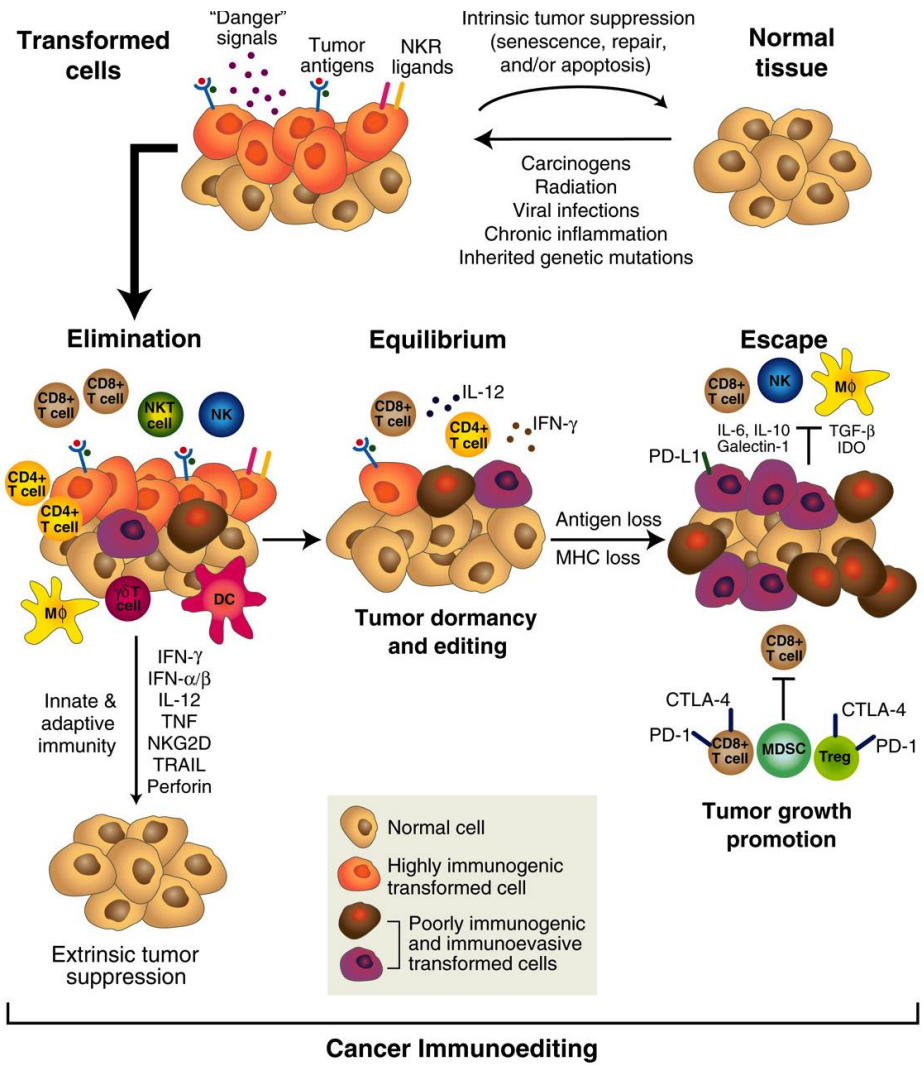
Engineering Chimeric Antigen Receptor Modified T Cells Of Defined Composition for Cancer Therapy

Fred Hutchinson Cancer Research Center

Local and Systemic Tolerance Mechanisms Prevent An Effective T Cell Response To Cancer



From Schreiber R, Old LO, Smyth MJ Science 2011

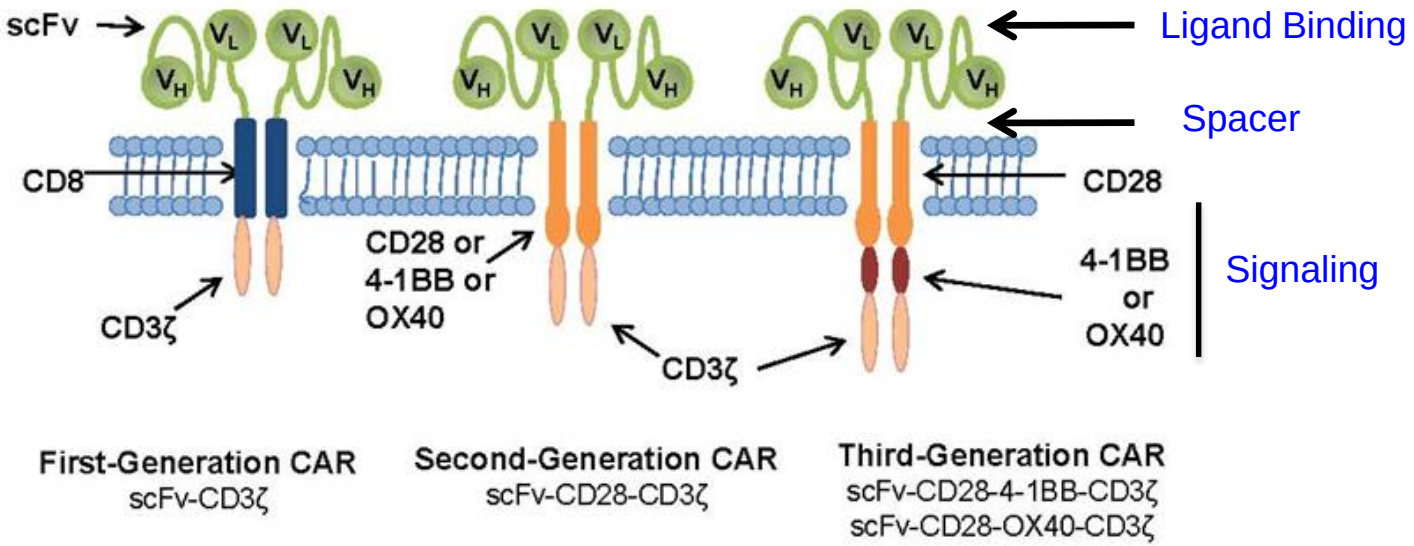
Multiple Mechanisms For Tolerance

- Insufficient functional tumor-specific T cells
  - Deletion
  - Exhaustion/functional suppression (PD-1, CTLA-4, TGF-b, IDO, others)
- Antigen or HLA loss on tumor cells
- Inhibition of T cell migration

Genetic Modification To Overcome Tolerance

- Introduce high affinity TCR or chimeric antigen receptor (CAR)-modified tumor-reactive T cells
- **TCRs** – target molecules involved in malignant transformation/progression
- **CARs** – HLA independent
- Gene transfer enables control **of the T cell subsets used for therapy**

