CAR-T Cell Immunotherapy:
Opportunities and Challenges For Memory T Cells

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Memory T Cells Officially Join the Stem Cell Club

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Immunity. 2014. 41:7-9

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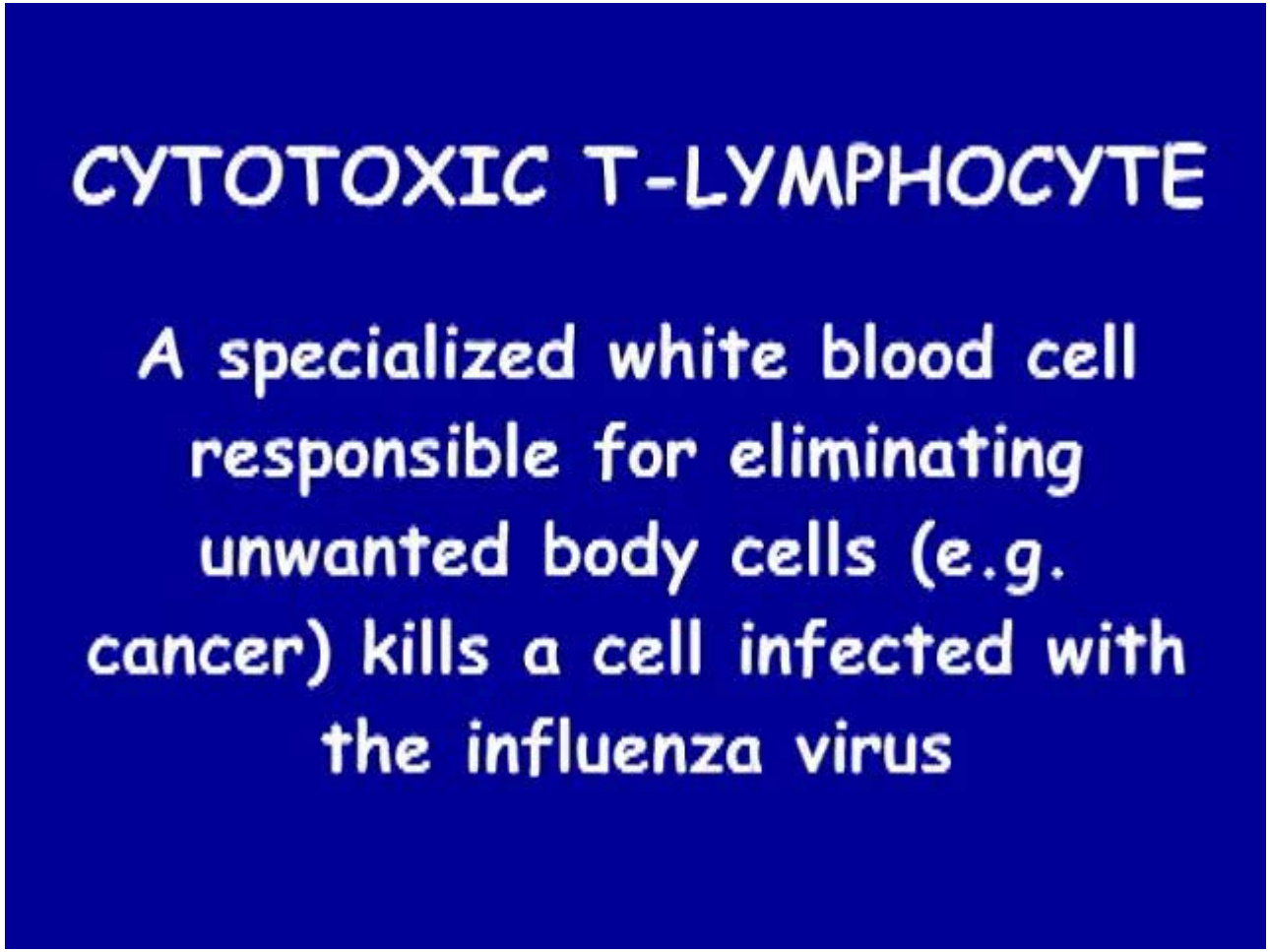
Harnessing the Potency & Specific of T cells for Cancer Therapy



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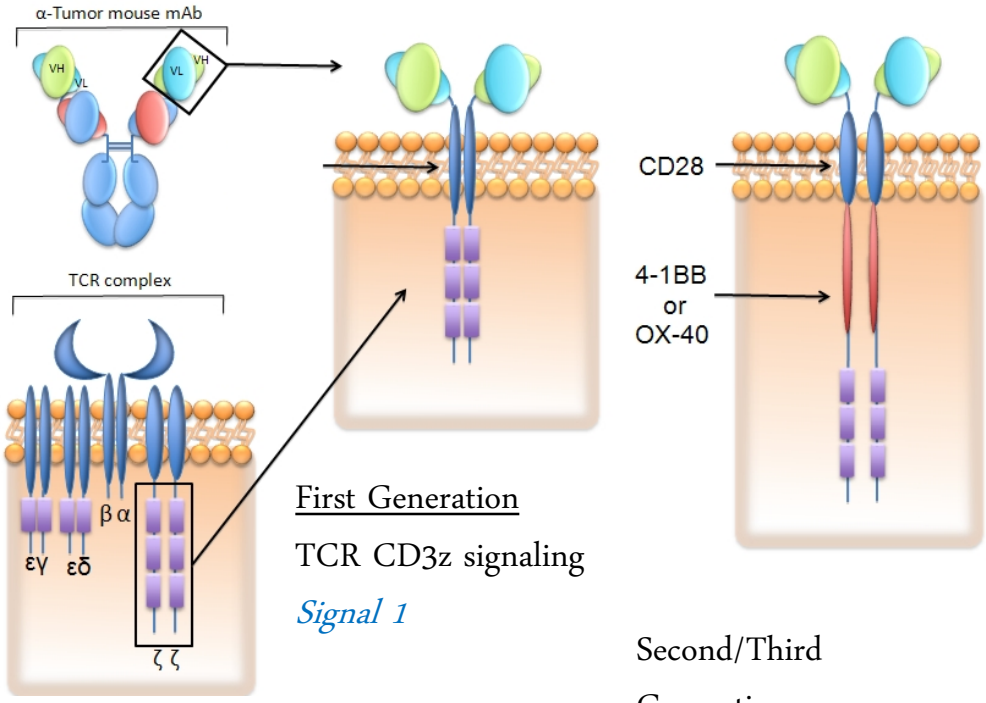
Chimeric Antigen Receptor (CAR) T Cell Therapy



First Generation

TCR CD3z signaling

Signal 1

Second/Third Generation

Costimulatory signaling

CD28, 41BB, OX40

Signal 2

Advantages of CARs

- Modular design
- HLA-independent antigen recognition
- Functional in both CD_4 and CD8 T cells
- Significant numbers of tumor specific T cells can be rapidly generated
- The potential to generate long-term antitumor immunity

Challenges

- Single antigen specificity
- Primarily restricted to extracellular antigens
- On-target and off-target toxicities

Chekmasova & Brentjens; Discovery Today; 2010

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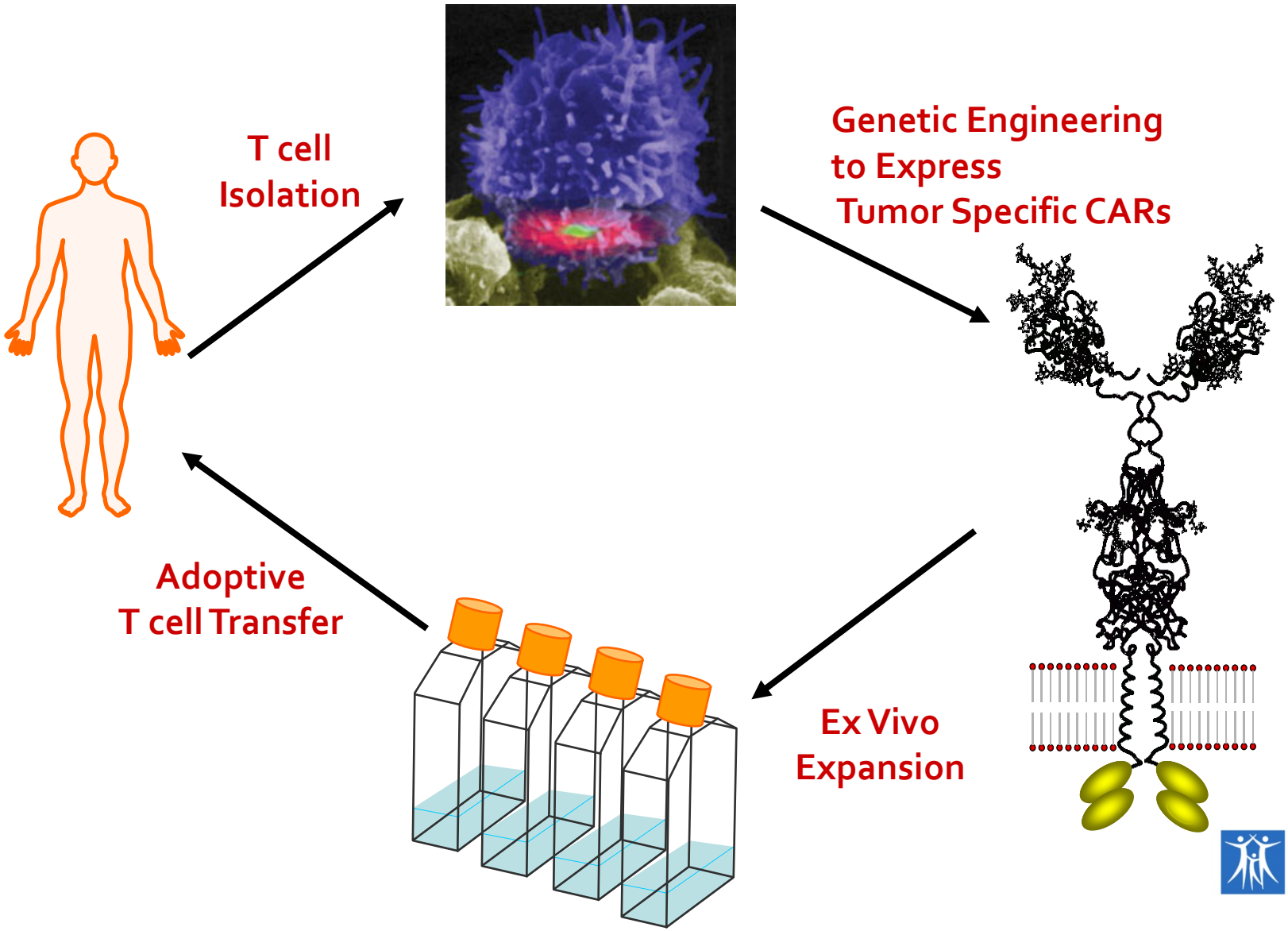
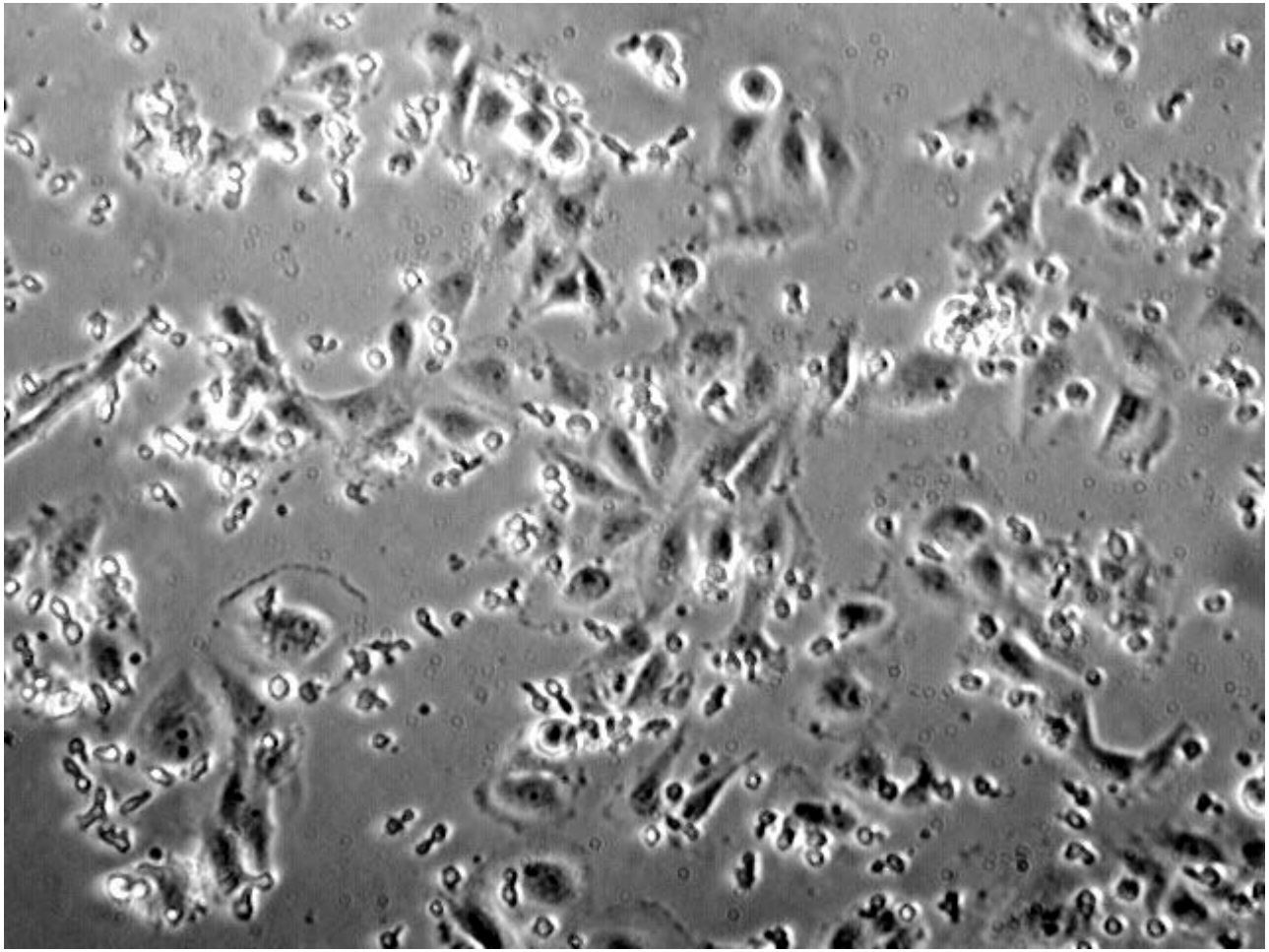
Adoptive Therapy using CAR-engineered T cells



