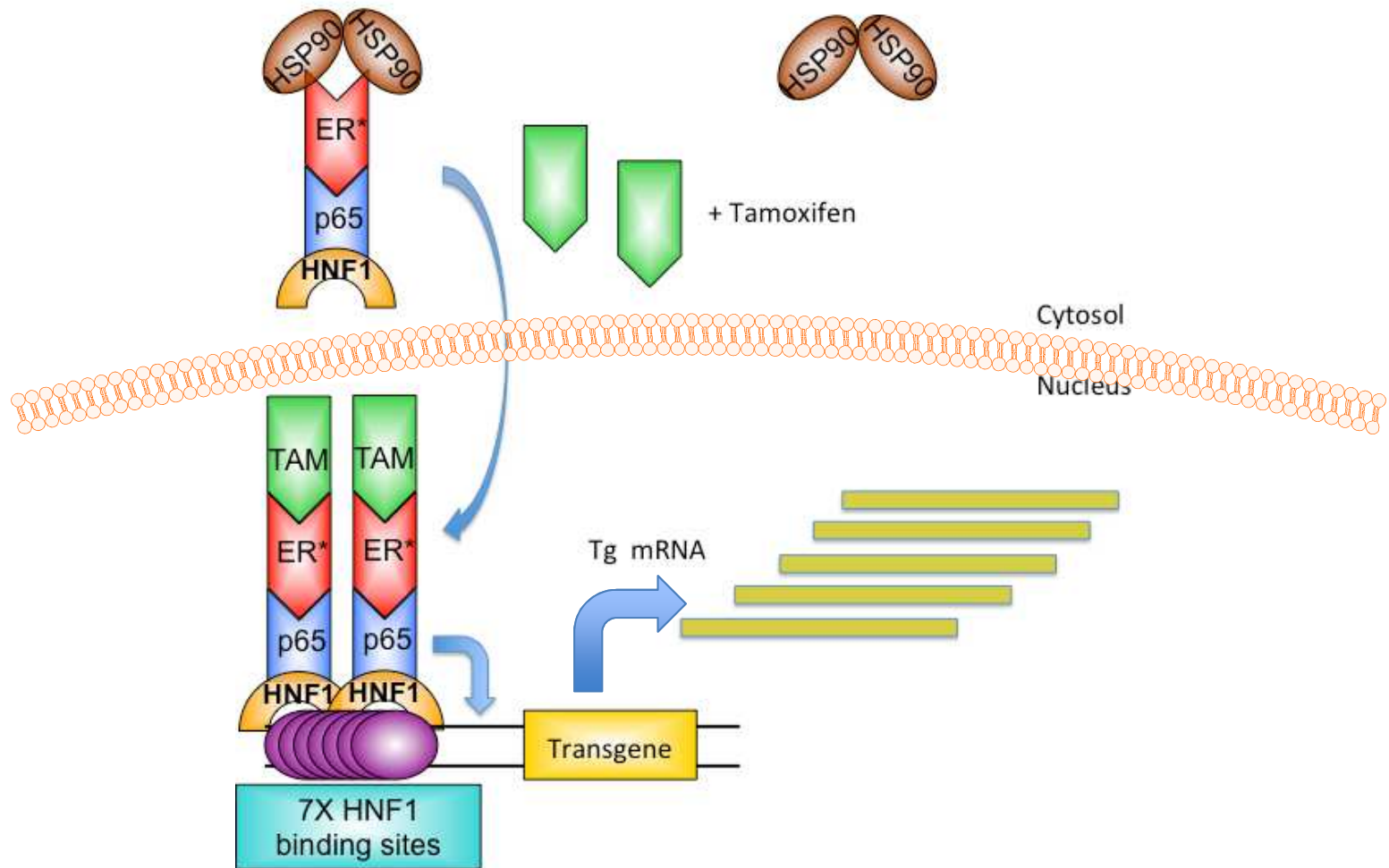
7X huAlb promoter
HNF-1 α Binding Motif



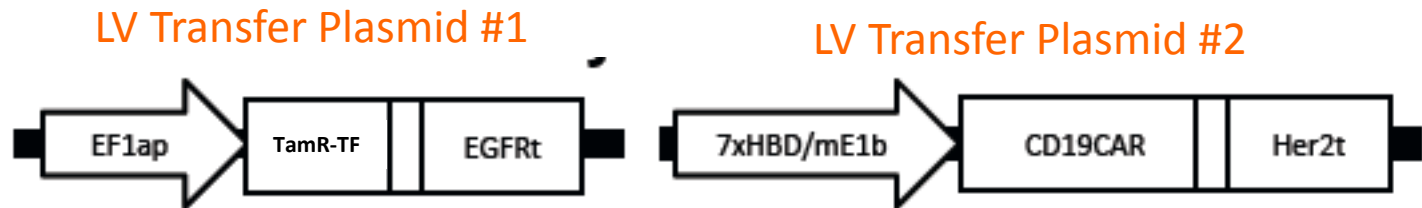
Adenovirus
E1b mp/TATA

Prototype described by Roscili et al., 2002

TamR Transcriptional Control System



TamR-Transcription Platform- *LV Device*



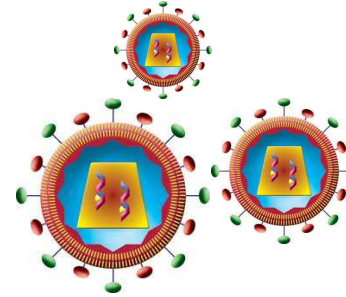
LV Transfer Plasmid #1



LV Transfer Plasmid #2



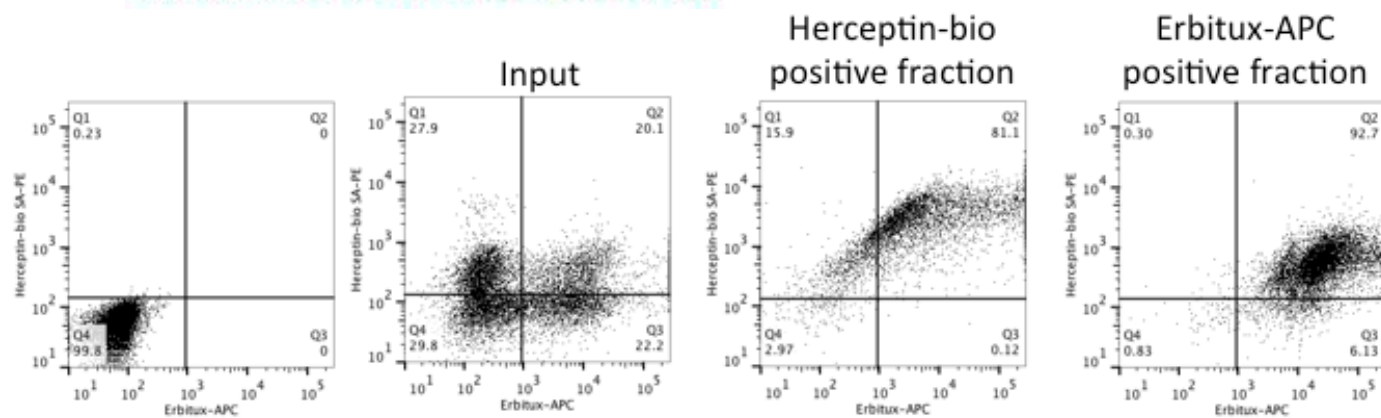
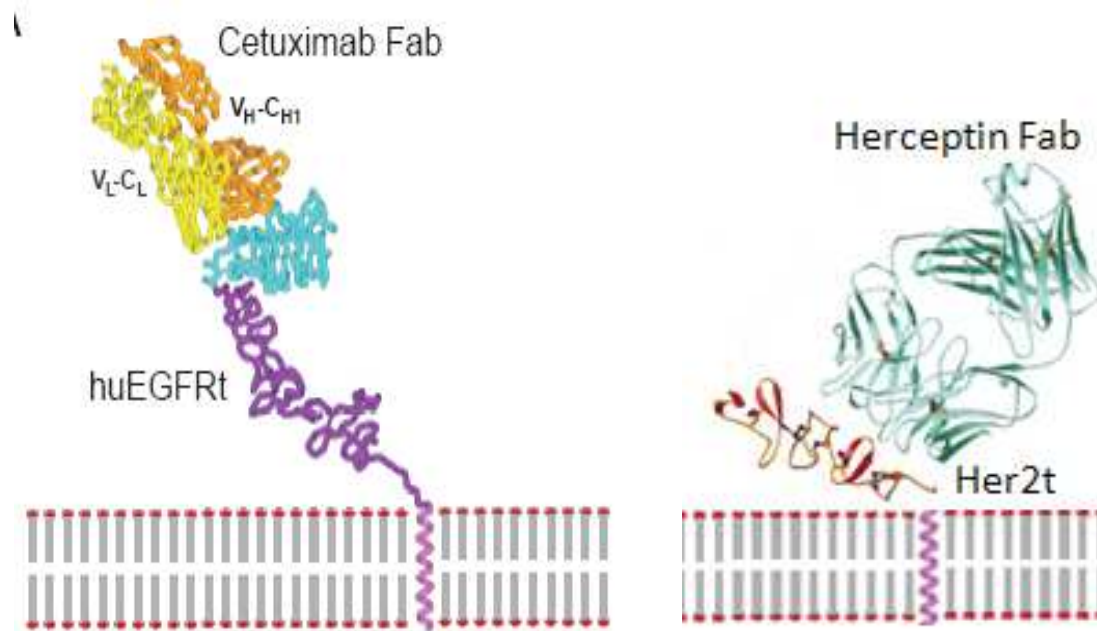
“Dual Packaged LV”