Features of chimeric antigen receptor (CAR) design

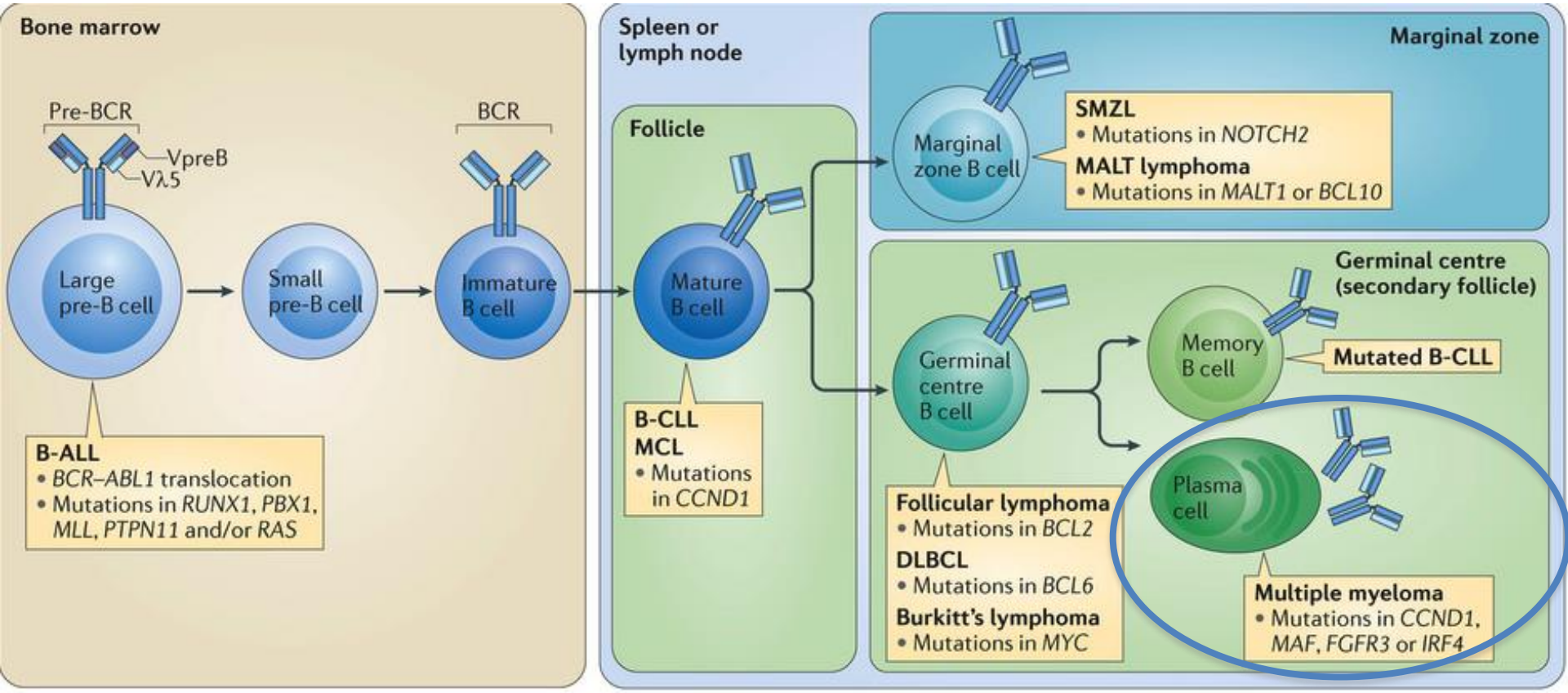


Tumor cell surface target molecules

- **CD19**
- CD20
- GD2
- Mesothelin
- Folate R
- **ROR1**
- Others

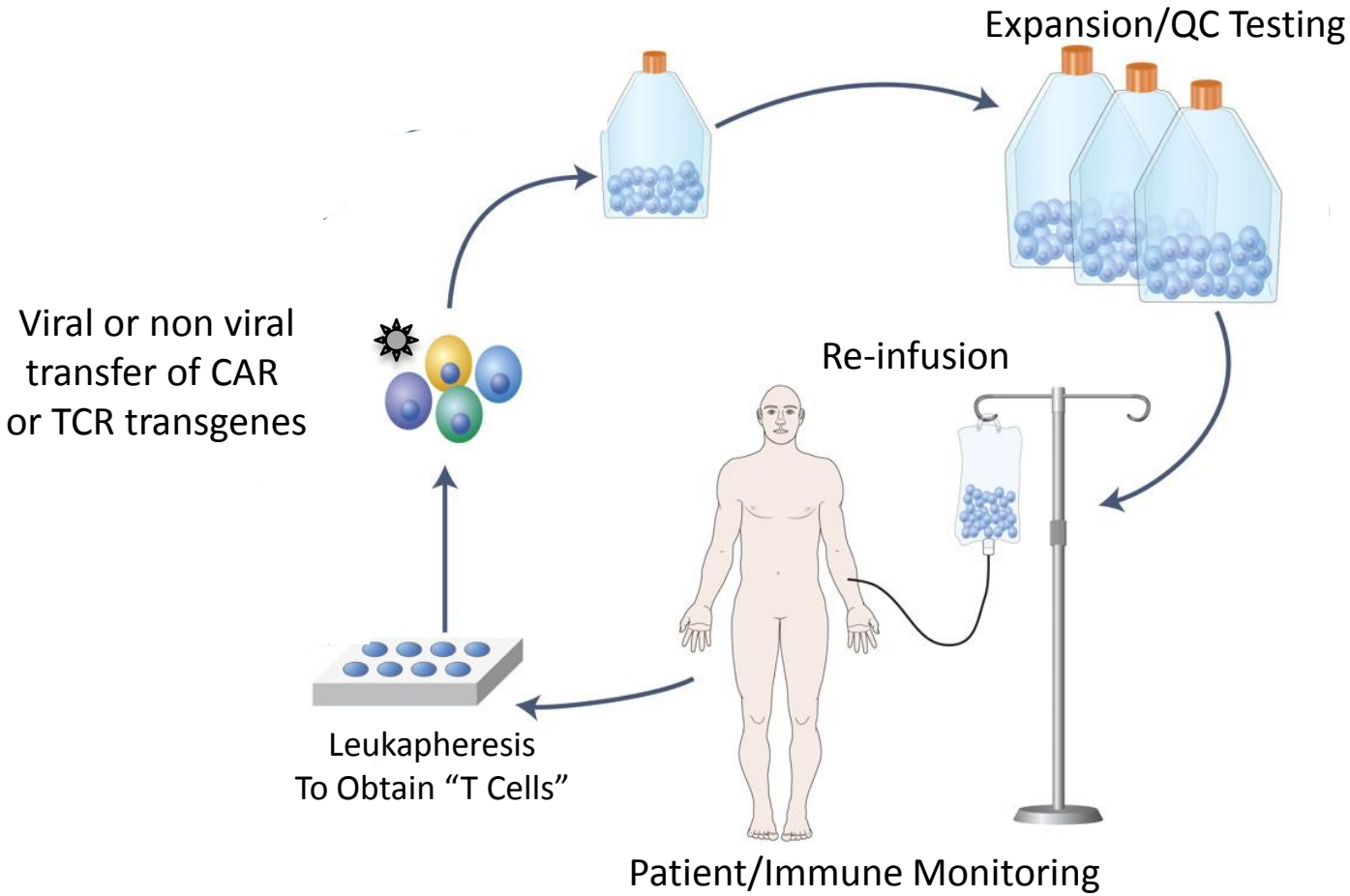
Targeting the Lineage Specific CD19 Molecule on B-Cell Leukemia (ALL, CLL) and Non-Hodgkin Lymphoma

- **CD19 is expressed at all stages of B cell development until the mature plasma, and is retained on malignancies that arise from various stages of B cell maturation**



Nature Reviews | Immunology

Without Selection of T Cells – Composition of CAR - Modified T Cell Products Will Be Highly Variable



- T cells are present in distinct subsets with different proliferative capacity/function
- Frequency of T cell subsets varies dramatically in individuals (especially cancer patients)
- Decline in telomere length with progressive differentiation of T cells which limits in vivo persistence

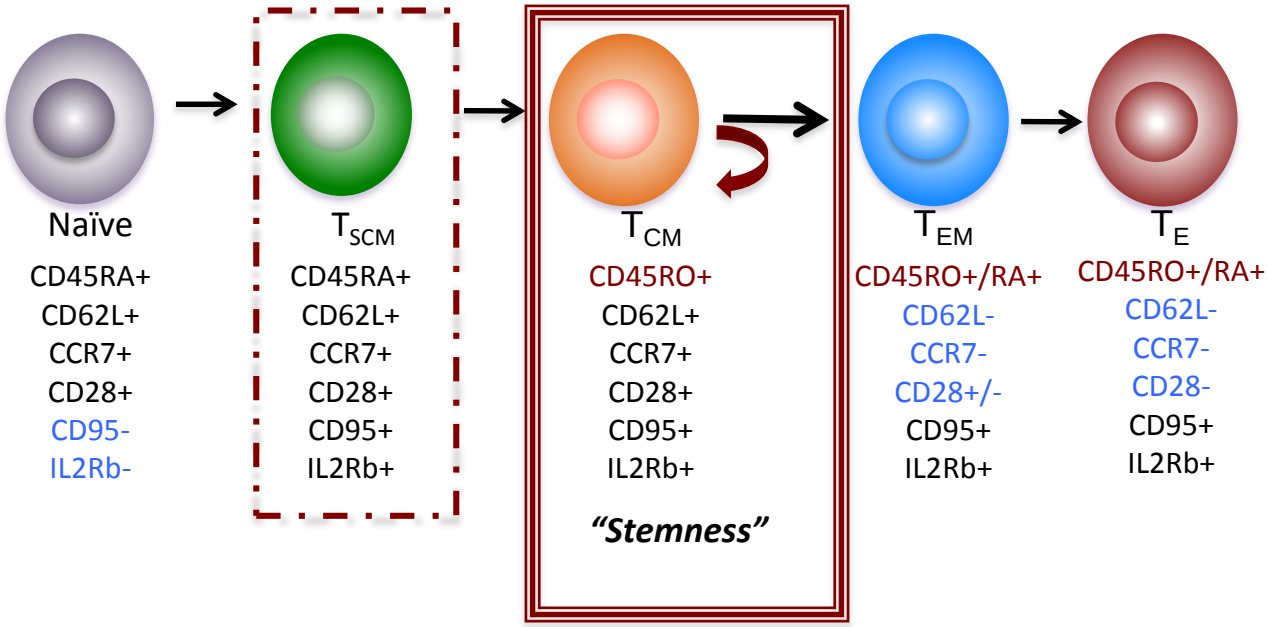
Phenotype of Infused 19-28z–Transduced T cells – MSKCC Trial

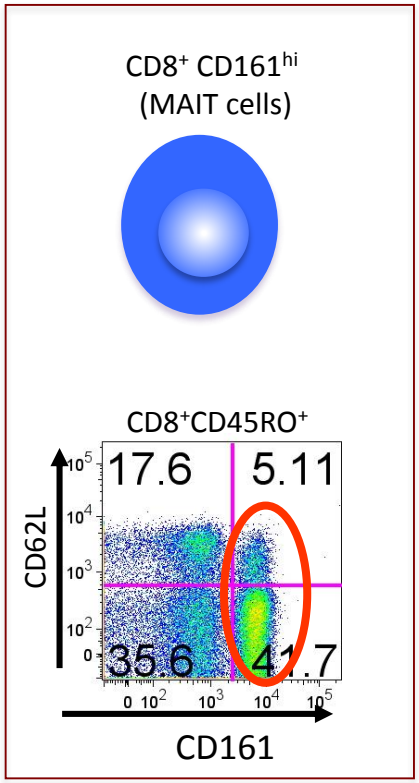
Patient	CD4+	CD8+	CD28+CD27−	CD28+CD27+	CD62L+	CCR7+	CD25+CD4+Foxp3+	Days in culture
CLL-1	94	5	83.2	11.5	9.1	ND	1.2	17
CLL-2	96	5	86.1	6.1	15.5	ND	0.61	19
CLL-3	93	8	40.6	2	17.7	ND	2.4	18
CLL-4	**99**	**0.7**	86.9	5.9	34.4	ND	0.9	15
CLL-5	87	12	56.6	20.8	40	7.7	0.7	18
CLL-6	79	21	45.8	30.8	51.5	3.4	1.3	15
CLL-6	78.6	20.8	57	19.3	52	3.7	ND	ND
CLL-7	58	27	41.3	13.5	63.4	1.1	1.2	15
CLL-8	91.5	8.5	81.7	9.1	33.1	19.6	ND	16
ALL-2	**52**	**48**	5.2	84.8	94.8	47.4	ND	13