CARs Efficiently Redirect T cell Killing in an HLA-Independent Manner



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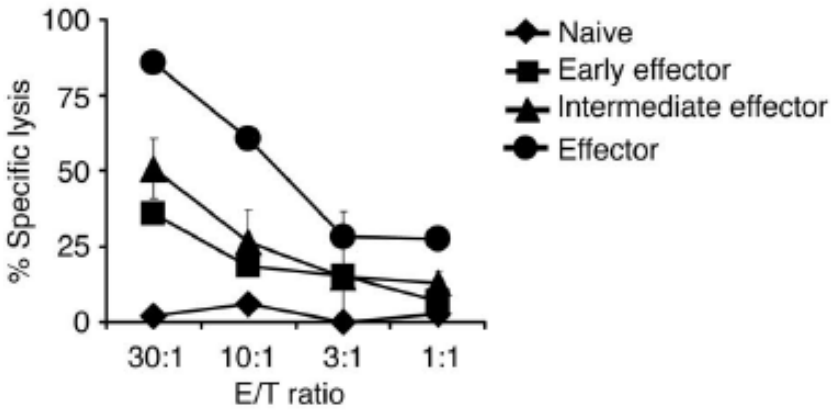
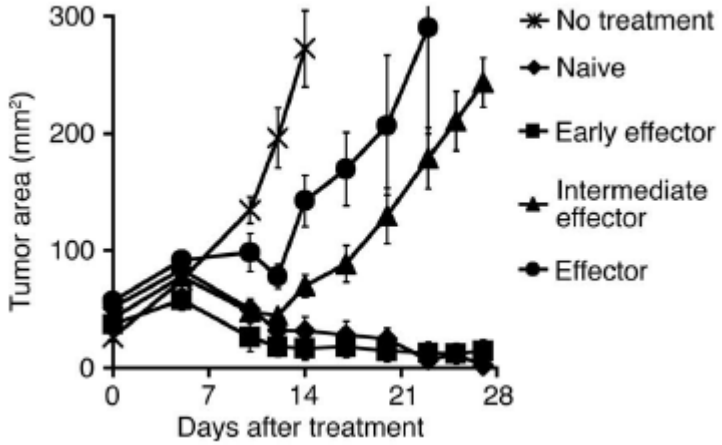
In Vitro Killing Does Not Predict *In Vivo* Antitumor Potency

IN VITRO



IN VIVO



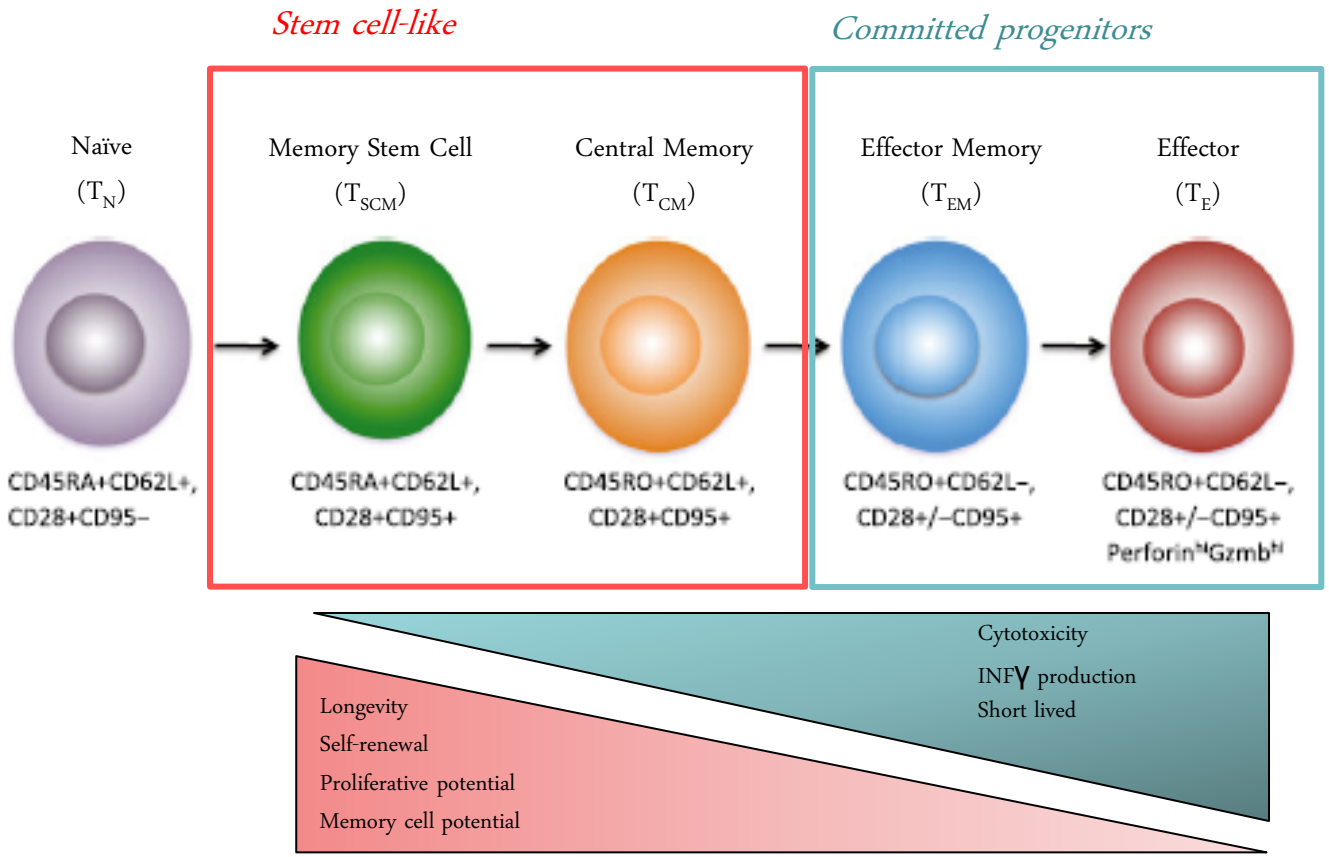
❖ *Less differentiated T cells are more therapeutically effective*

(1) Higher proliferative potential and survival capabilities

(2) Differentiate into short-lived effectors with potent effector function

Gattinoni. 2005. JCI. 115:1616

The T Cell Compartment Consists of T cell Subsets At Different Stages of Differentiation



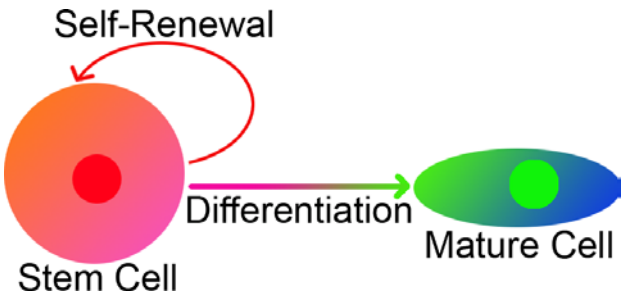
Gattinoni et al. Nat Rev. Cancer 2012

Memory T cells as Stem Cells

Definition of a Stem Cell:

(1) Cells that are able to self-renew (can create more stem cells indefinitely); and

(2) Differentiate into specialized, mature cell types.