>15Kb Payload
Capacity

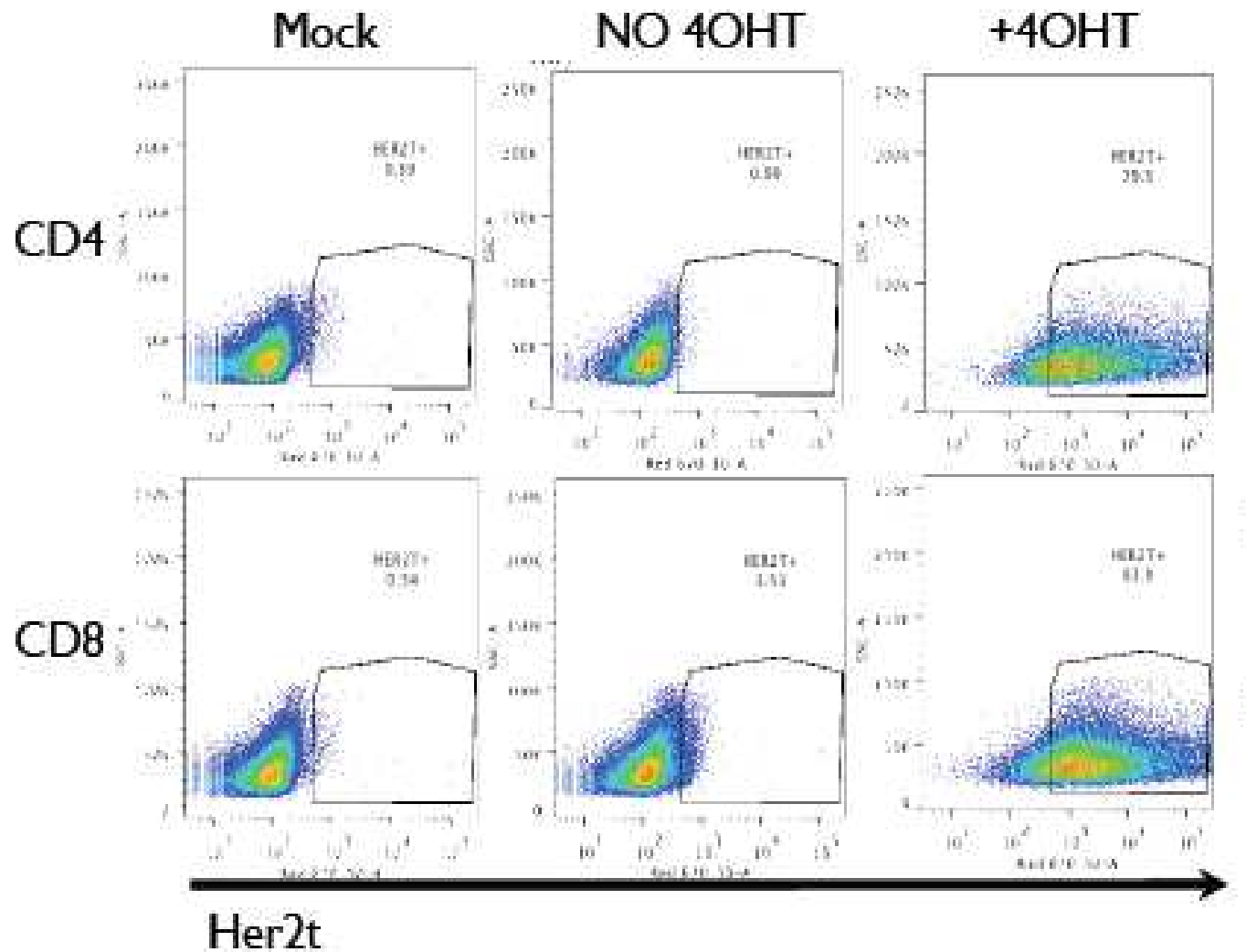
TamR-Transcription Platform- *Parts II*

Cell Surface Barcoding Tags

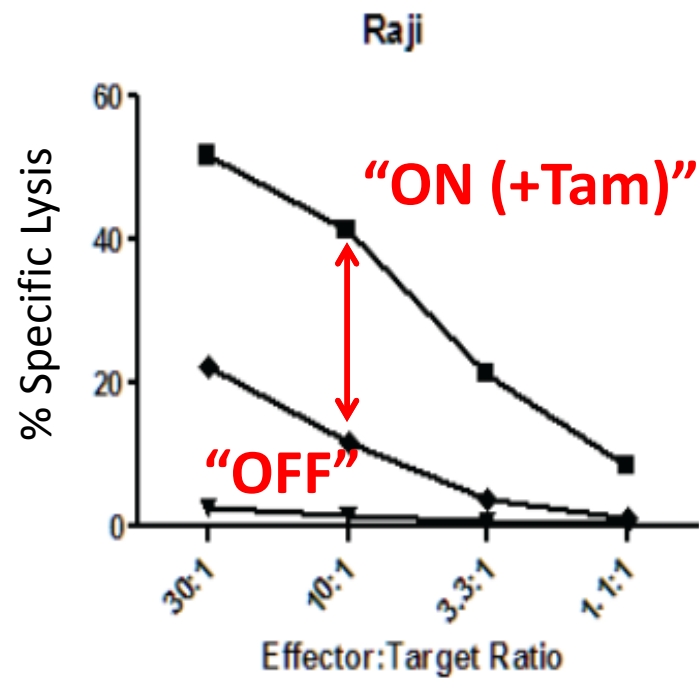
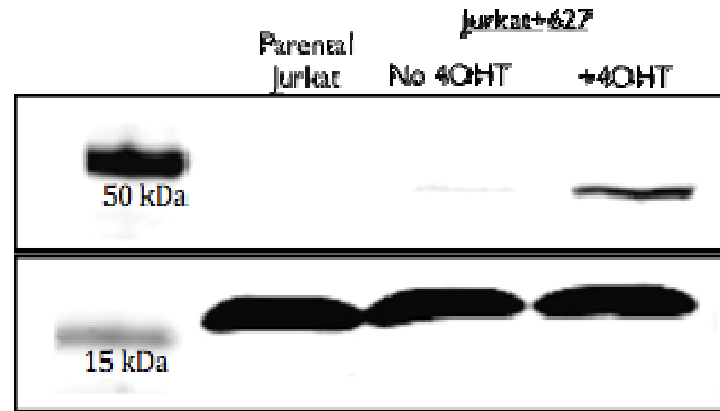