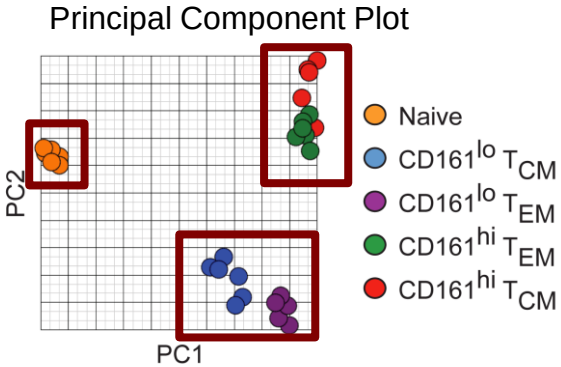
Adapted from

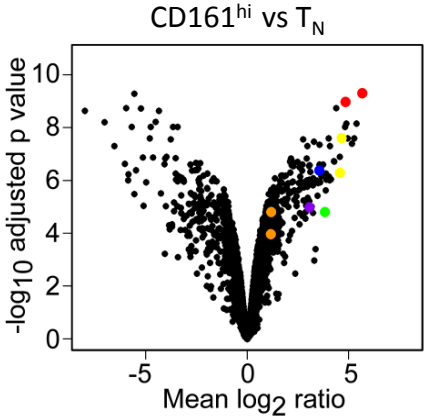
Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias **Brentjens et al Blood, 2011**

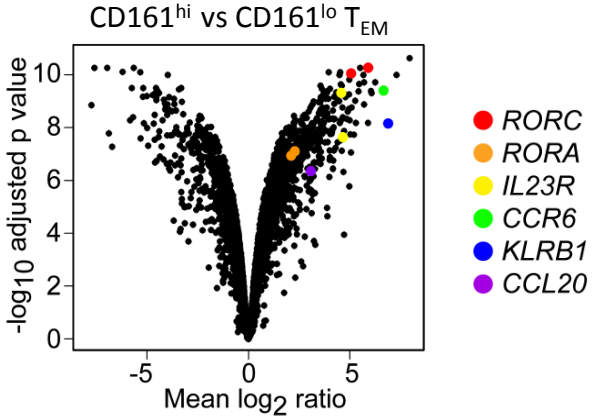
Heterogeneity of T Cell Subsets – CD8 T Cells







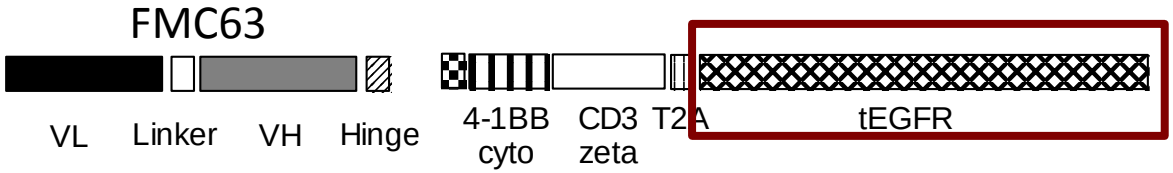




Turtle et al. Immunity, 2009; Blood 2011

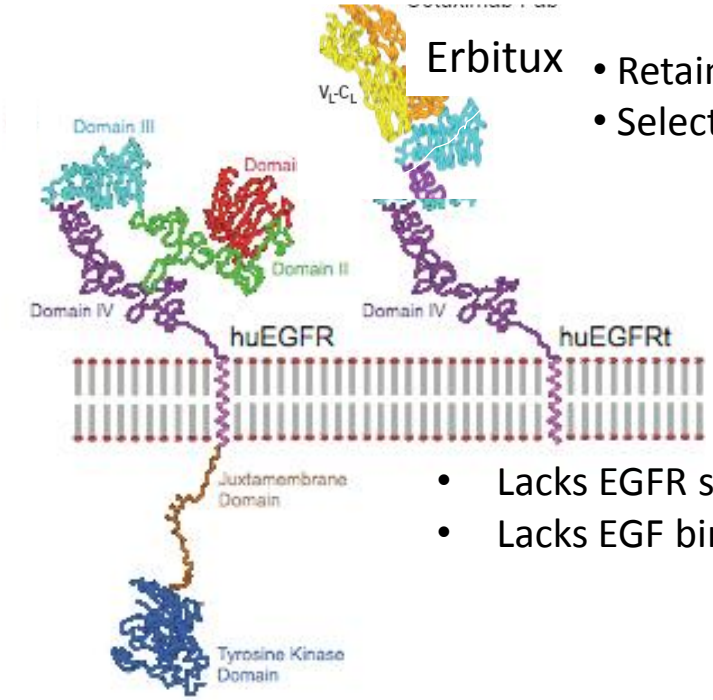
CD19 CAR Design To Facilitate Analysis of Cell Product Composition

CD19 CAR In Phase1/2 Trial

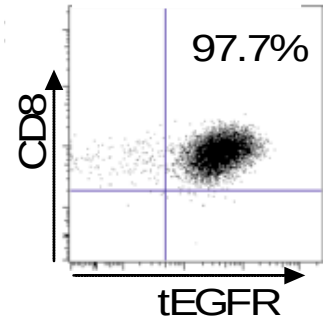


'short/4-1BB'

Truncated EGFR



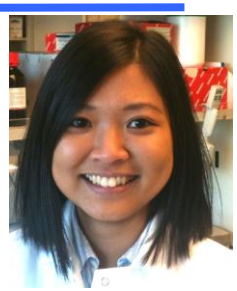
- Retains Erbitux binding
- Selection and tracking of T cells



- Lacks EGFR signaling
- Lacks EGF binding

Wang et al Blood, 2011

CD8+ Central Memory and Naive T Cells Modified With A CD19 CAR Are Superior to Effector Memory T Cells in Eradicating Disseminated Lymphoma in NSG Mice

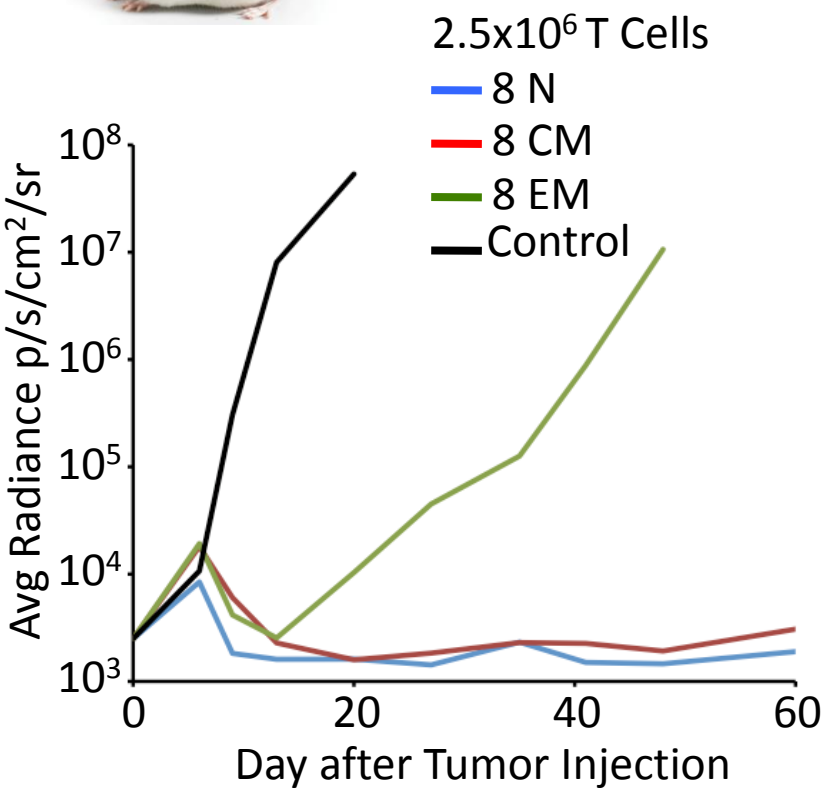


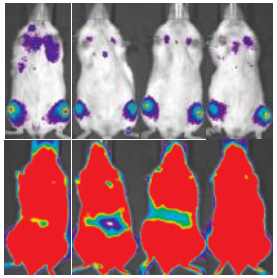
Tumor Inoculation
($5x10^5$ Raji ffluc IV)

T cell transfer

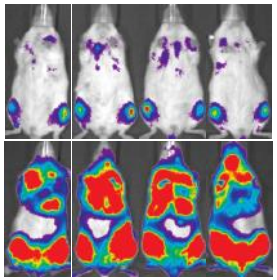
Serial imaging

0 d7 t

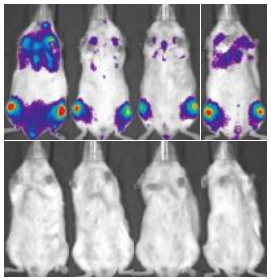




Control T Cells



CD19 CAR T_{EM}



CD19 CAR T_{CM}

Antitumor Activity of Titrations Of Purified CAR-T Cells Derived from CD8+ T_{CM}

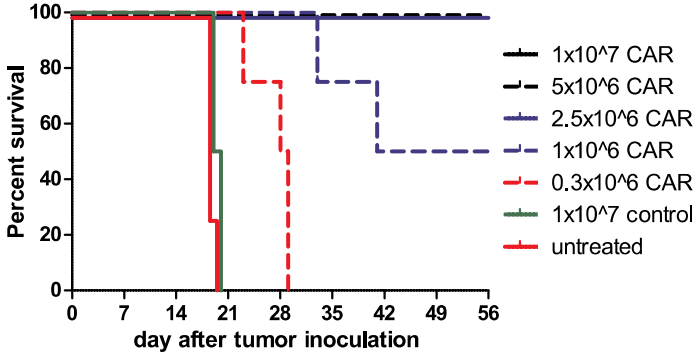
NSG/Raji tumor model:

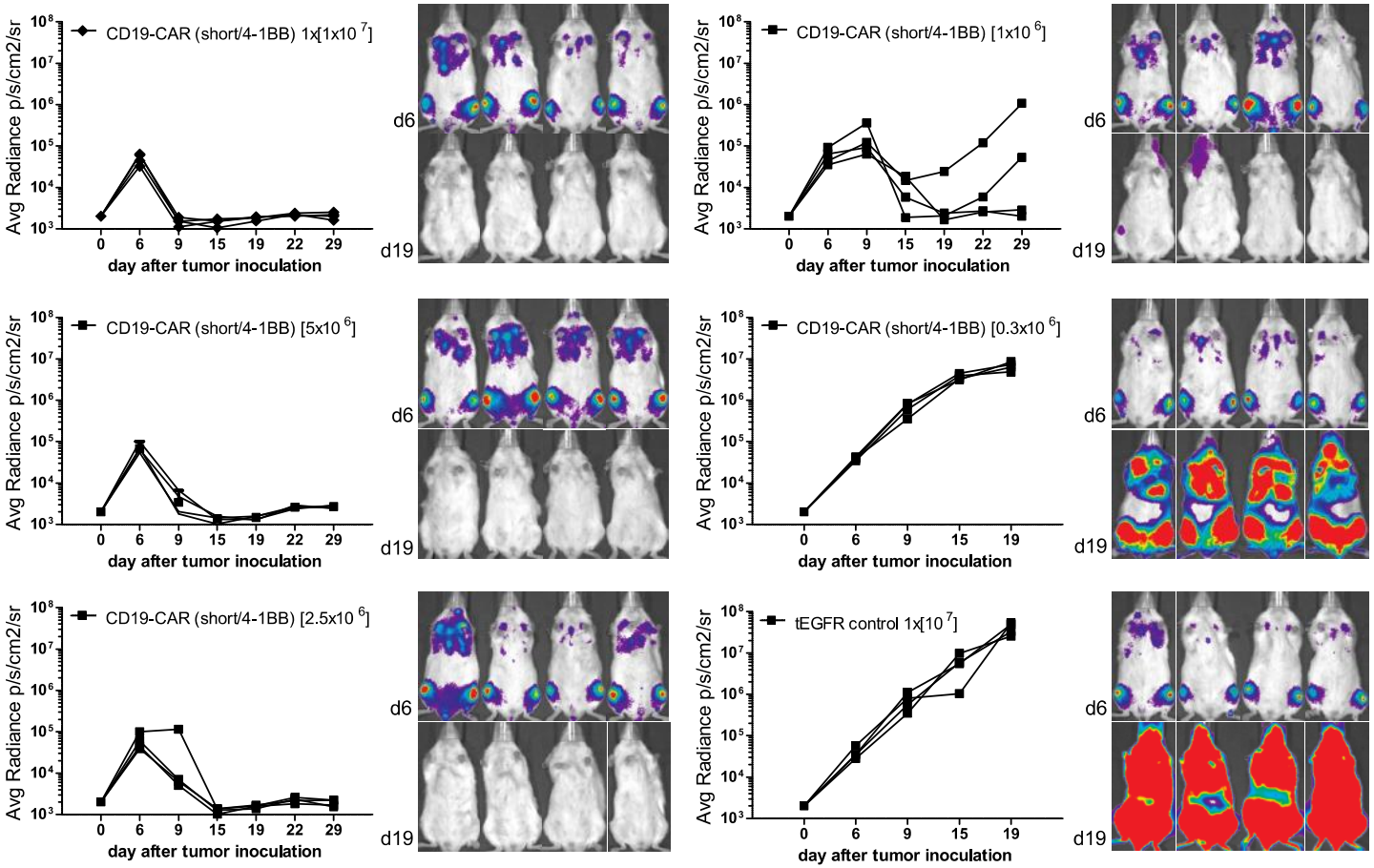
Tumor inoculation: 0.5×10^6 Raji-ffluc i.v.

T-cell transfer: 0.3 - 10x10^6 CTL i.v.

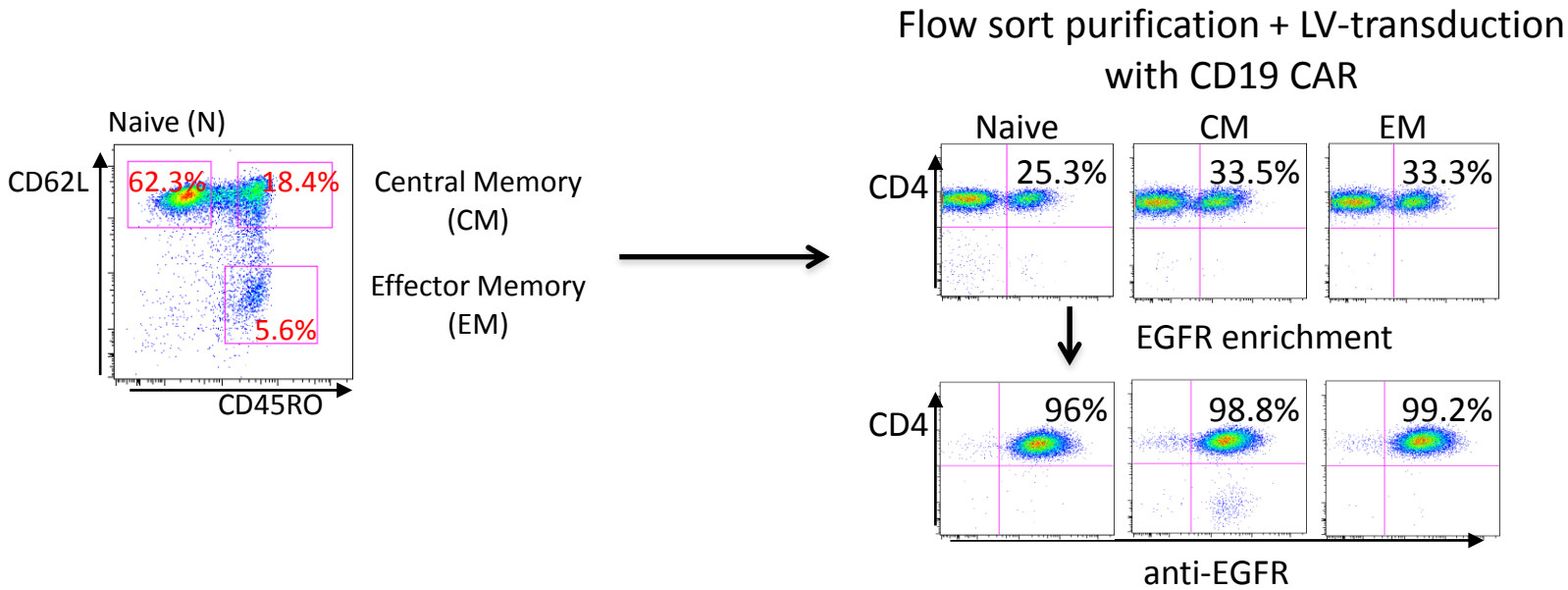
Serial tumor imaging

d0 d7 t



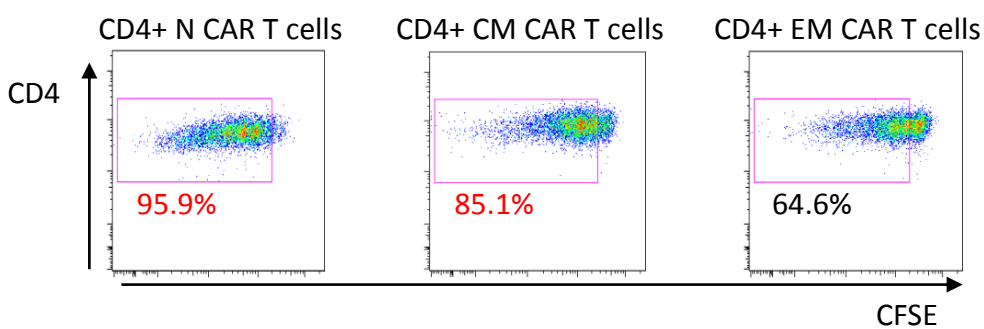


$CD4^+$ CAR-T cells derived from naive, central and effector memory subsets exhibit different activity in vitro



Cytokines: CAR modified $CD4^+$ $T_{N/E}$ and $T_{CM/E}$ produce greater IL-2, IFN-γ, and TNF-α than $T_{EM/E}$

CFSE assay – stimulation with K562/CD19



$CD4^+$ CAR-T cells derived from naive, central and effector memory subsets exhibit different activity in vivo

NSG/Raji tumor model:

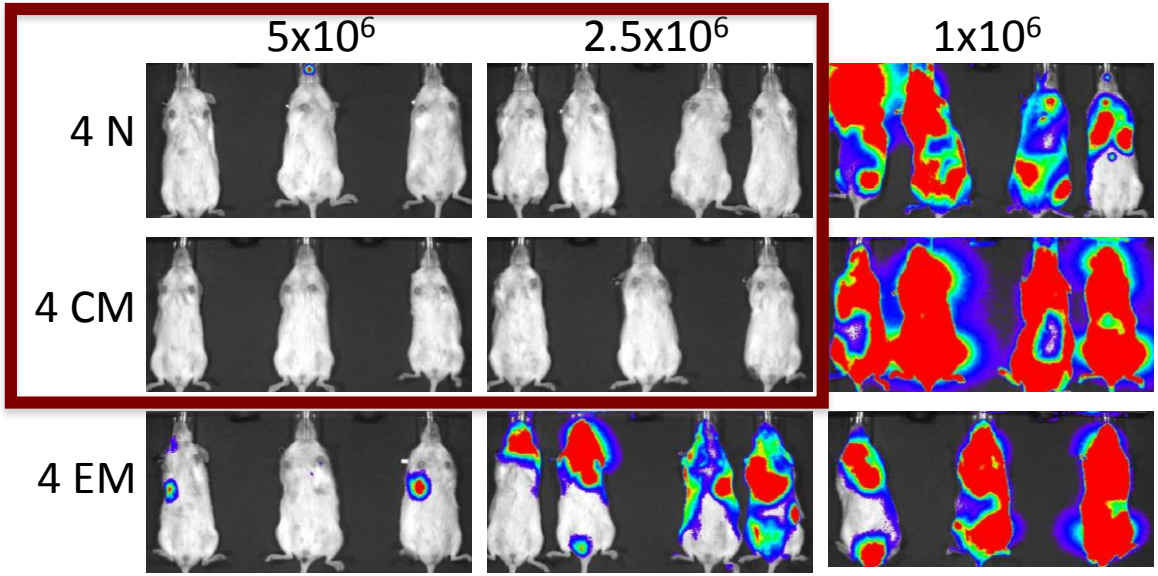
Tumor inoculation: $0.5x10^6$ Raji-ffluc i.v.

T-cell transfer: 0.3 - 10x10^6 CTL i.v.

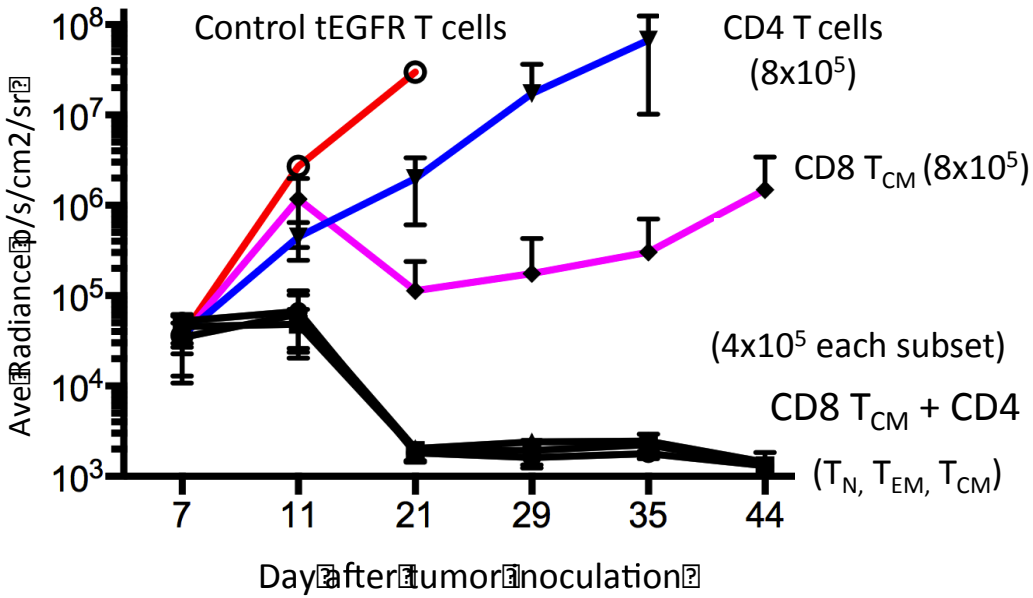
Serial tumor imaging

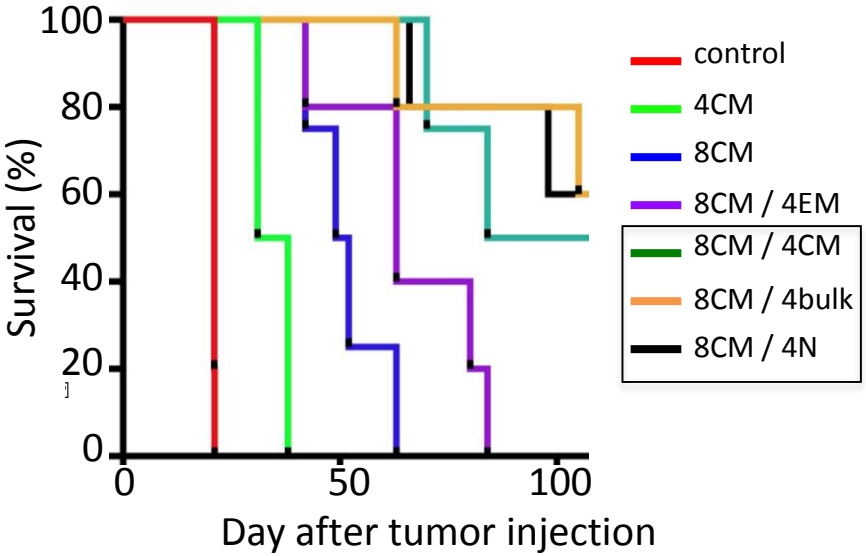
d0 d7 t

Day 35 Post Tumor injection



Defining Optimal Composition – Synergism Between CD8⁺ TCM and CD4⁺ Th Cells

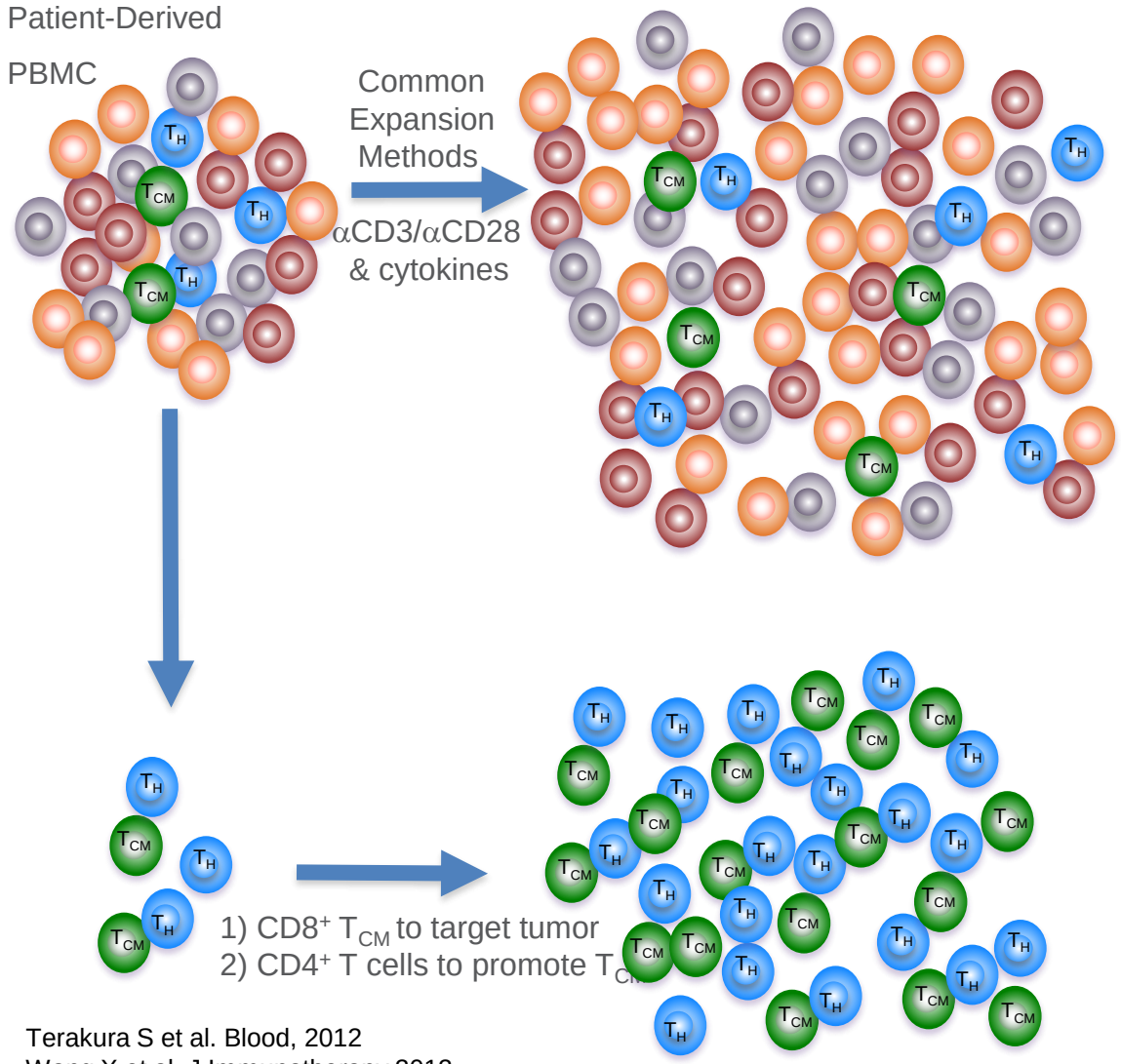




Mechanism: CD4 T cells sustain the proliferation and persistence of CD8⁺ T cells in the presence of large and rapidly progressive tumor burden

Relevance: Synergism of CD4⁺ T cells with CD8⁺ T_{CM} appears to be both CAR and tumor model Independent (CD19 – Lymphoma and ALL, ROR1 – Lymphoma and breast cancer)

Introducing Tumor-Specific Receptors Into Defined T Cell Subsets



Provides:

- Highly variable cell product
- Variable % of CAR T cells
- Requires high cell doses
- May cause toxicity

Provides:

- Defined cell products
- Higher % CAR T cells
- Permits lower cell doses
- Improves efficacy
- Lower toxicity

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

FHCRC Protocol #2639 – First Trial of CD19 CAR T Cells of Defined Composition For Advanced B Cell Malignancies

Disease Cohorts

1. Chronic Lymphocytic Leukemia
2. Indolent NHL/Mantle Cell
3. Aggressive
   - Diffuse Large B-Cell Lymphoma
   - Acute Lymphocytic Leukemia

Trial Objectives

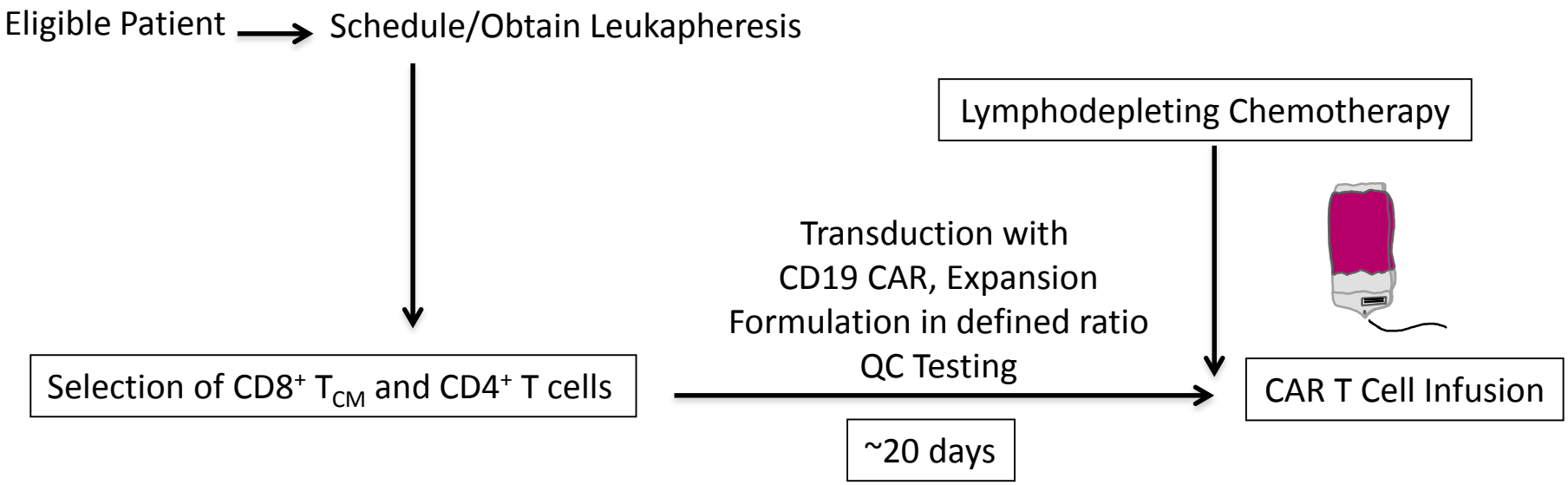
Primary objective

1. Feasibility and safety of adoptive T cell therapy using *ex vivo* expanded **autologous $CD8^+$ T_{CM} and $CD4^+$ CD19 CAR-T cells** for $CD19^+$ B cell malignancies.

Secondary objectives

1. Duration of *in vivo* persistence and phenotype of transferred T cells.
2. Trafficking of transferred T cells to bone marrow
3. In vivo function of CD19 CAR T cells (Depletion of normal $CD19^+$ B cells)
4. To determine if the adoptive transfer of CD19 CAR-T cells has antitumor activity

FHCRC Protocol #2639 – CD19 CAR T Cells of Defined Composition For Advanced B Cell Malignancies



Two Stage Treatment Plan

Stage 1 – Dose Escalation/De-escalation (not cohort specific, standard 3x3 design)

Dose level 0:	up to $2x10^4$ $EGFR^+$ cells/kg
Dose level 1:	**up to $2x10^5$ $EGFR^+$ cells/kg** (Starting dose level)
Dose level 2:	up to $2x10^6$ $EGFR^+$ cells/kg
Dose level 3:	up to $2x10^7$ $EGFR^+$ cells/kg

Stage 2 – Treatment of 15 patients in each of the three cohorts (45 patients) at dose level established in Stage 1

Resolution of Advanced Nodal Involvement with Lymphoma with CD19 CAR T Cells of Defined Composition (Patient #1)

Cell dose of $2x10^5$ cells/kg

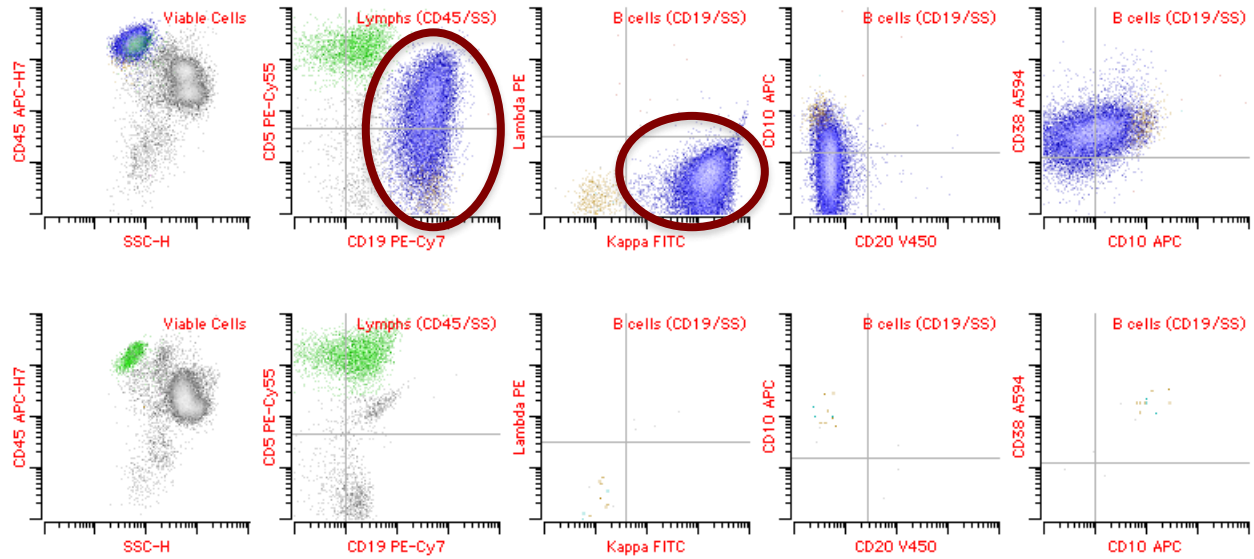
Pre CD 19 CAR-T cell infusion

28 Days Post CD 19 CAR-T cell infusion

Resolution of Bone Marrow Involvement with Lymphoma with CD19 CAR T Cells of Defined Composition (Patient #1)

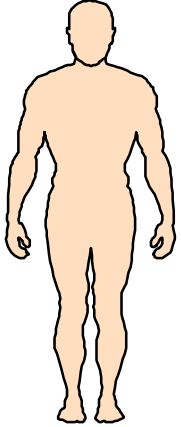
Pre CD19 CAR T Cells

Day 28 Post CD19 CAR T Cells



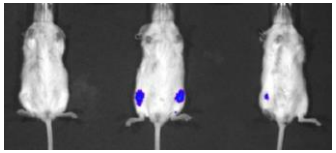
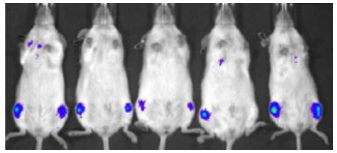
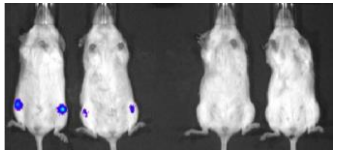
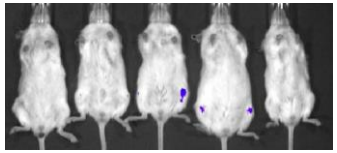
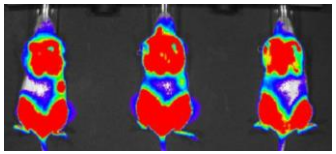
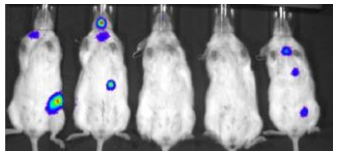
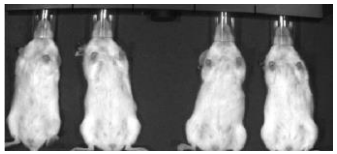
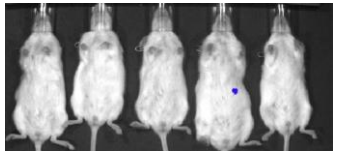
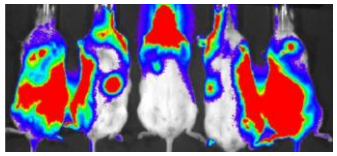
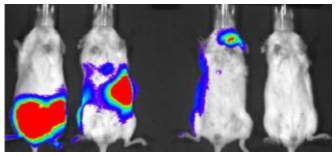
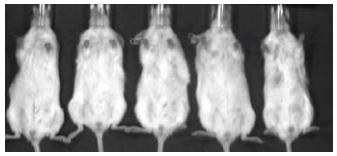
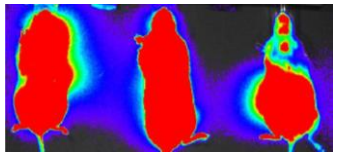
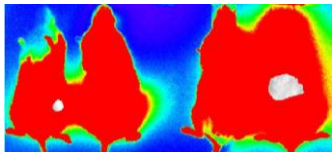
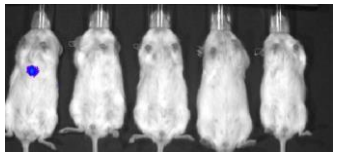
Toxicity