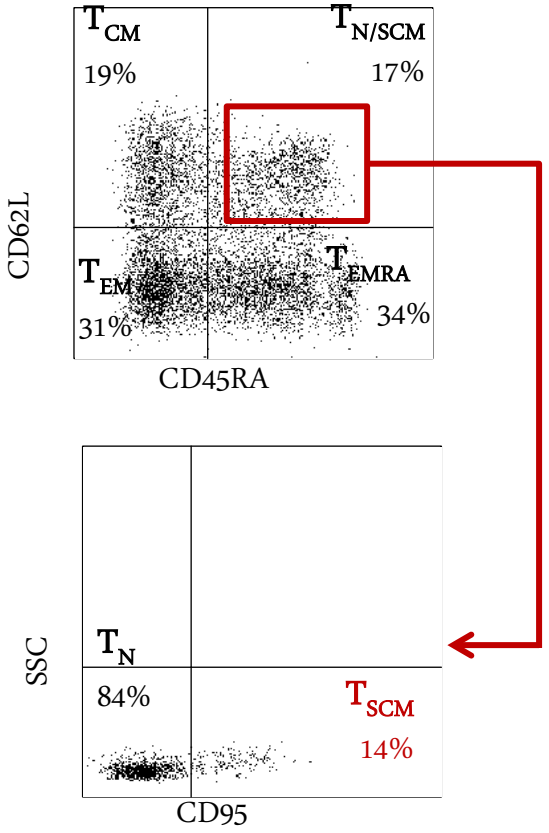


Memory T cells as Stem Cells:

- Recombination of T cell receptor and thymic selection requires that T cell immunologic memory be generated from T cells with stem cell properties and not hematopoietic stem cells (HSCs)
- Memory T cells display functional attributes of "stem cells"
  - (1) Self-renew to maintain immunological life-long memory
  - (2) Differentiate into effector memory (T_{EM}) and effector T cells (T_E) to reconstitute immune competence

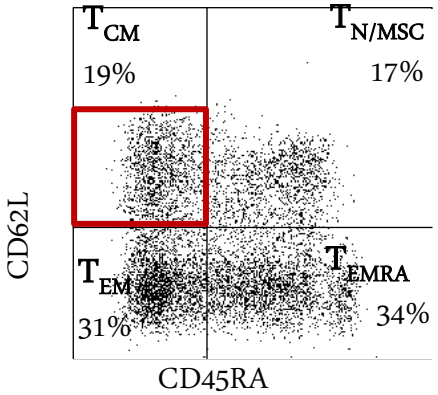
Memory Stem T Cells – T_{SCM}

- T_{SCM} are the least differentiated memory T cell subset with the greatest self-renewal and proliferative potential.
- T_{SCM} resemble naïve T cells (T_N), expressing CD45RA+, CD62L+, and can be can be differentiated from T_N by expression of CD95.
- Whether T_{SCM} represent a "stable" T cell subset is still a point of discussion.
- T_{SCM} are in relatively low abundance, and therefore their isolation and expansion can be challenging.
- T_{SCM} can be generated from T_N by stimulation with CD3/CD28 beads in the presence of IL-7 and IL-15, or also can be expanded in the presence of Wnt/β-catenin pathway activation (Cieri et al. Blood. 2013. 121:573; Gattinoni et al. Nat Med. 2011. 17:1290).



Central Memory T Cells – T_{CM}

- T_{CM} are a well-defined memory T cell subset with high self-renewal and proliferative potential.
- T_{CM} are identified by expression of CD45RO+ CD45RA- CD62L+ and CD95+.
- T_{CM} are more frequent in PBMC compared to T_{SCM}.
- T_{CM} persist following adoptive transfer better than T_{EM}
  - Effector T cells (T_E) derived from T_{CM} persisted long-term (>1 yr), and were capable of responding to viral antigen in non-human primates. By contrast, T_{EM} derived effectors persisted for less than 7 days. (Berger. JCI. 2008. 118:4817)
  - Similarly, human T_{CM} demonstrate greater persistence and antitumor activity than T_{EM} in NSG mouse models. (Wang et al. Blood. 2011. 117:1888)
- T_{CM} can be efficiently enriched from PBMC for T cell therapy manufacturing based on their CD45RA- CD45RO+ CD62L+ phenotype. (Wang et al. J Immunother. 2012. 35:689)

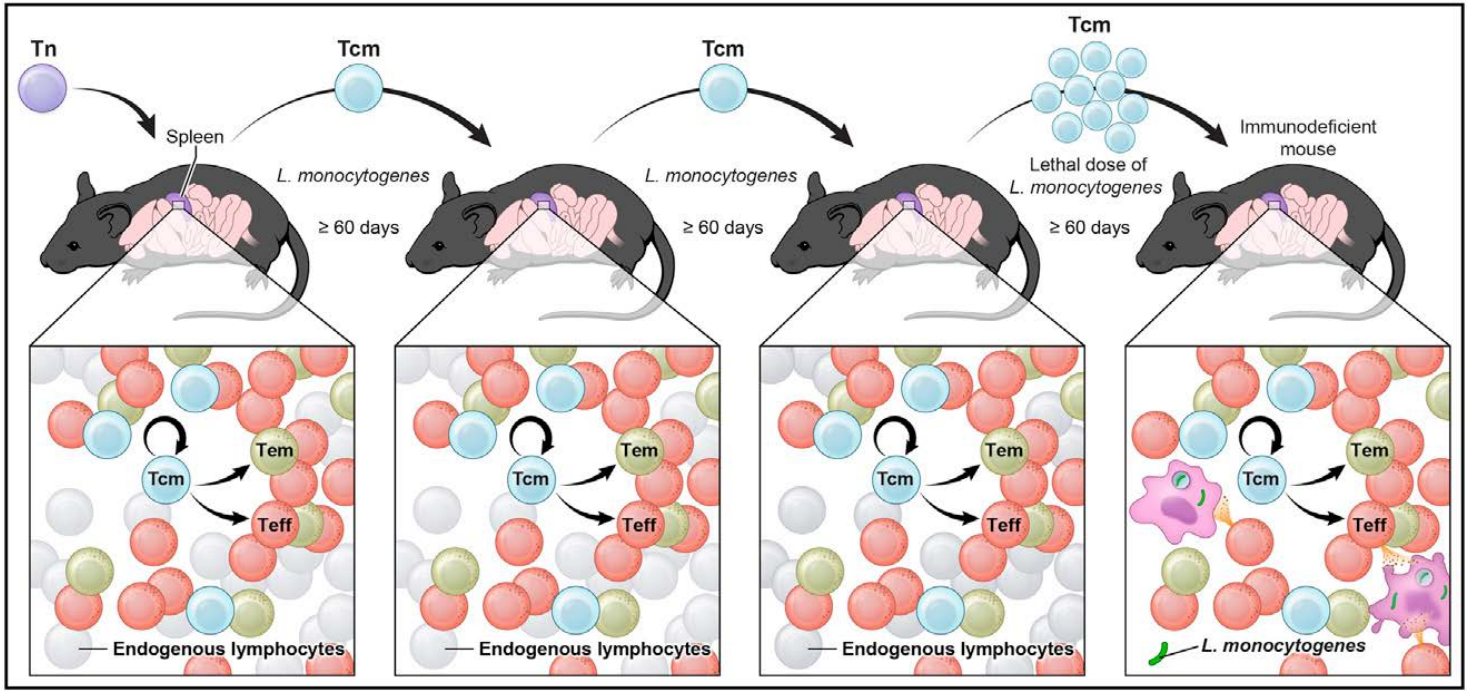


Central Memory T cells as Stem Cells

Immunity. 2014. 41:116

Serial Transfer of Single-Cell-Derived Immunocompetence Reveals Stemness of CD8$^+$ Central Memory T Cells

Patricia Graef,[1,10] Veit R. Buchholz,[1,10] Christian Stemberger,[1,2] Michael Flossdorf,[3,4] Lynette Henkel,[1] Matthias Schiemann,[1,5] Ingo Drexler,[6] Thomas Höfer,[3,4] Stanley R. Riddell,[2,7,8] and Dirk H. Busch[1,2,5,9,*]



Gattinoni. Immunity. 2014. 41:7

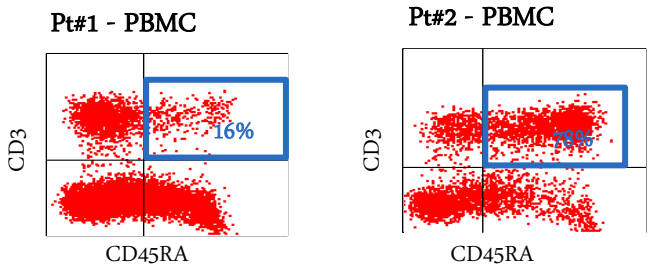
Opportunities and Challenges for Memory T cell Populations For CAR T cell Therapy

Potential Benefits

- Pre-selecting or expanding T_{MEM} for CAR-engineering may provide products capable of the greatest *in vivo* proliferation and persistence.

  Higher Potency per CAR T Cell Dose

- Frequency of naïve and memory T cells vary significantly from patient-to-patient. Enriching specific subsets may reduce product variability.



Challenges

- More complex manufacturing processes may be more difficult to transfer the technology for broader patient availability.
- More complex manufacturing processes will be more costly.
- Less abundant memory subsets (i.e., T_{SCM}) may require extended *ex vivo* expansion which could limit their utility.