TamR-LV Transcription Platform-

Performance in Primary Human T Cells



TamR-LV CAR Functional Outputs

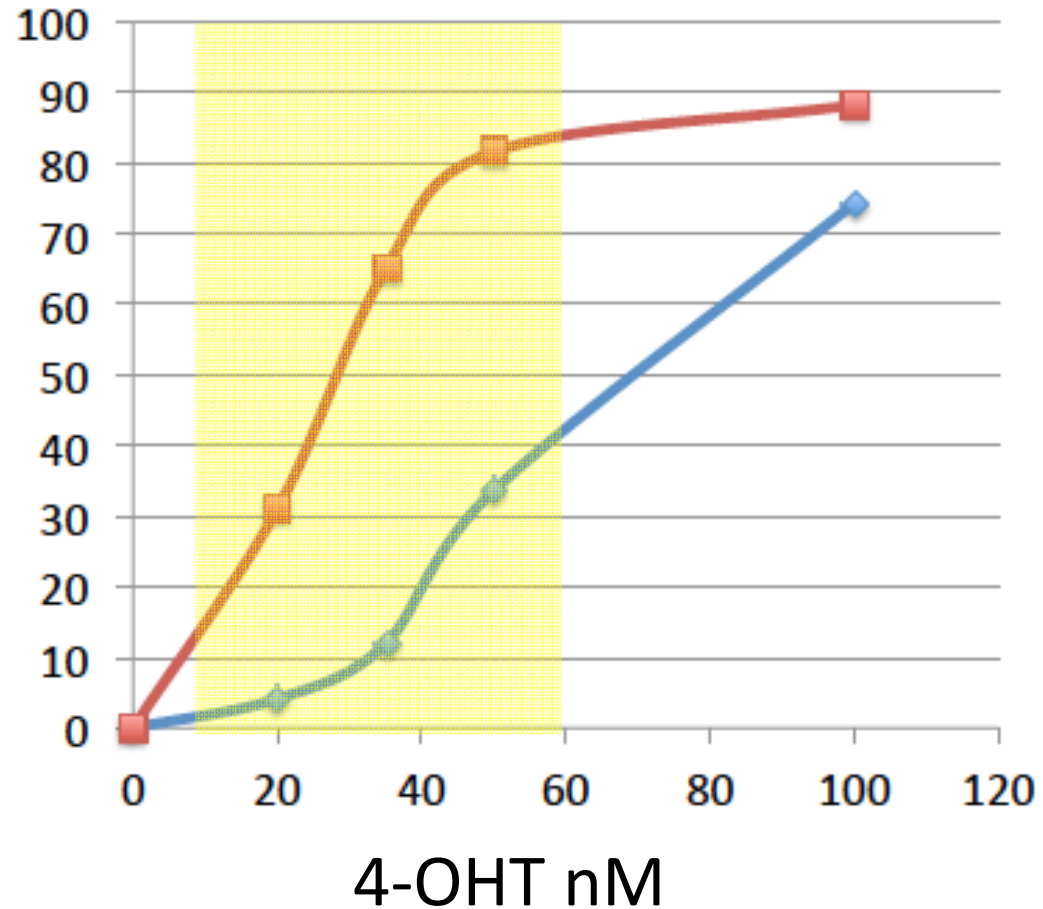


TamR-LV tf Tuning For High Versus Low Regulated Outputs

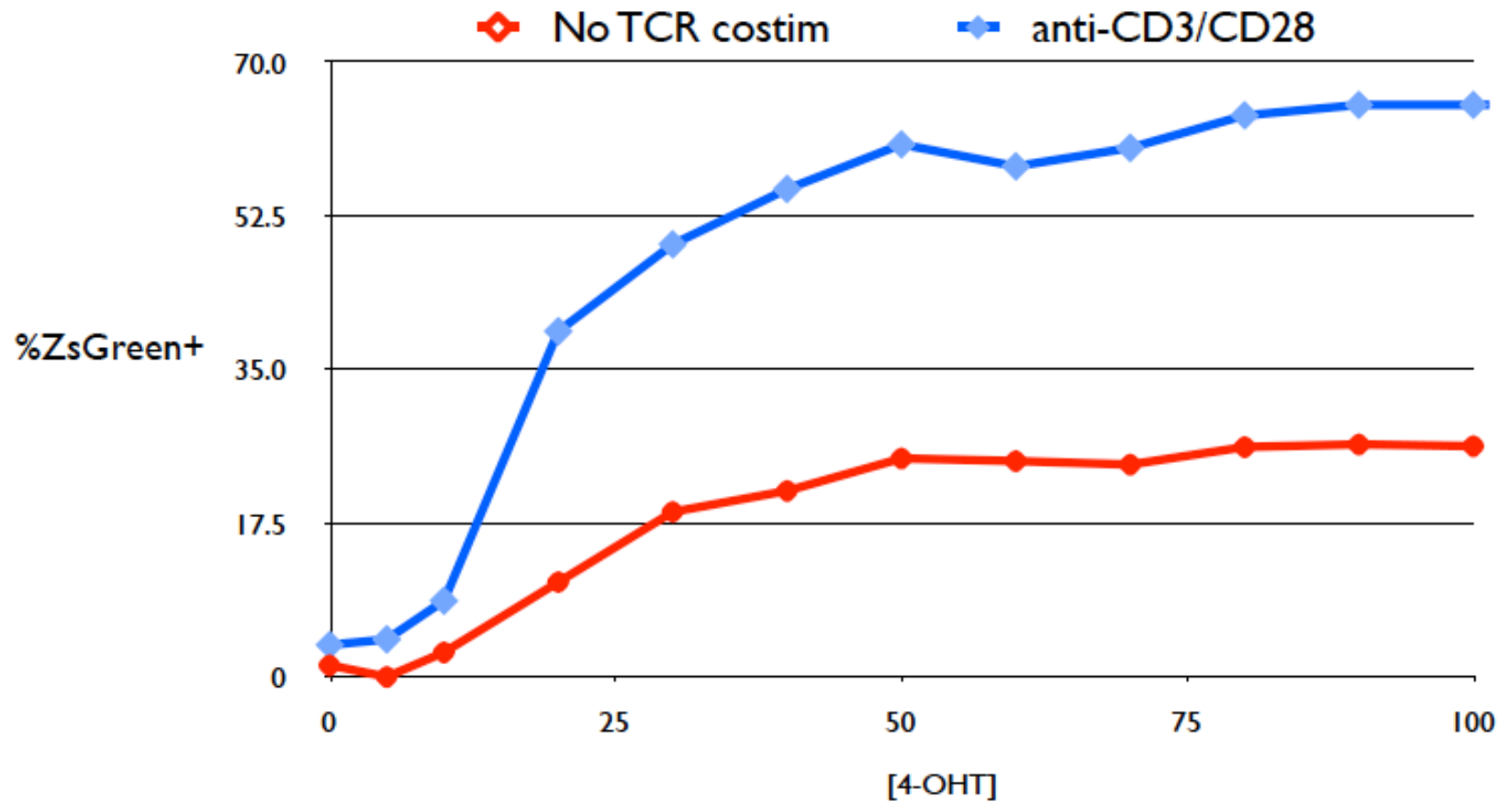
% of maximal
ZsGreen induction

TamR-tf^{high}

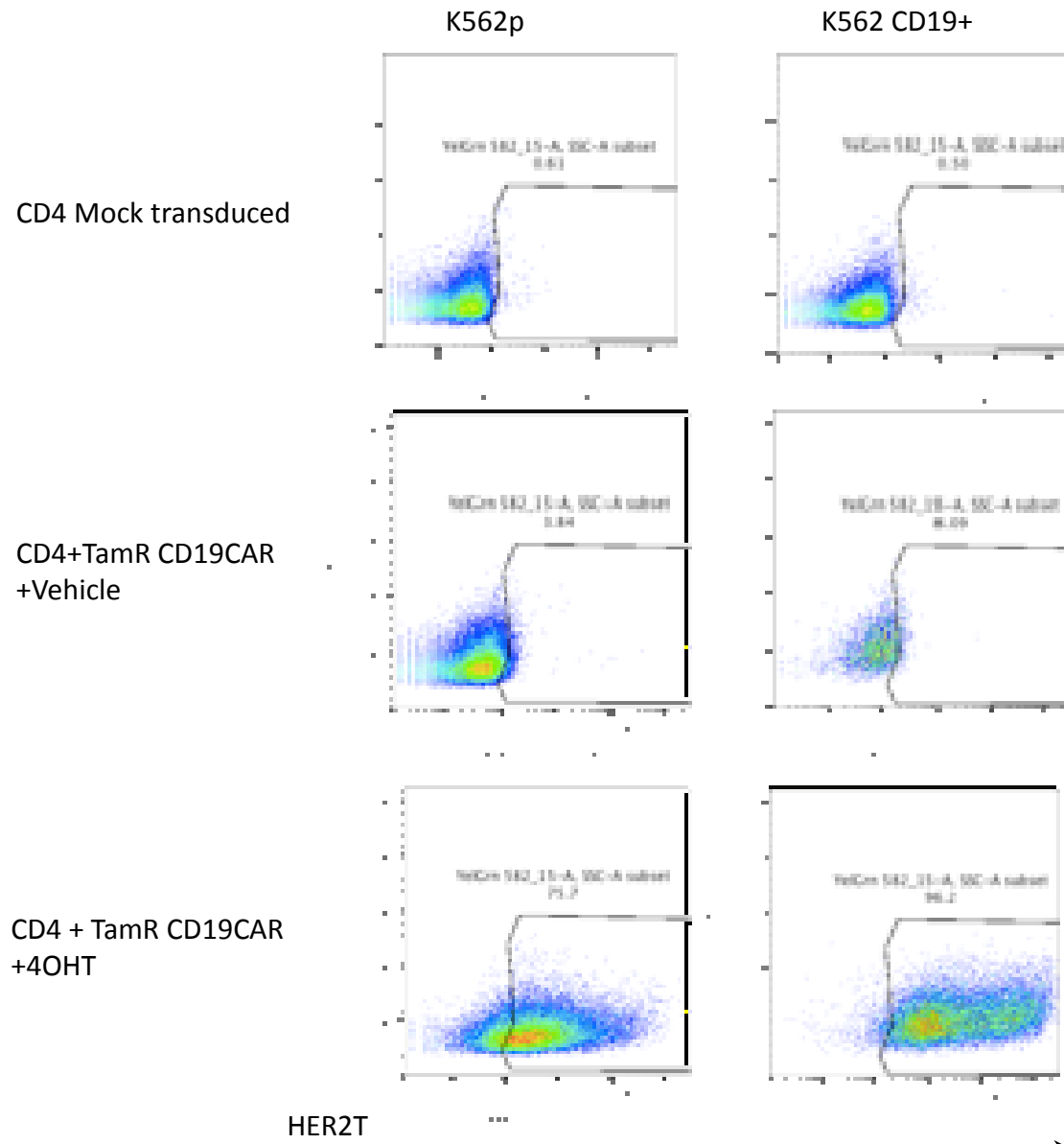
TamR-tf^{low}



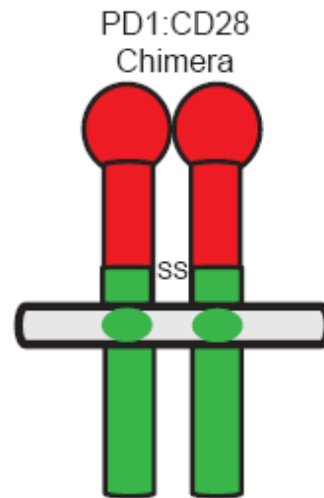
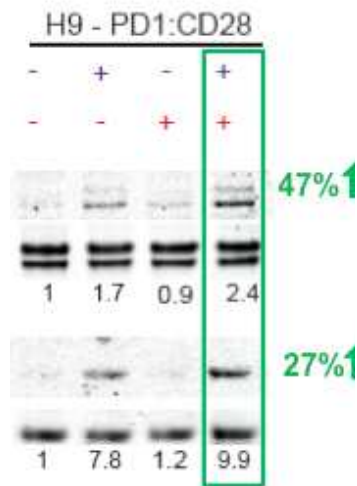
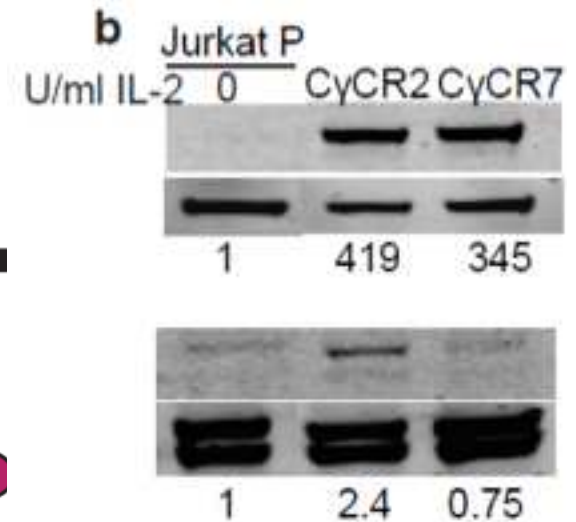
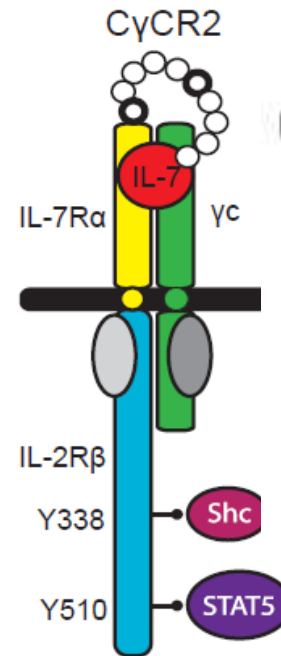
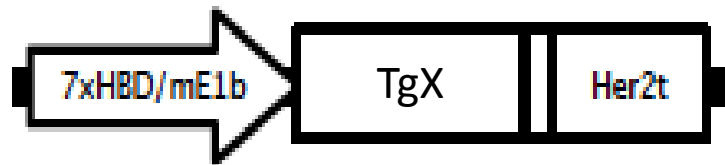
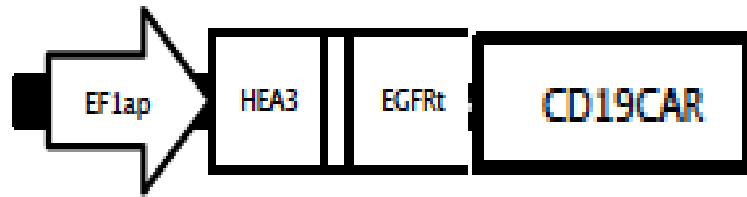
T Cell Activation Amplifies Tam-Dependent TamR LV Transgene Expression Outputs