- **Fever from day 7-11, mild hypotension (IV fluids)**
- **Mild elevation in IL-6 (3-fold), Ifn-γ (10-fold), IL1RA (5-fold), Mip1β (5-fold)**
- **Duration of cytokine elevation ~4 days**



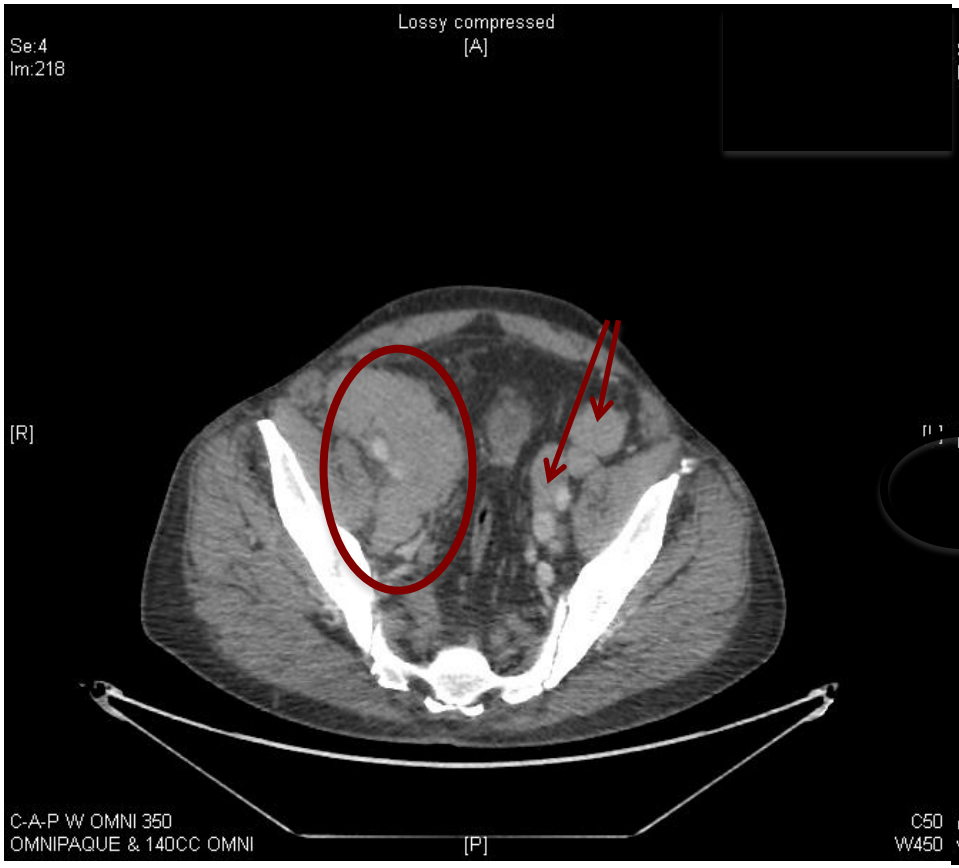
A Cell Product of Defined Composition Is More Potent (Antitumor Activity of Patient #1 T Cells in NSG Mice With Human Lymphoma Xenograft)

Total CAR T cell dose $1x10^6$

Day	tEGFR (Control)	PBMC	8CM	8CM/CD4 (Defined Ratio)
6				
12				
26	Sacrificed			
47				

Resolution of Advanced Nodal Involvement with Lymphoma with CD19 CAR T Cells of Defined Composition (Patient #4)

Cell dose of $2x10^6$ cells/kg



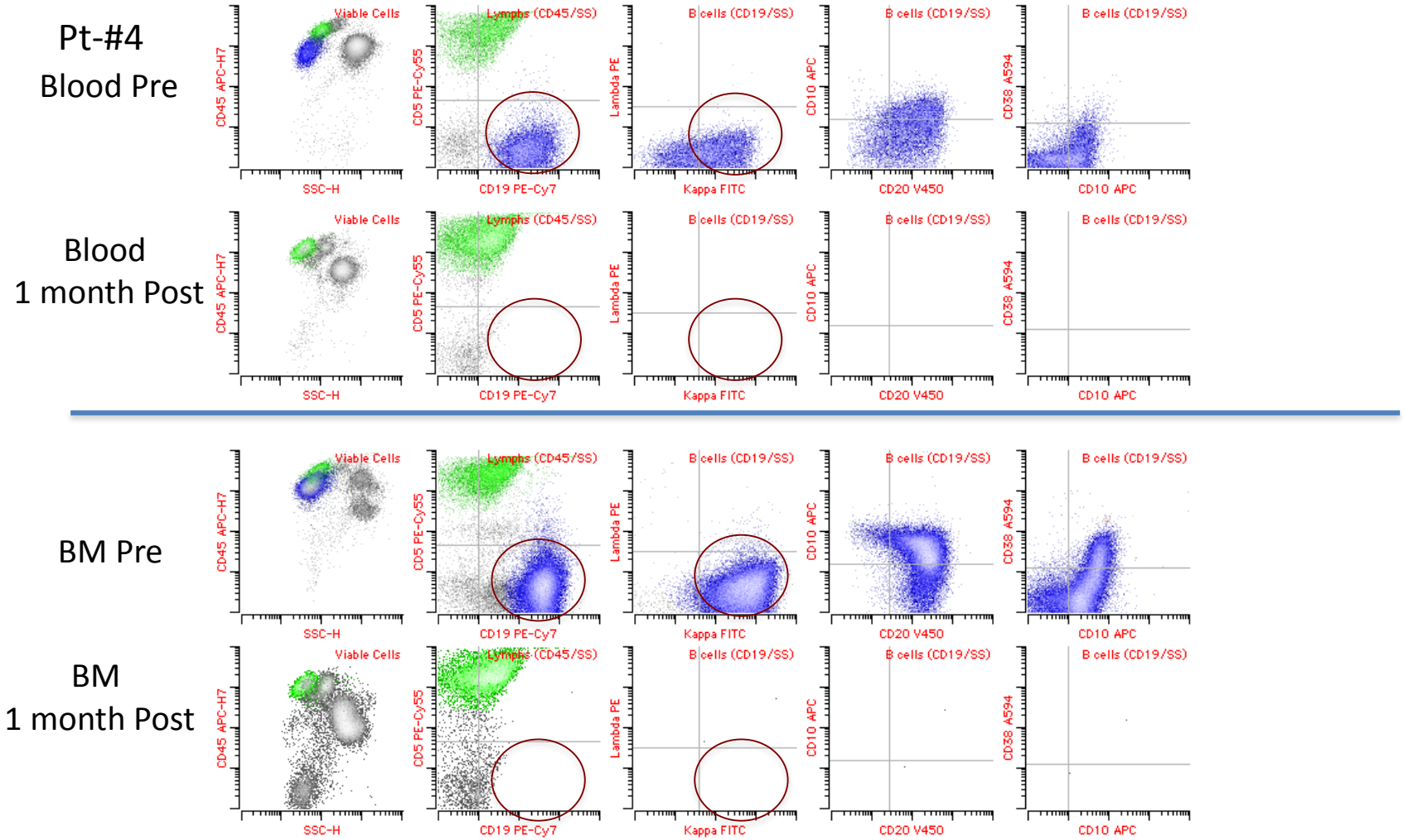
Before CD19 CAR-T cells

Bone Marrow -60% lymphoma

Day 27 after CD19 CAR-T cells

(PET Scan Negative)

Bone Marrow -0% lymphoma



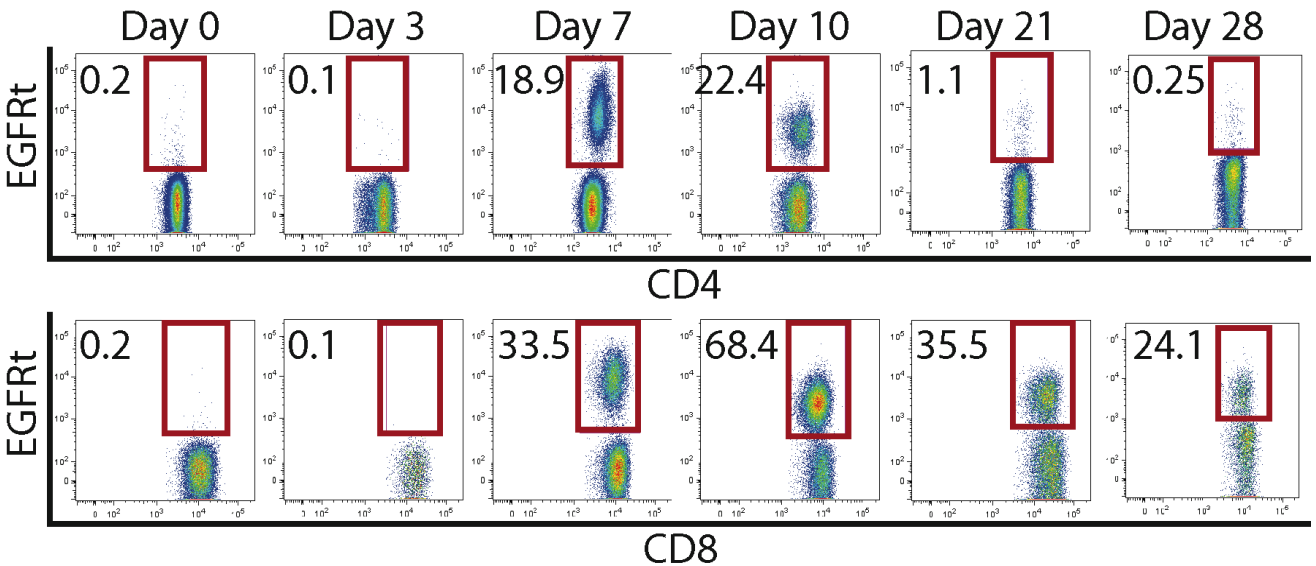
Toxicity (Pt #4)