Targeting CD19 with CAR T cells

Maus et al. Blood (2014)

Antigen	Cancers	Gene transfer	CAR signaling domain	Phase/ID	Sponsor	Cell type/selection/drug combination	Reference
CD19	Pediatric B-cell leukemia and lymphoma	Lentivirus	4-1BB–CD3ζ	1/NCT01626495	CHOP/University of Pennsylvania		37
CD19	$CD19^+$ malignancies	Lentivirus	4-1BB–CD3ζ	1/NCT01029366	University of Pennsylvania		34
CD19	ALL (post–allo-HSCT)	Lentivirus	4-1BB–CD3ζ	1/NCT 01551043	University of Pennsylvania	T cells from donor	
CD19	CLL (randomized to 1 of 2 doses)	Lentivirus	4-1BB–CD3ζ	II/NCT01747486	University of Pennsylvania		
CD19	CLL	Retrovirus/ lentivirus	CD28-CD3ζ; 4-1BB–CD3ζ	1/2/ NCT00466531	MSKCC/University of Pennsylvania		
CD19	ALL		CD28-CD3ζ	1/NCT01044069	MSKCC		32
CD19	Auto-HSCT for NHL followed by T-cell infusion	Retrovirus	CD28-CD3ζ	1/NCT01840566	MSKCC		
CD19	Relapsed ALL post–allo-HSCT	Retrovirus	CD28-CD3ζ	1/NCT01430390	MSKCC	CD19 CAR-transduced EBV-specific CTLs from donor	
CD19	CLL (resi[illegible] pentostatir	[illegible]	[illegible]	[illegible]	MSKCC		30
CD19	Pedi[illegible]						
CD19						[illegible]	
CD19						[illegible] 2 wk after	
CD19	ALL, CLL, N[illegible]					[illegible]cific CTLs [illegible]m donor	23
CD19							
CD19	Leukemia/ly[illegible]						
CD19	B-cell m[illegible]						
CD19	B-cell malignancies post–auto HSCT	Transposon	[illegible]	[illegible]	MD Anderson Cancer Center	Low- and high-dose cohorts with and without IL-2	
CD19	Pediatric leukemia and lymphoma	Retrovirus	CD28-CD3ζ	1/NCT01593696	National Cancer Institute		
CD19	CLL, small lymphocytic lymphoma; MCL, follicular lymphoma, large-cell lymphoma	Retrovirus	CD28-CD3ζ	1/2/ NCT00924326	National Cancer Institute	IL-2	
CD19	B-cell malignancies relapsed post–allo-HSCT	Retrovirus	CD28-CD3ζ	1/NCT01087294	National Cancer Institute	T cells from donor	29
CD19	Auto-HSCT for NHL followed by T-cell infusion (day 2 or 3)	Lentivirus	CD3ζ	1/2/ NCT01318317	City of Hope	T_{CM}-enriched $CD8^+$ T cells	
CD19/EGFRt	Auto-HSCT for NHL followed by T-cell infusion (day 2 or 3)	Lentivirus	CD28-CD3ζ	1/NCT01815749	City of Hope	T_{CM}-enriched T cells (cetuximab as possible suicide system)	
CD19/EGFRt	Pediatric ALL	Lentivirus	CD28-CD3ζ	1/NCT01683279	Seattle Children's Hospital		
CD19	Relapse/refractory CLL, NHL, or ALL	Lentivirus	CD3ζ	1/2/ NCT01865617	Fred Hutchinson Cancer Research Center		
CD19/EGFRt	ALL, DLBCL, MCL, NHL, CLL relapsed post–allo-HSCT	Lentivirus	CD28-CD3ζ	1/2/ NCT01475058	Fred Hutchinson Cancer Research Center	Donor-derived, CMV- or EBV-specific $CD62L^+$ T_{CM}	
CD19	Pediatric ALL post–allo-HSCT	Retrovirus	CD3ζ	1/2/ NCT01195480	University College, London	CD19 CAR-transduced EBV-specific CTLs from donor; 2nd cohort adds vaccination with irradiated EBV-LCL	
CD19	ALL, CLL, NHL	Retrovirus	CD137-CD3ζ and CD3ζ	1/2/ NCT01864889	Chinese PLA General Hospital		

CD19 is an ideal target for CAR T cell therapy

- Near universal expression on B-cell malignancies
- Limited normal tissue expression restricted to B cells
- Off-tumor targeting of B cells is well tolerated

Clinical Potential of CD19-CAR T cell Therapy

❖**CD19-CAR T cells mediate impressive clinical efficacy against CD19-malignancies across institutions, CAR designs, manufacturing platforms and trial designs.**

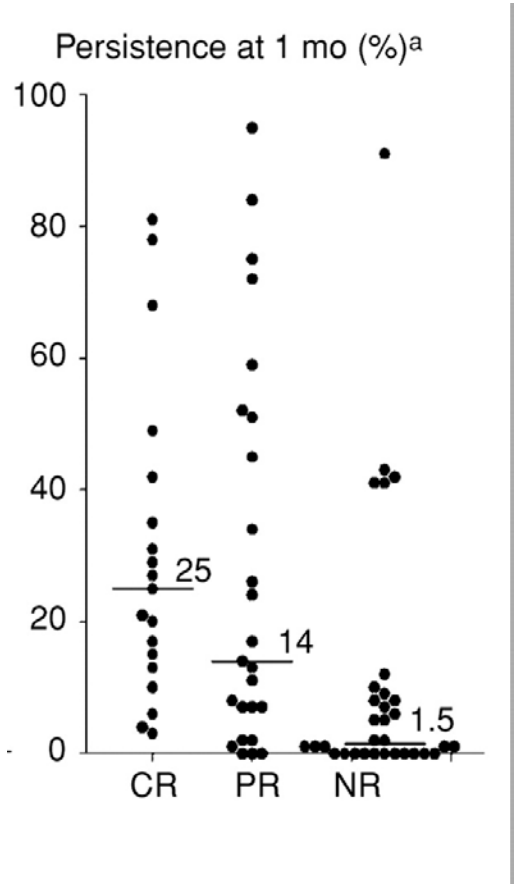
- More than 100 patients have been treated on clinical trials with CD19-CAR T cells for the treatment of B-cell maligancies.
- Complete response rates are reported for 70-90% of patients with ALL.
- In July 2014, FDA granted 'breakthrough therapy' designation to CTL019 Novartis/UPenn. CD19CAR-T are also being commercially developed by Kite Pharma/NCI and Juno Therapeutics/MSKCC&FHCC.
- ***However, not all patients respond to therapy.***
- ***The long-term durability is not yet established.***
- ***Successfully extending this therapeutic approach to solid tumors is an immediate goal.***

How can we further refine this promising therapy?

Reviewed by Gill and June. 2015. Immunological Rev. 263: 68.

Limited T cell Persistence is a Barrier to Robust Clinical Efficacy

T cell Persistence Correlates With Cancer Regression



Rosenberg S A et al. Clin Cancer Res 2011;17:4550-4557

Strategies to Improve T cell Persistence

- ***Incorporate lymphodepletion regimens prior to ACT*** *(Dudley et al. JCO. 2005. 23: 2346; Dudley et al. JCO. 2008. 26: 5233; Brentjens et al. Blood. 2011. 118:4817)*

- ***Optimize CAR design for improved co-stimulatory signaling*** *(Brentjens et al. Clin Cancer Res. 2007. 13:5426; Savoldo et al. JCI. 2011. 121:1822)*

- ***Reduce transgene immunogenicity*** *(Jensen et al. Biol Blood Marrow Transplant. 2010. 16:1245. Maus et al. Cancer Immunol Res. 2013. 1:26)*

- ***Engineer and expand T cell subsets with enhanced long-term persistence (i.e. T memory cells)***

CD19-CAR T cell Antitumor Responses Correlate with A Memory Phenotype

T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

Michael Kalos,[1,2*] Bruce L. Levine,[1,2*] David L. Porter,[1,3] Sharyn Katz,[4] Stephan A. Grupp,[5,6] Adam Bagg,[1,2] Carl H. June[1,2†]

2011. STM.

Persistent CAR T cells reacquired a central memory phenotype in patients.

Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15

Yang Xu,[1,2] Ming Zhang,[1] Carlos A. Ramos,[1,3,4] April Durett,[1] Enli Liu,[1] Olga Dakhova,[1] Hao Liu,[5] Chad J. Creighton,[5] Adrian P. Gee,[1,6] Helen E. Heslop,[1,3,4,6,7] Cliona M. Rooney,[1,2,6,7] Barbara Savoldo,[1,6,7] and Gianpietro Dotti[1,2,4,7]

2014. Blood.

Frequency of CD8+ CD45RA+ CCR7+ a subset closest to T_{SCM} correlates with CAR-T cell engraftment and expansion in lymphoma patients.

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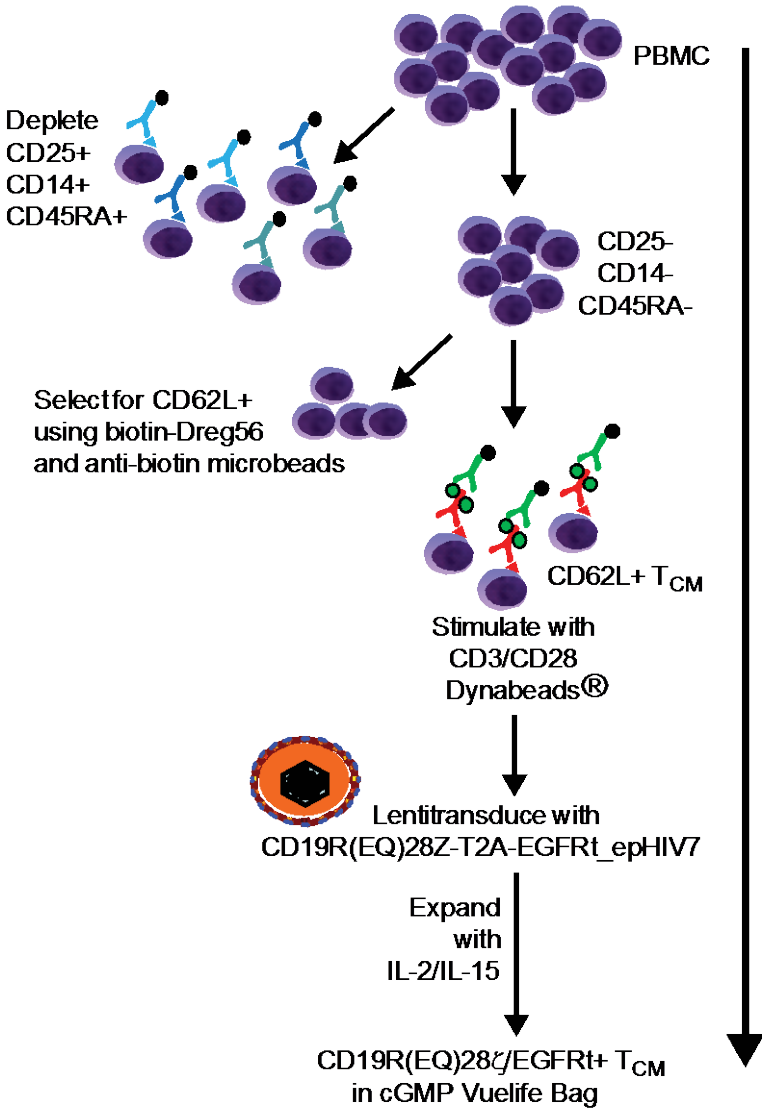
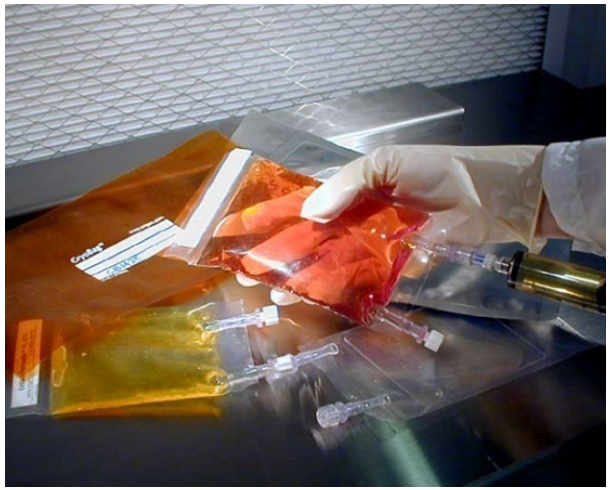
GMP Manufacturing Platform for T_{CM}-Enriched CAR+ T cells