T Cell Activation Amplifies Tam-Dependent CAR Expression



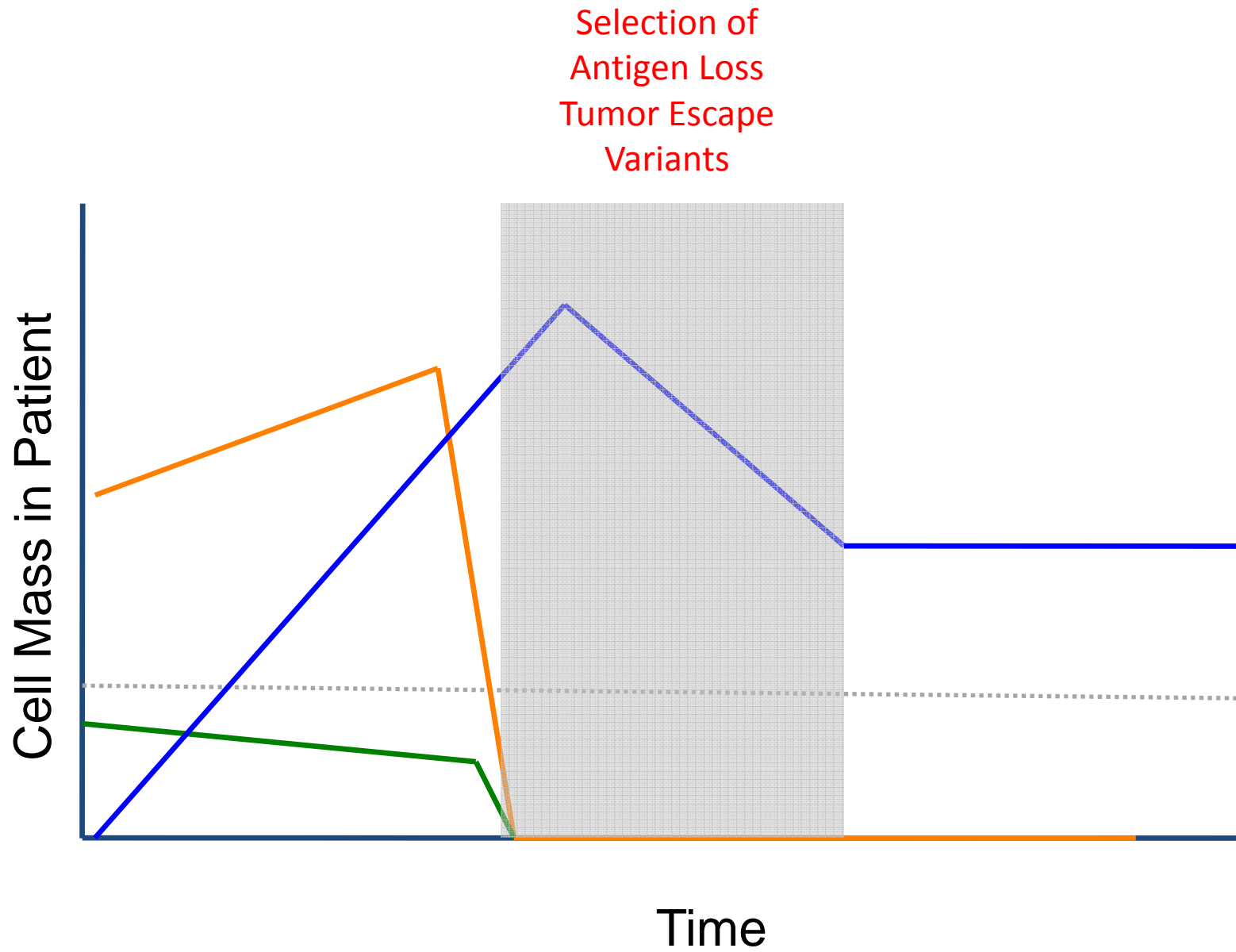
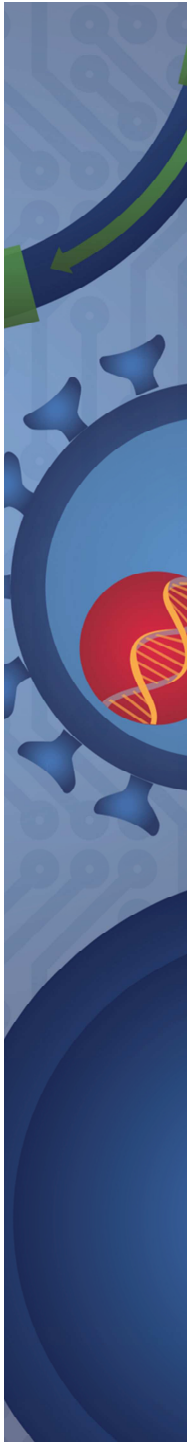
TamR-LV Formats For Regulated Expression of TransgeneX by CAR T Cells





Summary II:

- 1. TamR-LV transgene expression regulation system displays favorable attributes for clinical application.**
- 2. System has tunable features for output states (4-OHT sensitivity).**
- 3. System exhibits context specific (T cell activation) positive feedback.**



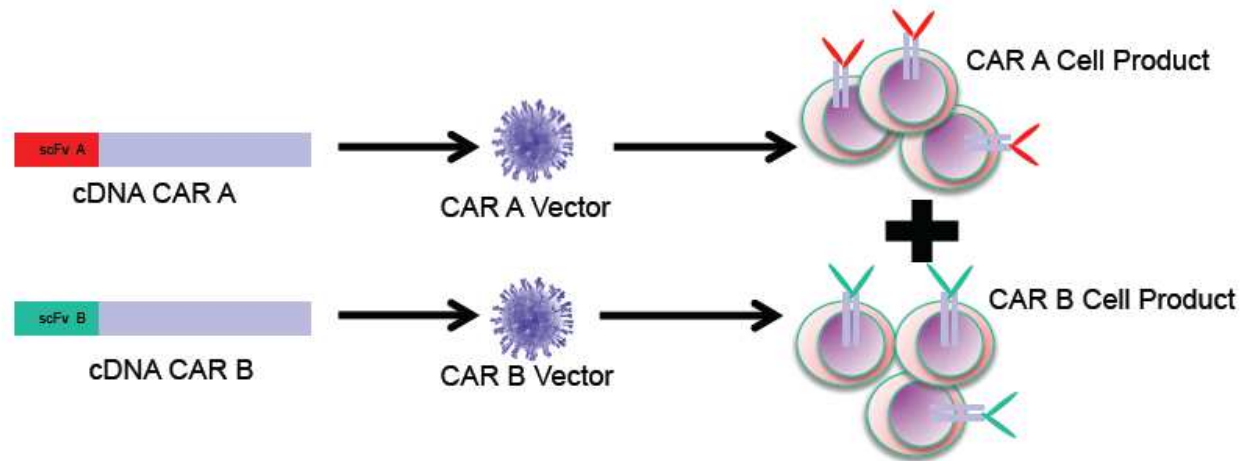


III. Multiplexing CAR T Cell Target Antigen Recognition

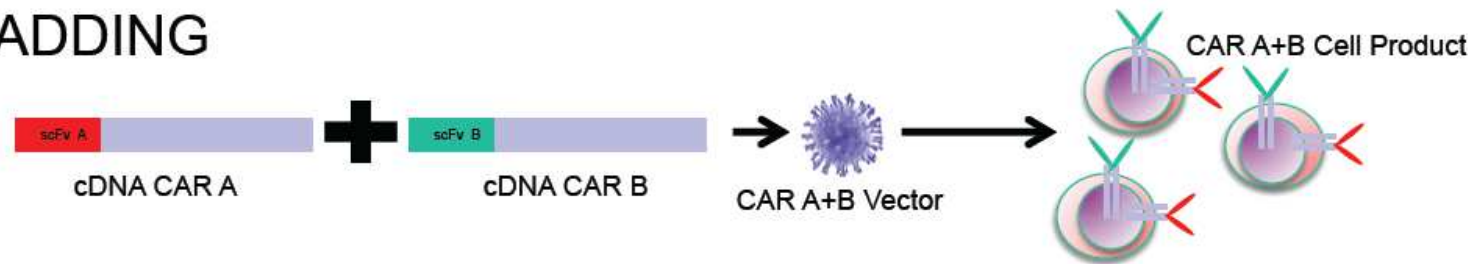
Aggregate data from CHOP, NCI, Seattle suggest CD19 epitope escape loss as etiology of treatment failure in approx. 10% of relapsing patients.