- **Fever from day 7-9, mild hypotension (IV fluids)**
- **Mild elevation in IL-6, Ifn-γ, IL1RA, Mip1β**
- **Duration of cytokine elevation ~4 days**

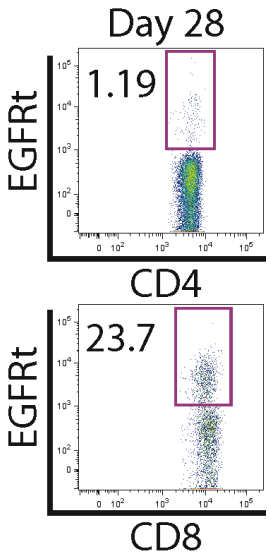
Marked In Vivo Proliferation of CD19 CAR T Cells of Defined Composition (Patient #4)

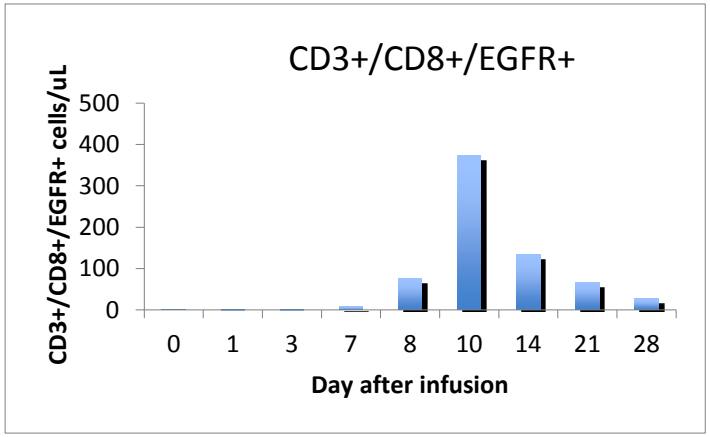
CAR-T Cell Frequencies At Intervals After Infusion

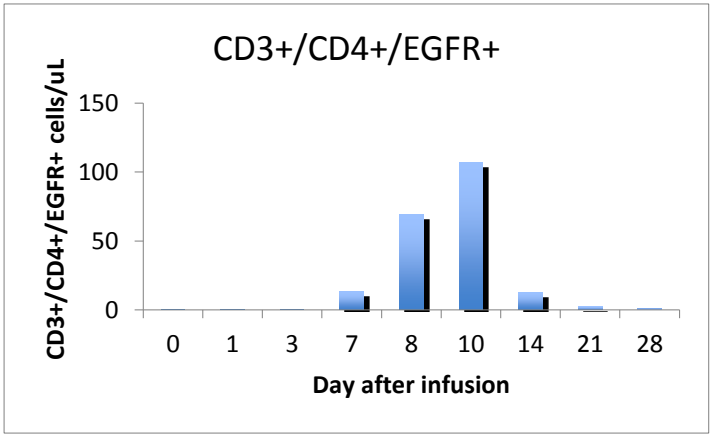
Peripheral)blood)



Bone Marrow







High Response Rate In Advanced Lymphoma With Low Doses of CD19 CAR T Cells of Defined Composition

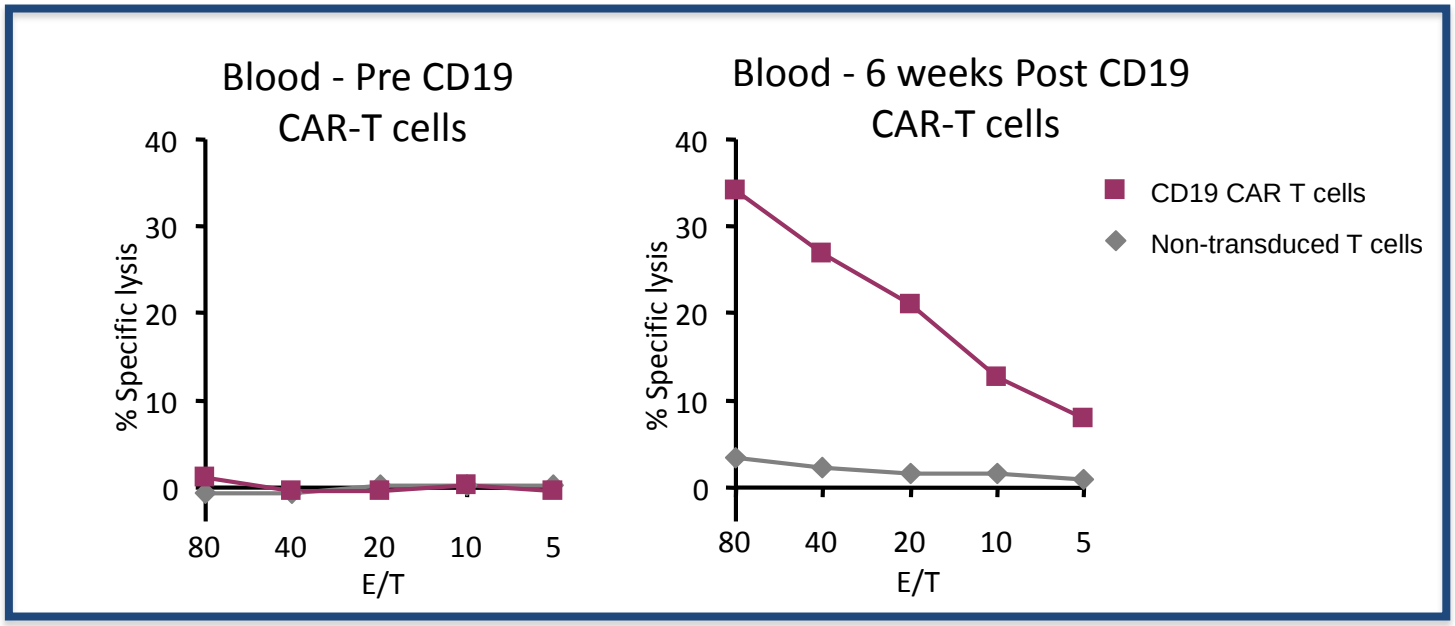
Pt ID	Age/ Sex	Diagnosis	Prior Tx	Disease Status	Disease Sites	B cell Depletion	Response 30 days
2639-1	58/M	Follicular Lymphoma	Chemo Auto HCT	Relapse	LN (bulky) BM (30%)	Yes	**CT resolution of LN, BM - CR**
2639-2	37/F	Ph+ ALL	Chemo/TKI	MRD flow/PCR	BM	Yes	**MRD -ve Flow and PCR**
2639-3	57/M	DLBCL (double hit)	Chemo	Relapse	LN (bulky) Extranodal, BM +ve	Yes	**CT resolution of LN, BM - CR Extranodal - PR**
2639-4	67/M	DLBCL	Chemo/Auto HCT	Relapse	LN (bulky) BM (60%)	Yes	**CT resolution of LN BM - CR**
2639-5	52/M	DLBCL	Chemo/ Auto HCT	Relapse	LN (bulky)	Pending	**Pending**

Potential Mechanisms of Resistance – Patient #3

1. Antigen escape – selection of CD19 loss variant
   - *Biopsy of extranodal site showed uniform CD19 expression*

2. Lack of durable T cell persistence/proliferation **(CD19 CAR T cells not detected in blood at day 28)**
   - **T cell exhaustion or deletion** due to persistent antigen load
   - Local immune suppressive tumor microenviroment (PDL1; $CD4^+$ T_{REG})
   - Loss of CAR-T cells due to immune response to "foreign" components of the CAR

     (Riddell et al. Nature Medicine, 1996 – immune response to transgene products in humans receiving gene-modified T cells)



Summary/Challenges

- **Engineering T cells with tumor-targeting receptors can overcome immune tolerance to tumors and eradicate large established tumors.**
- **Remarkably small doses of CAR T cells are effective and potency is enhanced by optimizing the cell composition**
- **Not all patients respond completely – and the mechanisms of failure need to be defined in non-responding patients.**
  - Failure of T cells to persist ***(Immunogenicity of the murine scFv)***
  - Suppressive tumor microenvironment (Tregs, MDSC, soluble inhibitors TGFb)
  - Immune checkpoints (CTLA-4, PDL-1)
  - Antigen loss variants
- **How durable are these responses and in what malignancies?**
- **What are the mechanisms of toxicity?**
  - Pathogenesis of macrophage activation/IL-6
  - Does cell composition affect frequency of toxicity?
  - Regulated CAR expression
  - Inhibiting cytokine networks

Fred Hutchinson Cancer Research Center

Riddell Lab

Cameron Turtle
Daniel Sommermeyer
Michael Hudecek
Stephen Liu
Carolina Berger
Paula Kosahih
Natalya Steevens
Michael Berger

David Maloney

Program In Immunology

Colette Chaney
Anna Marie Kane
Susan Lemmon

Cell Processing Facility

David Shank
Jason Carstens
Shelly Heimfeld

Seattle Children's Research Institute

Michael Jensen

TUM/IAS Clinical Cell Processing and Purification Focus Group

Dirk Busch
Christian Stemberger
Stephan Dreher
Jeannette Bet
Paulina Paszkiewicz

IAS TUM Institute for Advanced Study