Day : Bioprocess

Day 0: Leukapheresis

Day 1: CliniMACS Selection of T_{CM}
Dynabead stimulation
Lentiviral Transduction



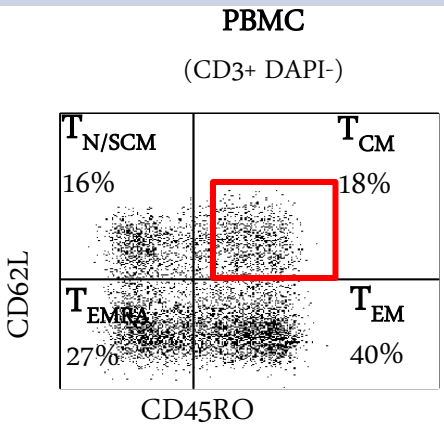
Day 7: Dynabead Removal

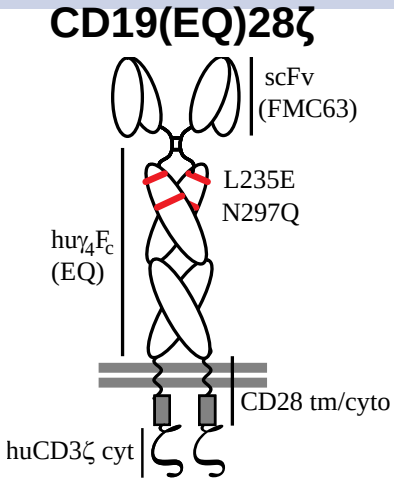
Day 7-16: Ex Vivo Expansion

Day 10-16: Cryopresservation

Wang et al. 2012. J Immunother. 35:689

PBMC versus T_{CM}-Enriched CAR T cell Products



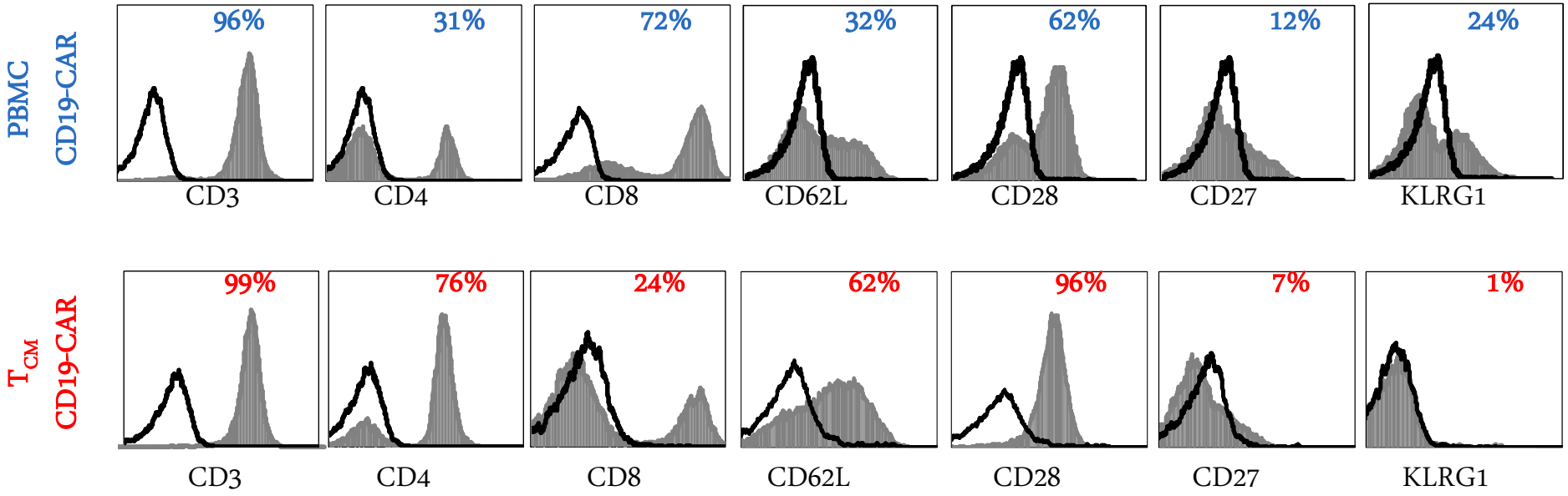


Manufacture CD19-CAR T cells

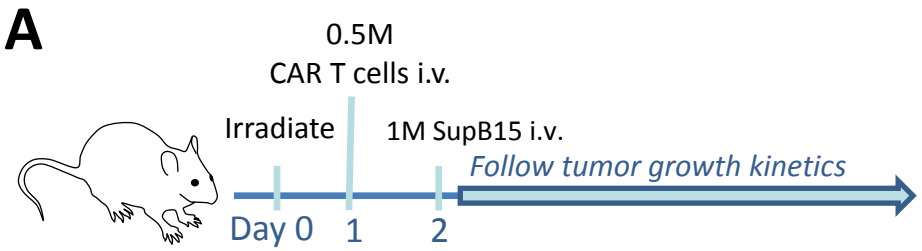
-CD3/CD28 Bead Stimulation

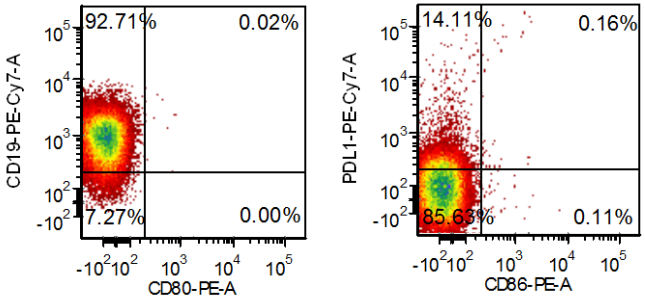
-Lenti transduce CD28-CAR

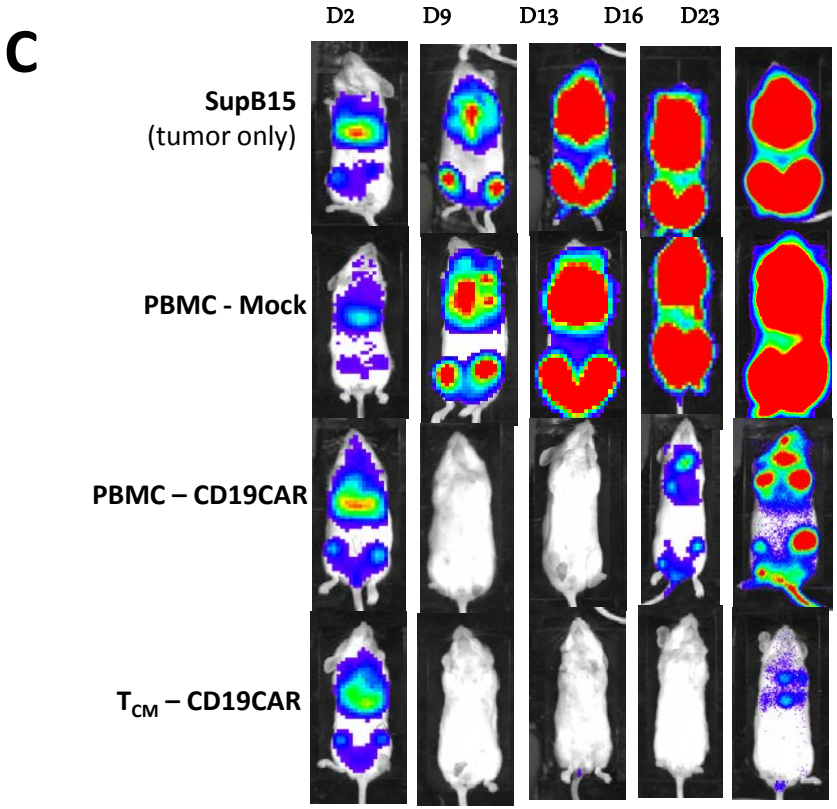
-Expand with low dose IL2/IL15

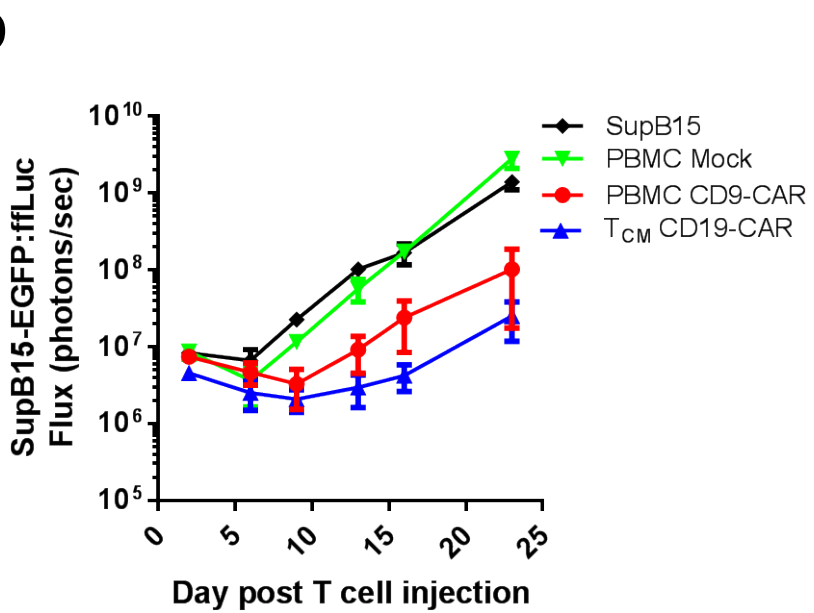


Comparison of PBMC versus T_{CM}-derived CD19-CAR T cells for Antitumor Efficacy



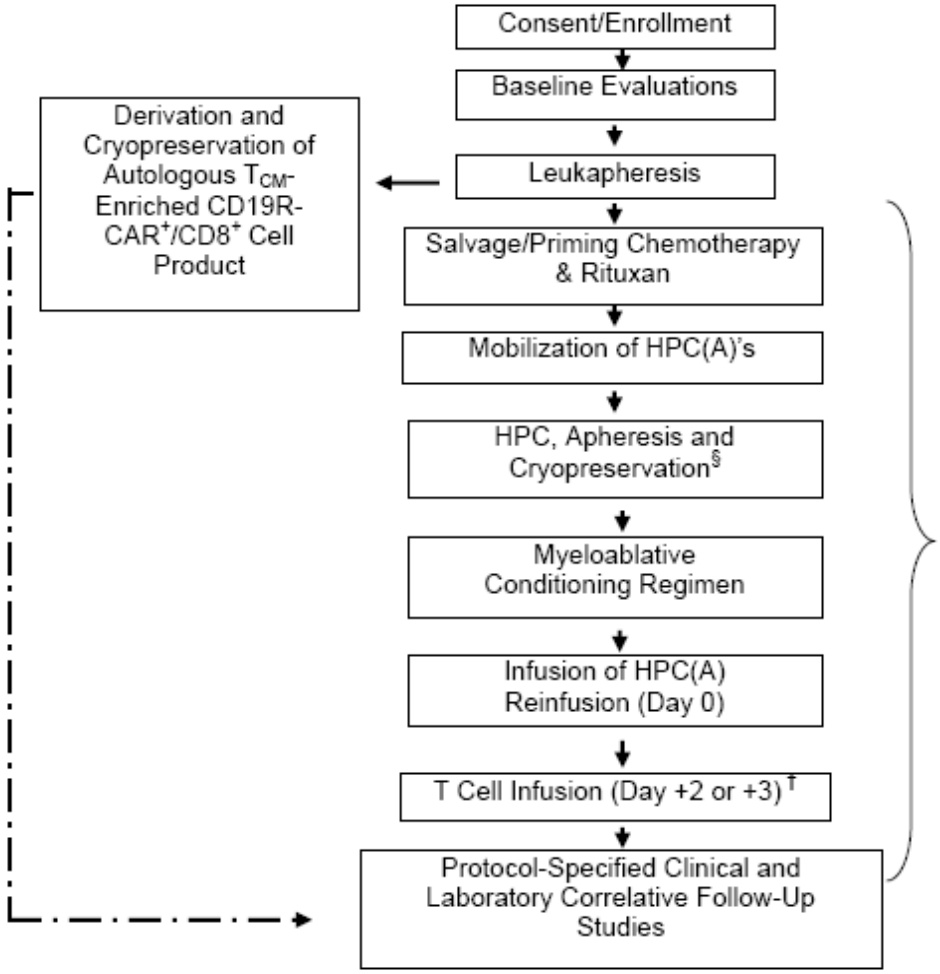






Phase I Clinical Trial Using Central Memory Enriched CD19-CAR T cells

Leslie Popplewell, M.D.; Clinical PI



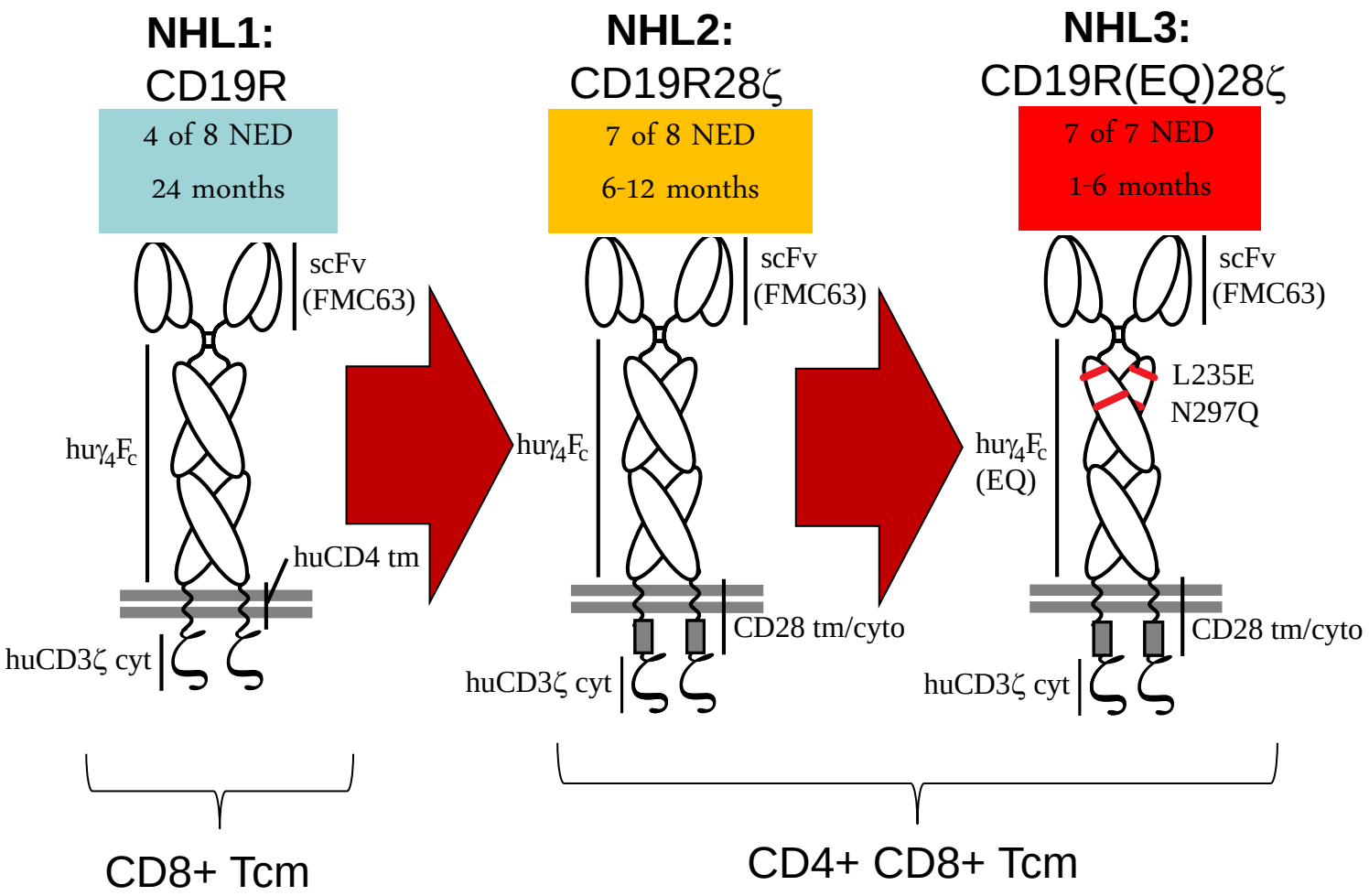
Enrollment:

- Relapsed B Cell Lymphoma (recurrent large cell & mantle cell lymphoma)
- High risk patient population for relapse with poor prognosis with auto-transplant