STRATEGIES TO GENERATE CAR T CELL PRODUCTS WITH 2X SPECIFICITIES

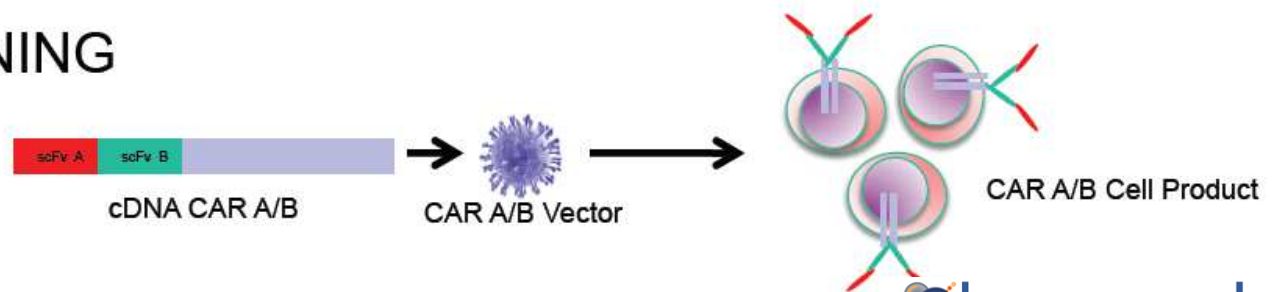
MIXING



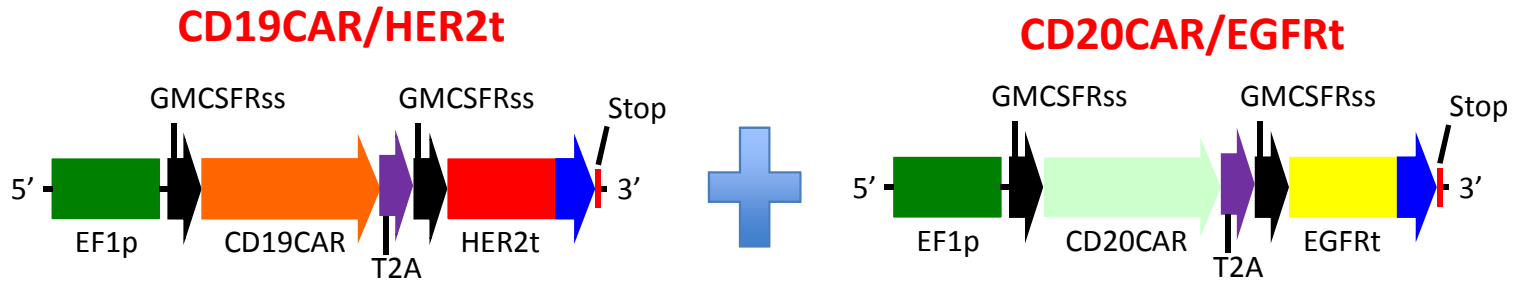
ADDING



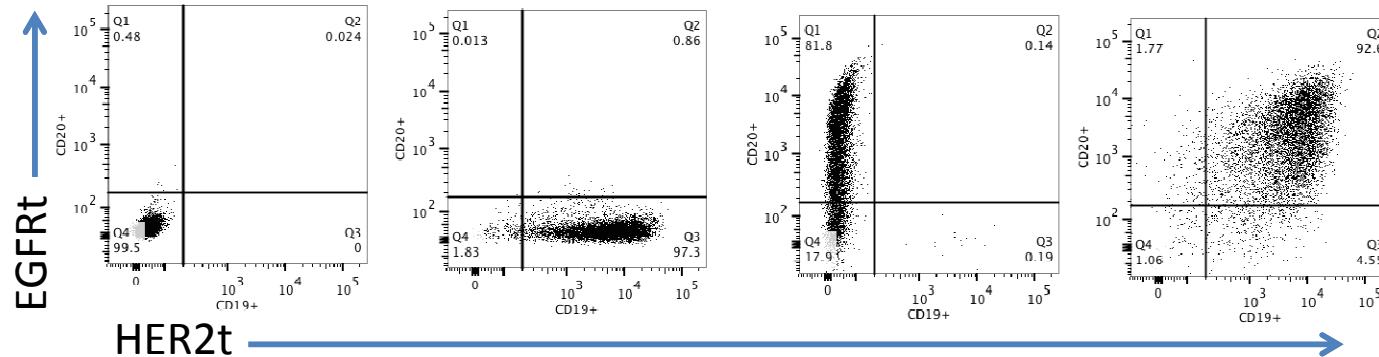
COMBINING



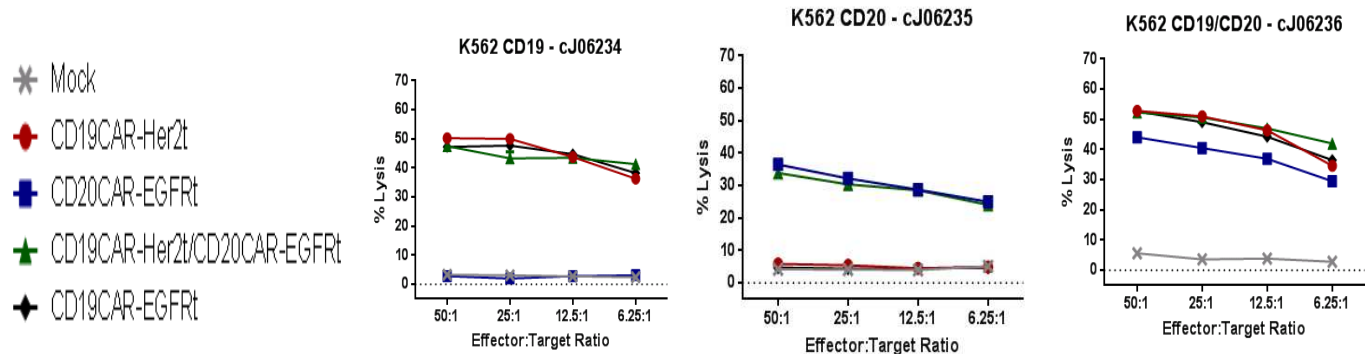
Dual CAR LV's ("Adding")



EGFRt/HER2t Expression by Transduced Human CD8⁺ CTLs

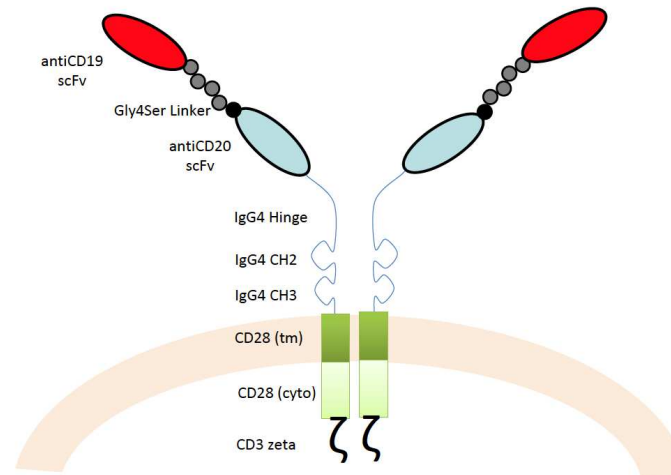


Redirected CD19 and/or CD20 Cytolysis by Human CD8⁺ CTLs



BiSpecific CAR (*“Combining”*)

Schematic of Bispecific antiCD19xCD20 Chimeric Antigen Receptor

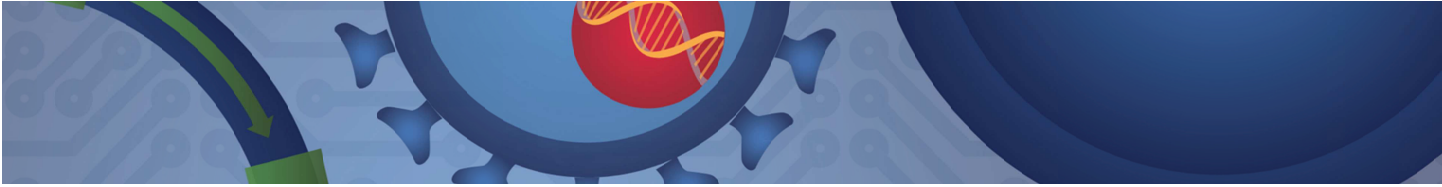


Bispecific Anti-CD19xCD20 CAR Components:

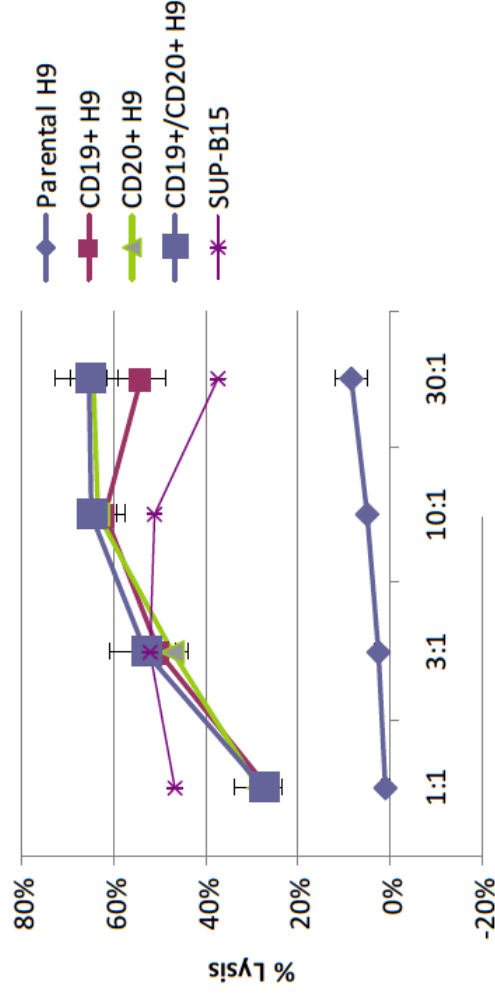


Complete cDNA packaged into epHIV-7 lentivirus vector transfer plasmid:

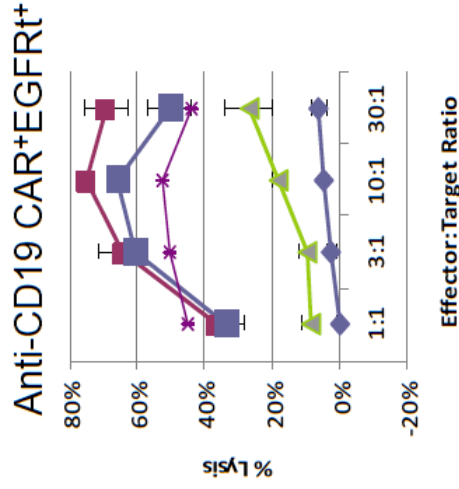




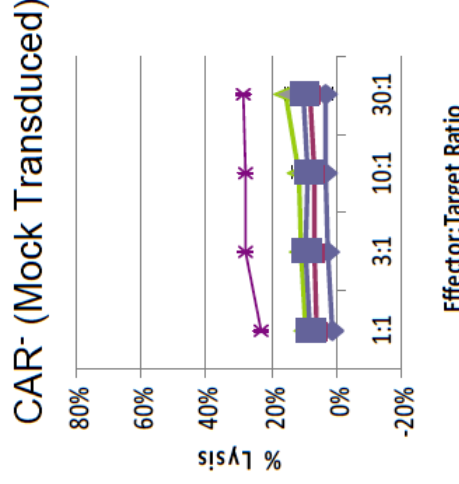
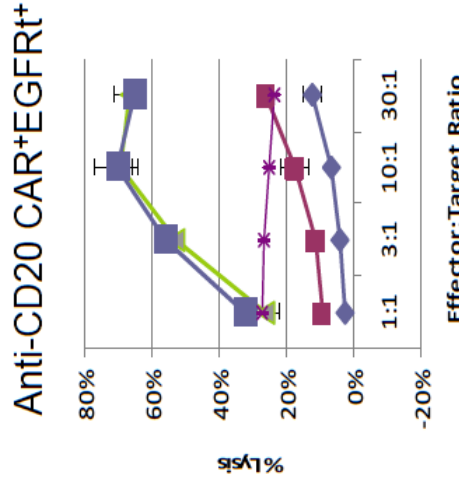
4-hr Chromium Release Assay: Anti-CD19xCD20 CAR+ T Cells Kill Both CD19⁺ and CD20⁺ Target Cells



Effector T Cells: Anti-CD19xCD20 CAR⁺ EGFR⁺



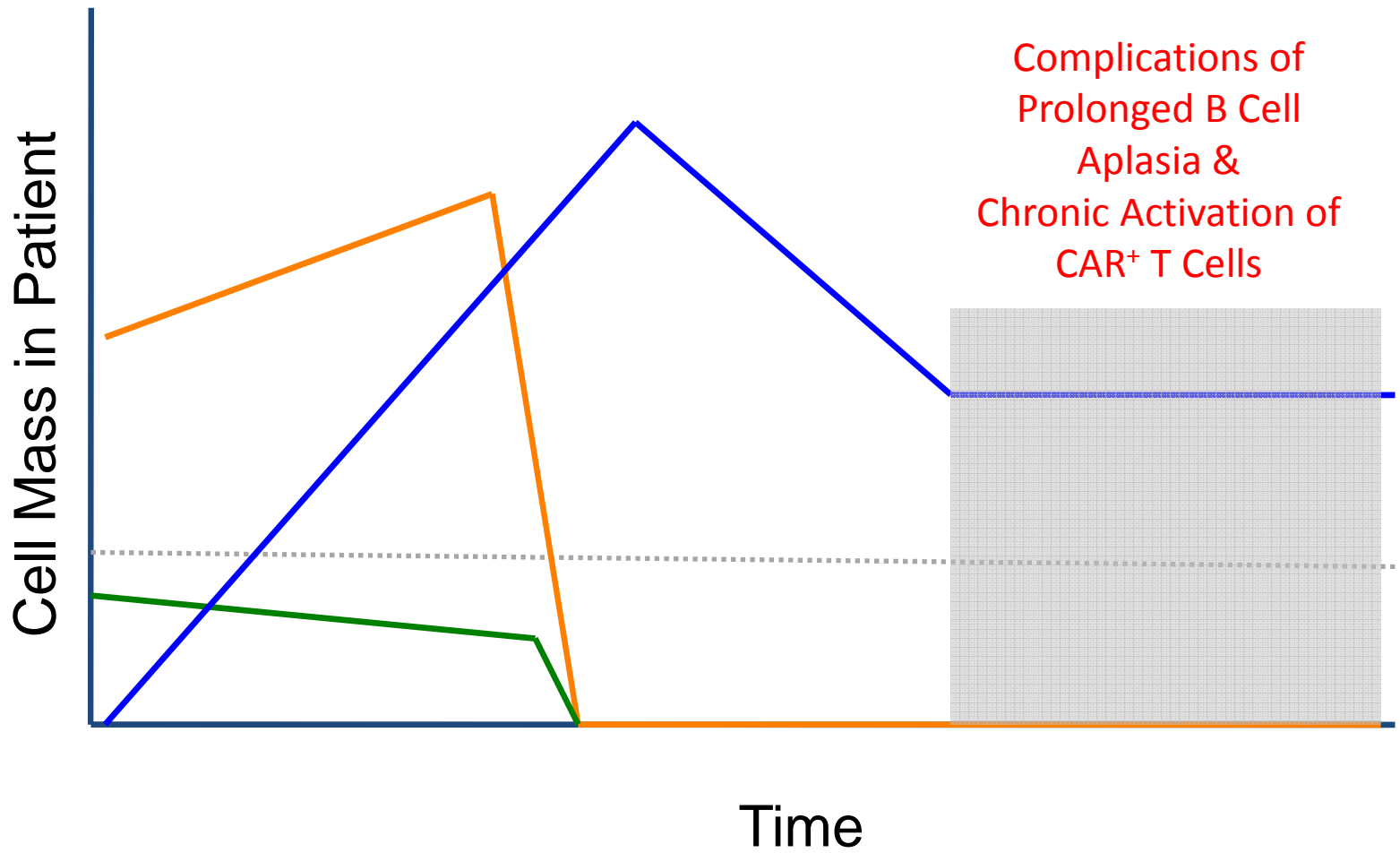
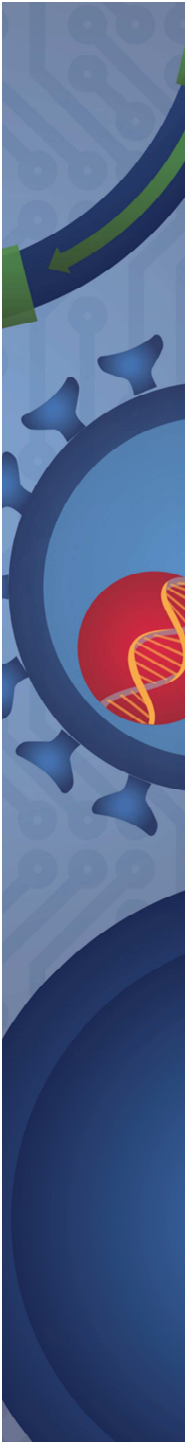
Effector T Cells:





Summary III:

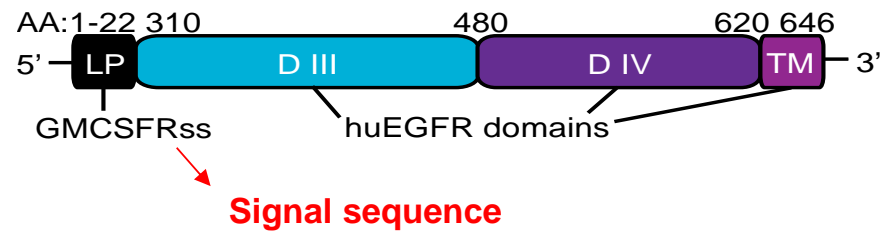
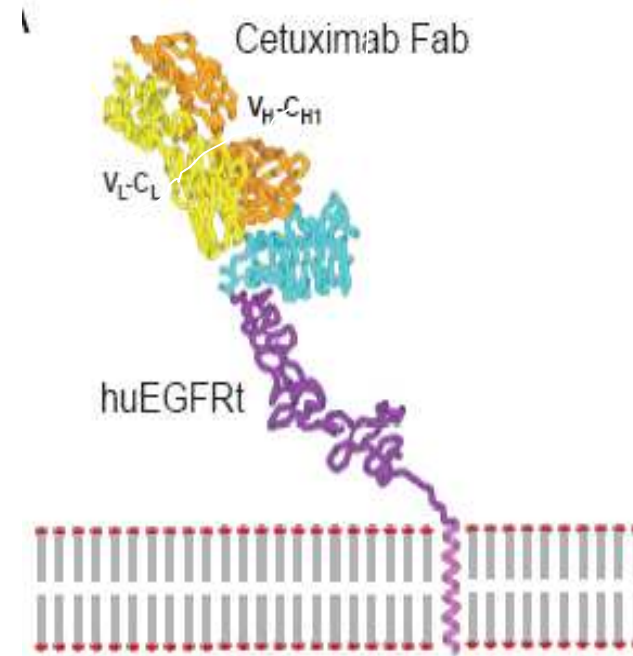
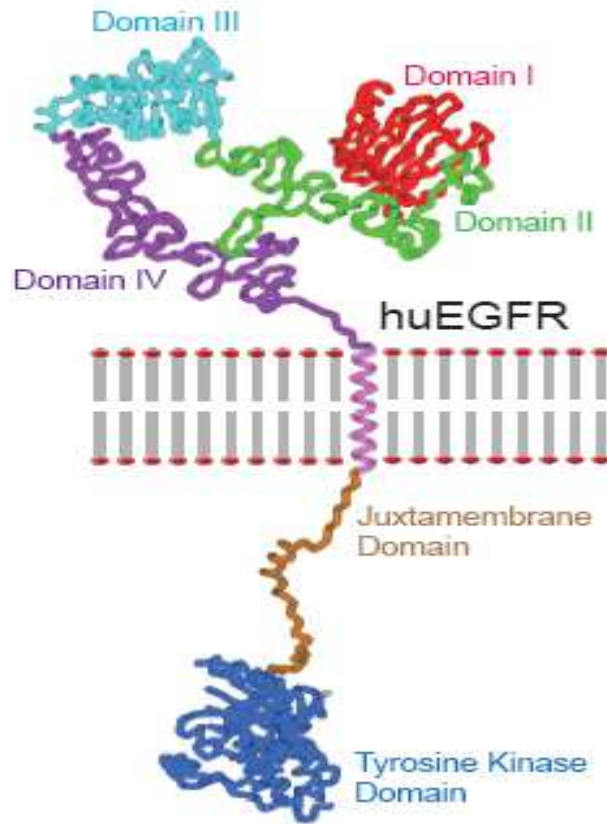
- 1. Multiplexed antigen specificity is feasible and can be accomplished in a single LV vector.**
2. Targeting 2 antigens on tumor cells expected to diminish antigen escape as etiology of treatment failure.



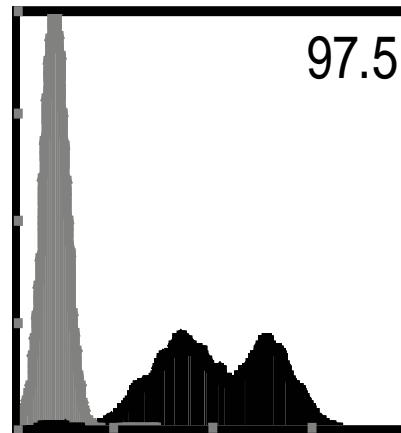


IV. Control of CAR T Cell Persistence

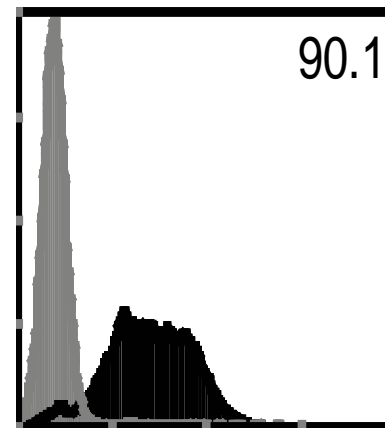
Construction of truncated human EGFR (huEGFRt) with retention of Cetuximab binding epitope



huEGFRt can be incorporated into lenti-viral vector for co-expression with CD19 chimeric antigen receptor (CD19CAR)

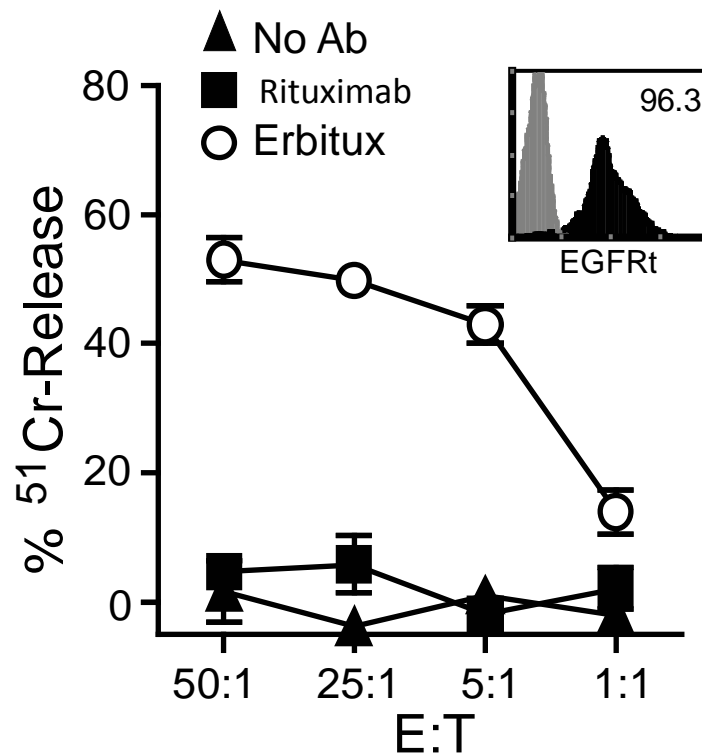


CD19CAR
(anti-Fc-Bio+SA-PE)



EGFRt
(Ctxmb-Bio+SA-PE)

huEGFRt sensitizes huEGFRt⁺ human T cells to Cetuximab mediated ADCC



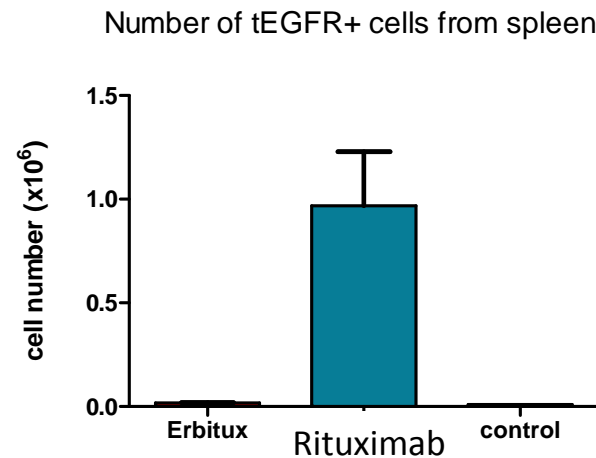
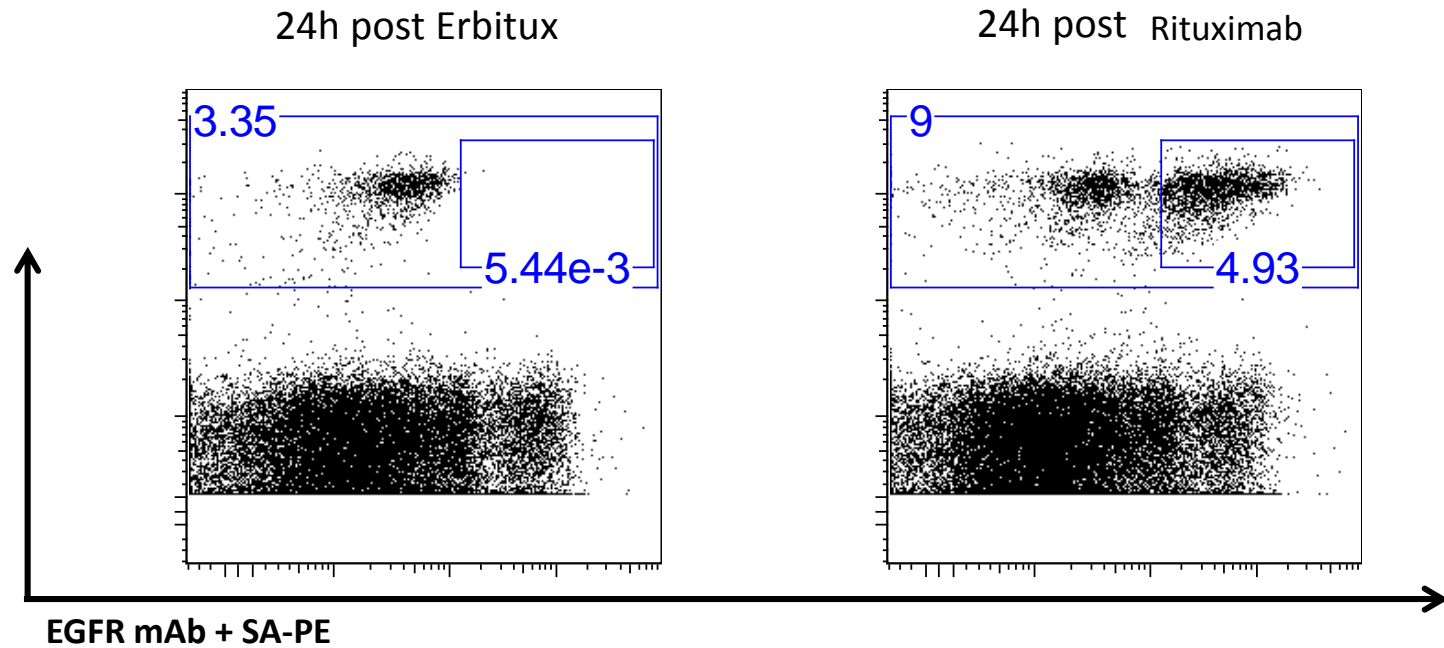
Targets: ⁵¹Cr-labeled huEGFRt⁺ T cells;