Clinical Design:

- Infuse cells on day +2/+3 after HSCT
- Lymphopenic environment to support homeostatic expansion
- Engraft cells as a component of the reconstituted immune system

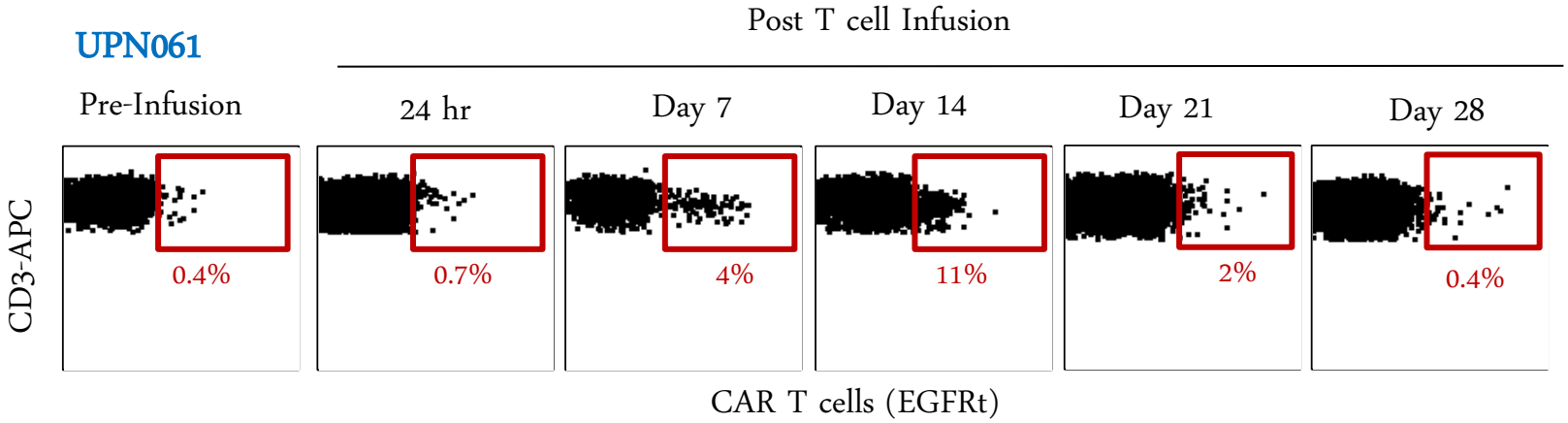
Optimizing CAR Design for Costimulation and Reduced Off-Target Interactions with Fc Receptors



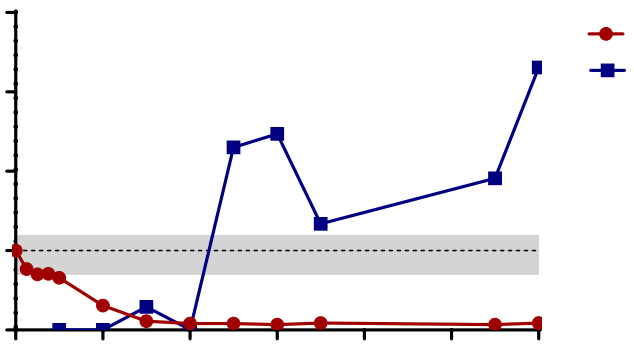
Jonnalagadda et al. Mol Ther. 2014

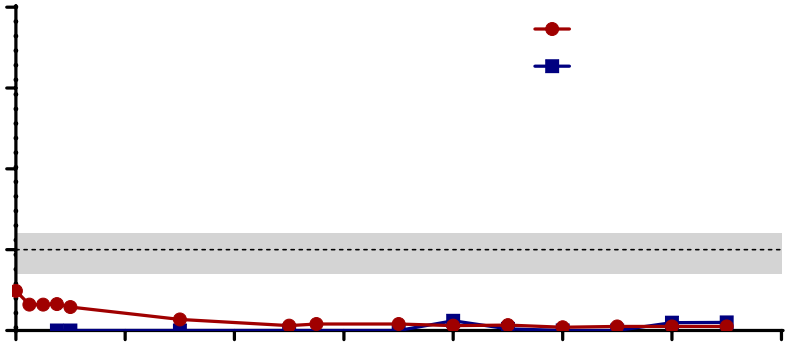
T cell Persistence and B Cell Aplasia Following CD19-CAR T_{CM} Therapy

CD19-CAR T_{CM} Persistence in Peripheral Blood is Transient



B Cell Aplasia Following CD19-CAR T_{CM} Therapy





NHL1/2/3 Lessons Learned To Date…….

- Feasibility of engineering and expanding CD19-CAR+ T_{CM}, even post salvage chemotherapy.
- Safety of CD19-specific CAR T cells when administered in conjunction with allo HSCT, with no delay in hematopoietic reconstitution and limited cytokine release syndrome in this MRD setting.
- Low levels of CAR T cells persist in peripheral blood for a subset of patients (peak at ~14day).
- Subset of patients display long-term B cell aplasia.

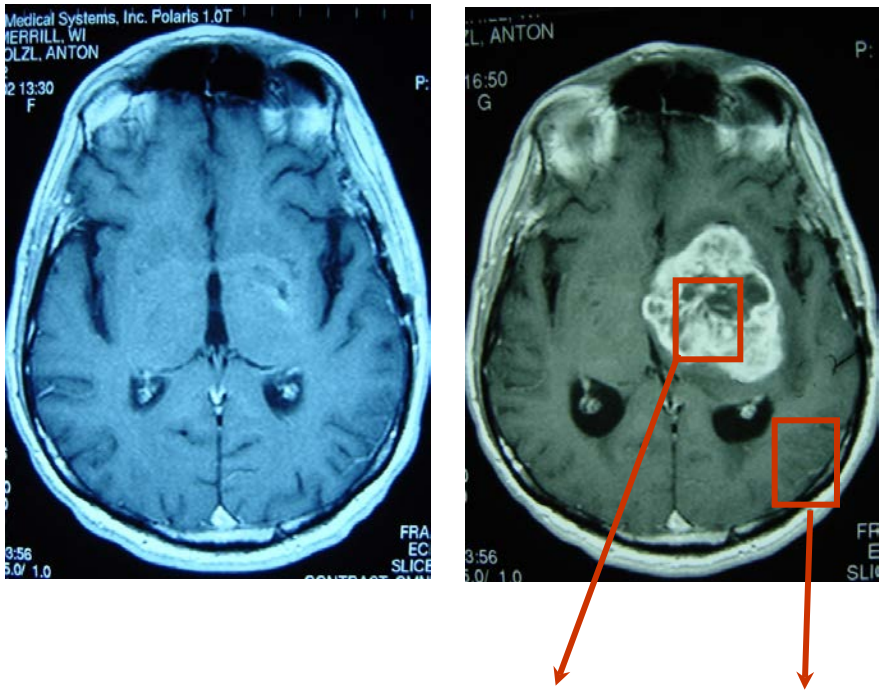
Understanding Limited CAR T cell Persistence in the Auto Transplant Setting

- Do CD62L+ T_{MEM} take up residence in secondary lymphoid tissue?
- Does the lack of antigen drive in this minimal disease setting affect persistence?
- Could inclusion of T_{SCM} subsets in the enriched memory pool increase persistence?
- Do CD28-CAR designs support long-term CAR T cell persistence?

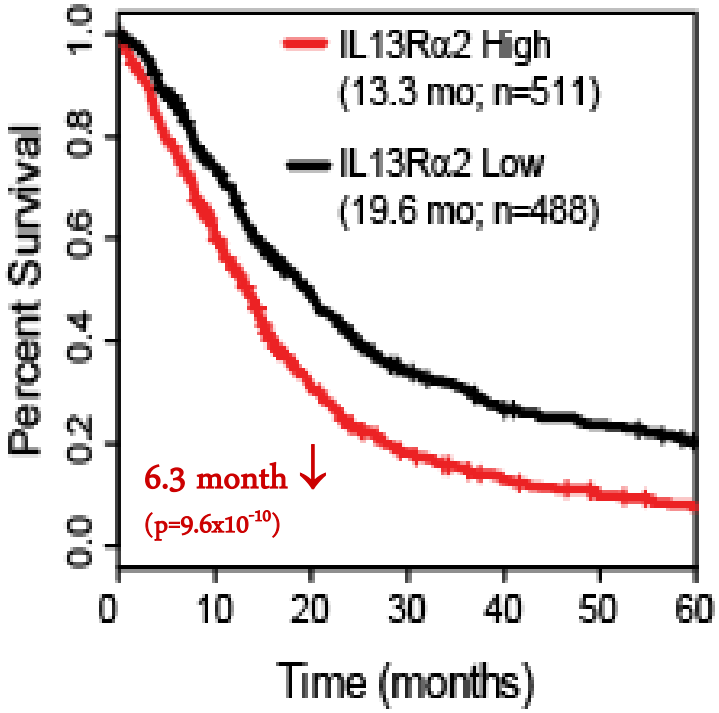
Adoptive T cell Therapy for Recurrent Glioblastoma

Glioblastoma is the most common and most aggressive type of primary brain tumor, and one of the least curable of all human cancers.

Dec 2002 **March 2003**



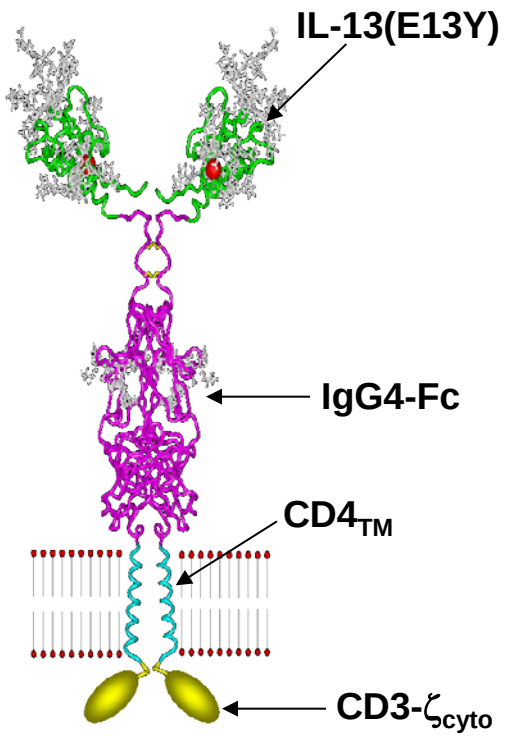
	GLIOMA	Normal Brain
IL13Rα2	>50%	neg



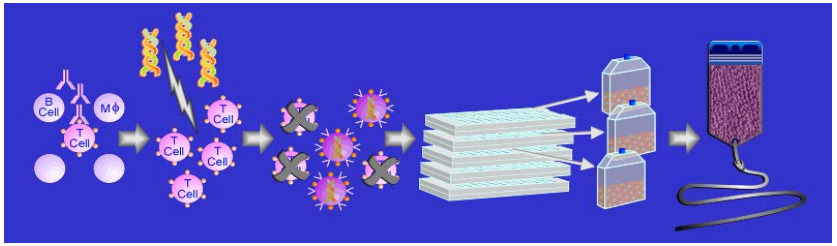
Brown et al., PlosOne 2013

First Generation CD8+ IL13-zetakine+ Effector T Cells for the Treatment of Glioblastoma

1st Gen IL13Rα2-specific IL13ζ CAR



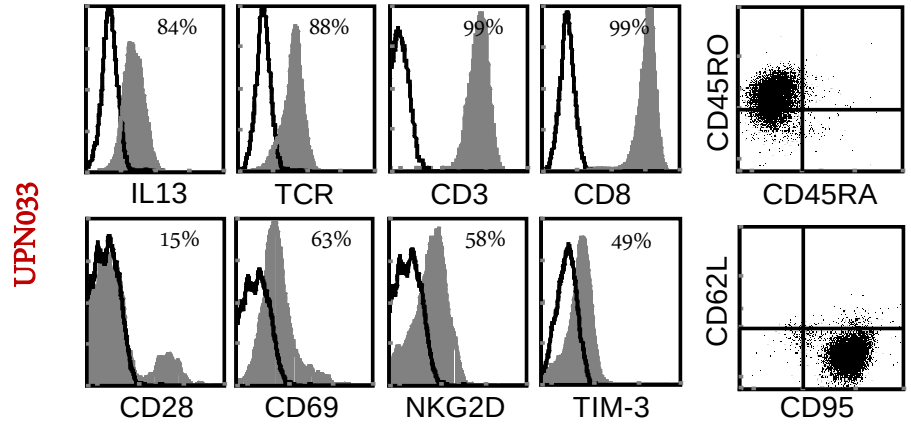
Effector CD8+ T cell Manufacturing Platform



Day 0	Day 3	Day 5	Day 14	Day 28+
Polyclonal Activation of T Cells with OKT3	Electroporation of OKT3-activated PBMC	Hygomycin drug selection	Cloning of surviving T Cells	Expansion of drug resistant clones

>1x10^9 IL13-zetakine+ CTL clone

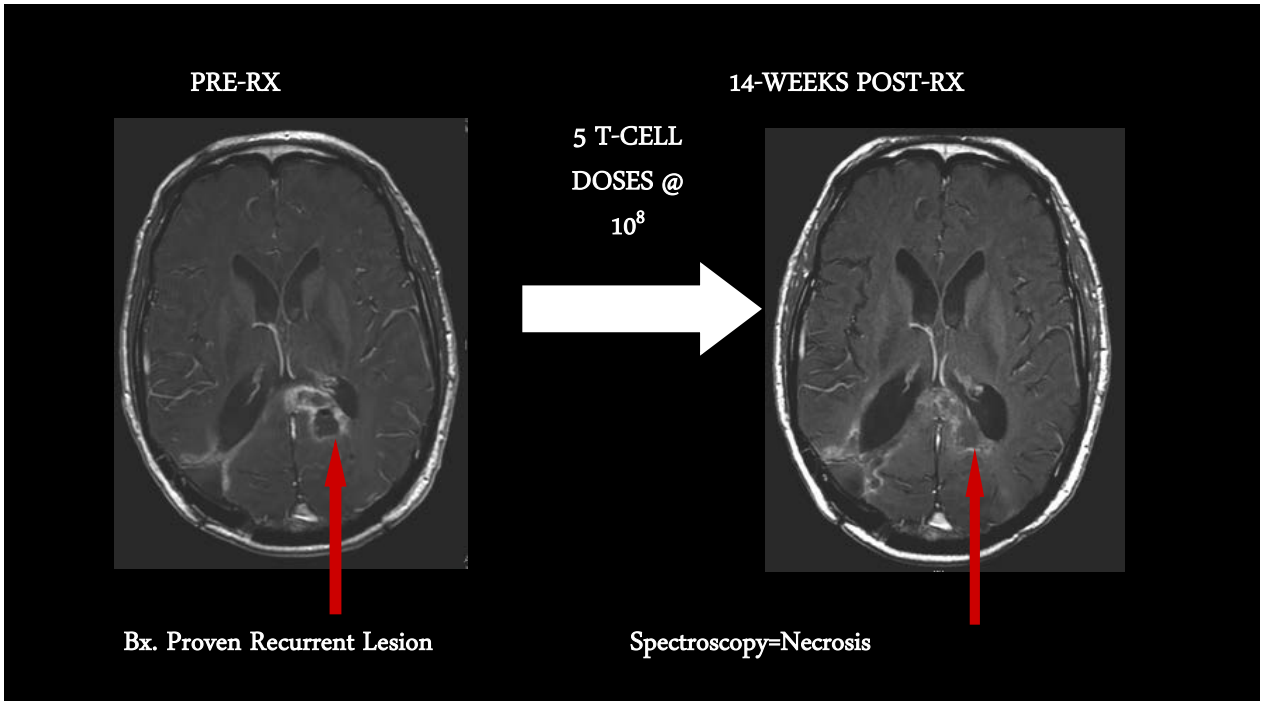
3-5 month production

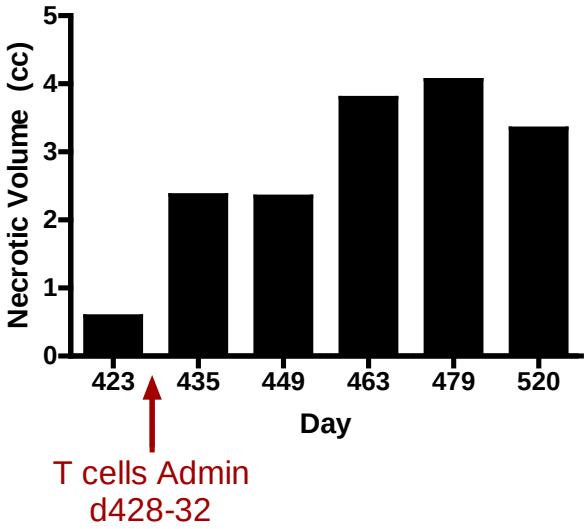


Kahlon et al.; Cancer Res; 2004
Brown et al; Cancer Res; 2012

Transient Regression of a GBM Lesion Following First Generation Autologous CD8+ IL13-zetakine+ T cell Therapy