Effectors: GM-CSF stimulated huPBMC

Mixed with 1ug/mL Cetuximab or Rituximab (anti-CD20) for 4hr

In vivo: Depletion of EGFRt+ cells

Frequency of transferred cells in blood (FACS analysis)



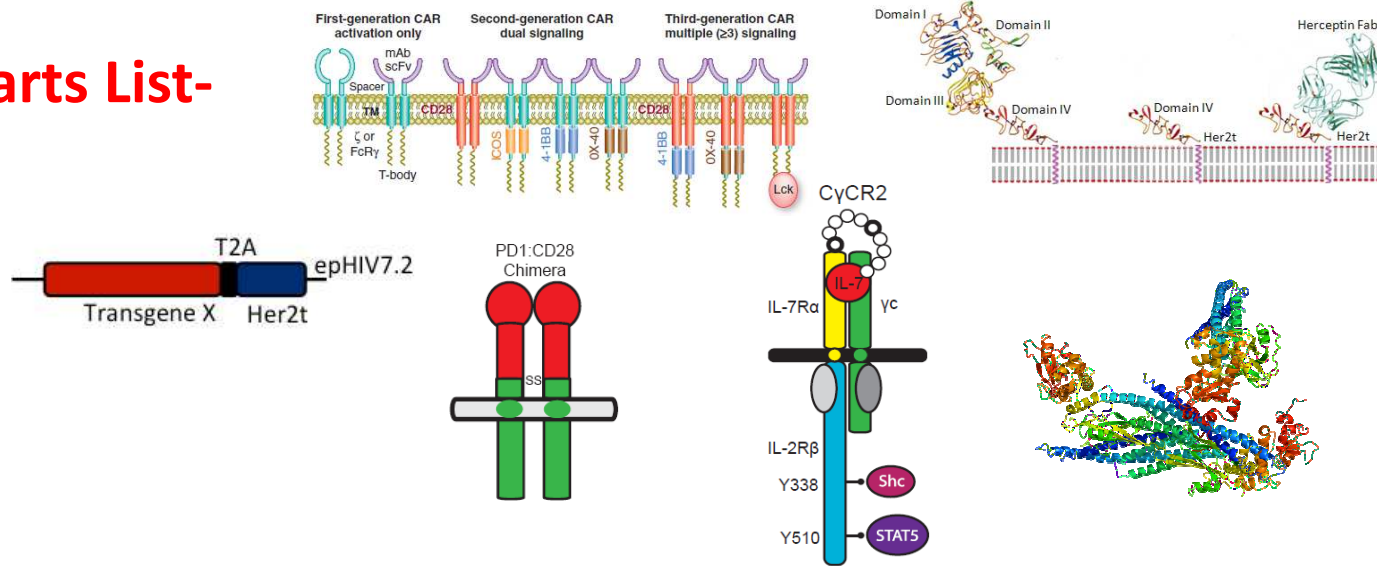


Summary IV:

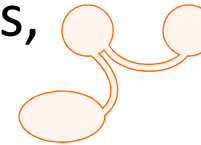
- 1. EGFRt can serve as a suicide construct based on in vitro and murine models.**
- 2. The efficacy of cetuximab mediated ablation of EGFRt⁺ T cells in humans is unknown.**

SynBio T Cells FUTURE STATE: **Version 3.0**

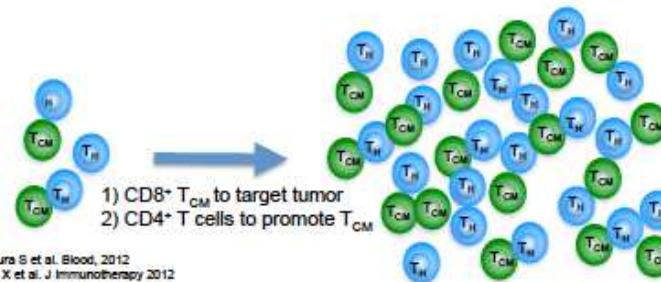
Parts List-



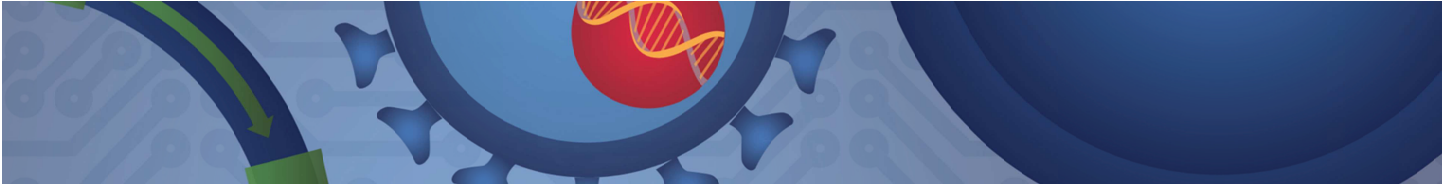
Devices- Expression Rheostats, Sensors, Logic Gated Bio-Circuits



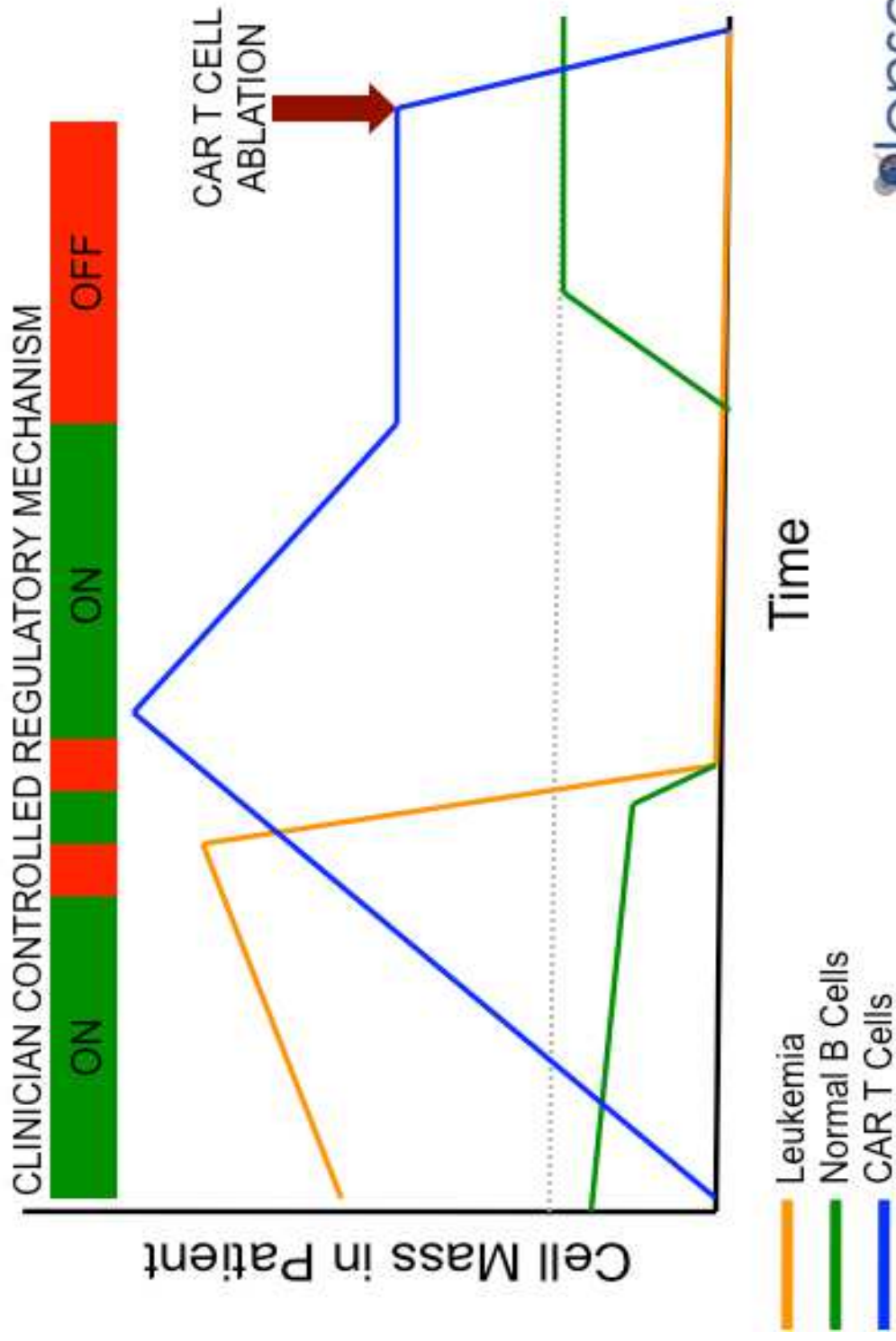
Chassis- Defined combinations of T cell subsets



Terakura S et al. Blood, 2012
Wang X et al. J Immunotherapy 2012
Stemberger C et al. PLoS One, 2012



Future State: Defined Cellular Composition/Multiplexed Antigen Recognition/Clinician Controlled Regulation/Effective suicide ablation





Jensen Lab

Advanced Lymphocyte Engineering



FRED HUTCHINSON
CANCER RESEARCH CENTER

A LIFE OF SCIENCE

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Annette Kuenkele, MD (L1-CAM CAR)

Kaileen Rohr (TamR-Tg)

Anne Silva, MD (spacer/bispecific)

Cindy Chang (mouse models)



Paulina Paszkiewicz (Busch Lab)
(EGFRt/Erbitux ablation)



Xiuli Wang, MD, PhD
(human Tcm/EGFRt)
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SU2C/St. Baldrick's Dream Team

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