UPN033



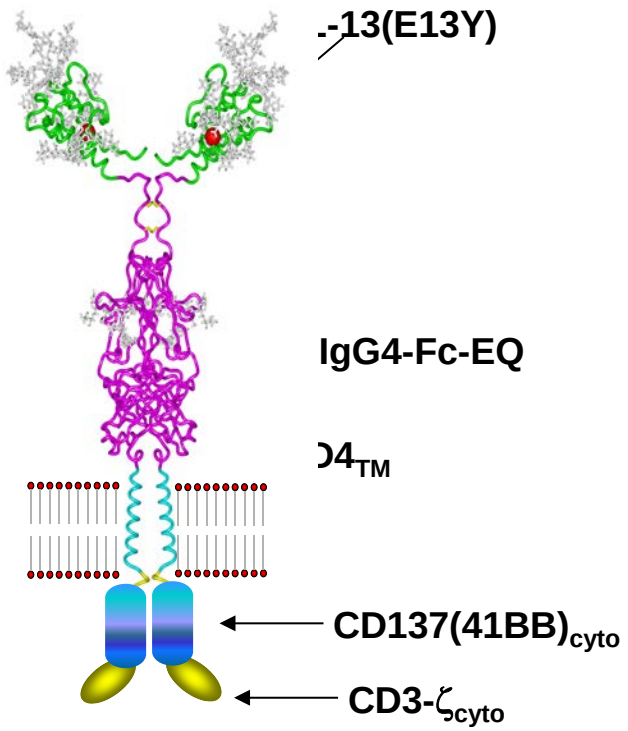


Clinical Development of a Central Memory, Second-Generation CAR T Cell Therapy for the Treatment of GBM

Optimize for T_{MEM}

- Optimize CAR design for costimulation.
- Engineer central memory T cells for improved persistence and self-renewal potential.
- Incorporate CD4 help.
- Limit ex vivo expansion to reduce T cell exhaustion and maintain a T_{MEM} phenotype.

2nd Gen IL13Rα2-specific IL13BBζ-CAR



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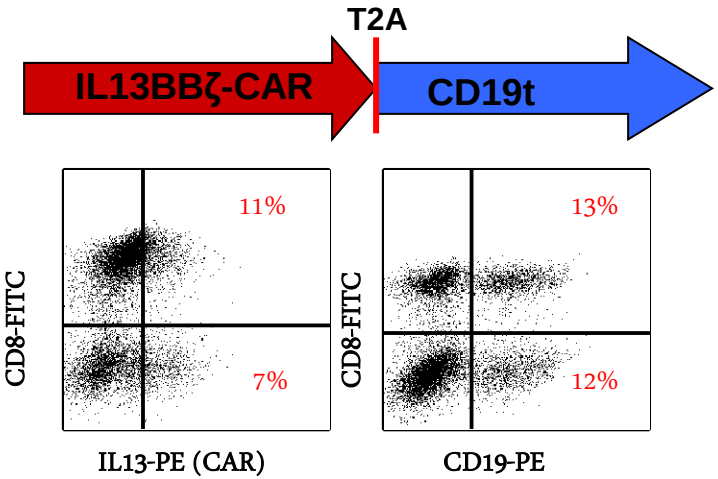
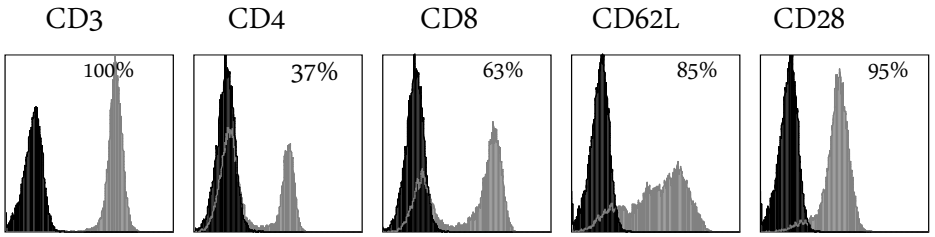
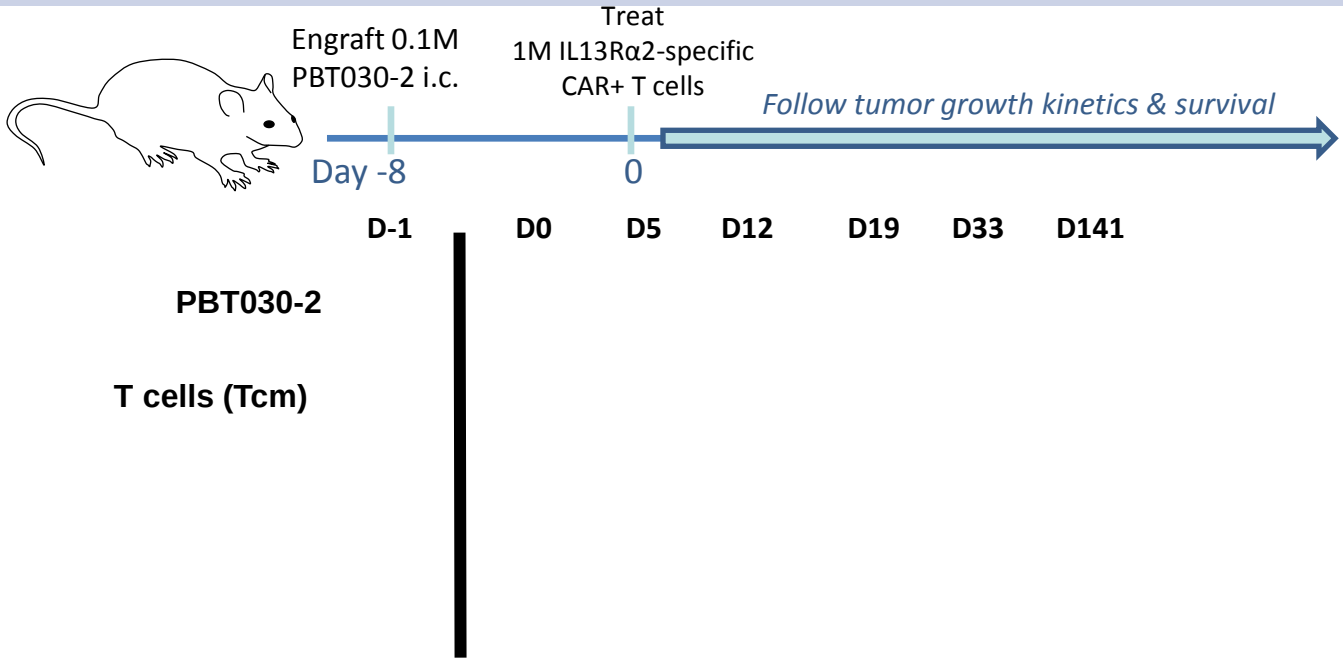
Lentiviral Cassette



CD62L+ Central Memory T cells (T_{CM})



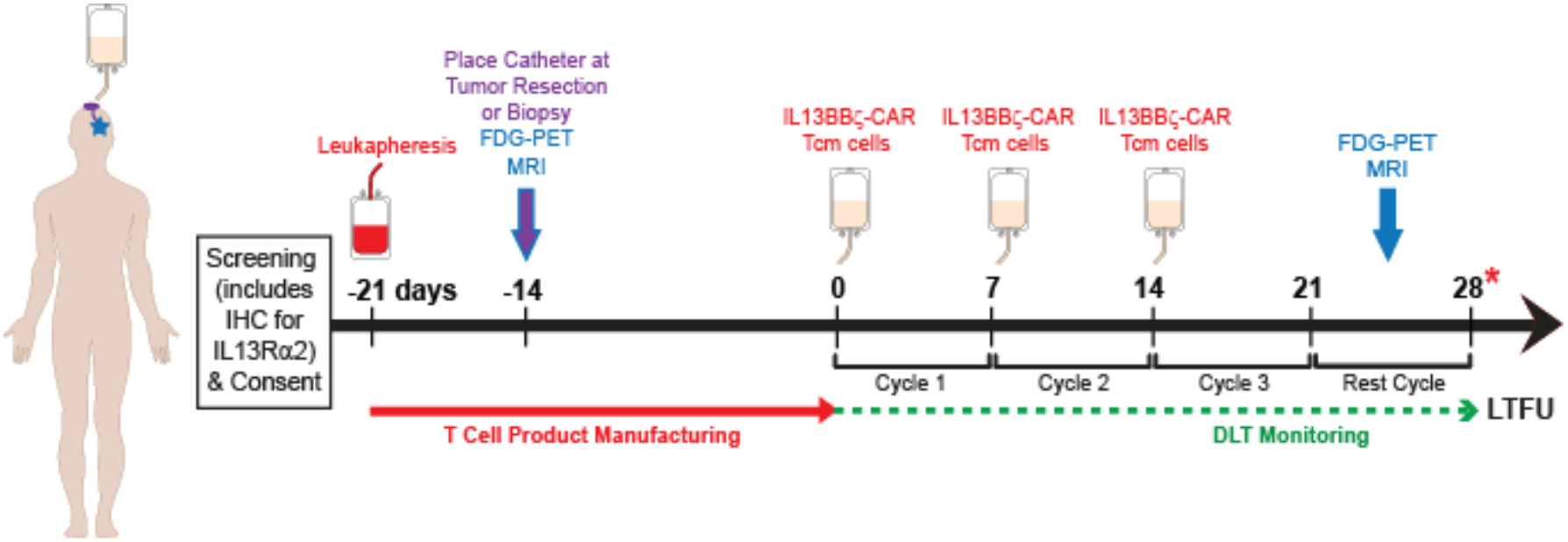
Next Generation IL13BBζ CAR Tcm Mediate Robust Antitumor Activity Against Tumor Sphere Initiated GBM



Phase I Clinical Trial with Optimized IL13Rα2-specific CAR T_{CM}

IND Activated March 2015

NCT	Patient Population	Agent	T cells Dose	Planned Enrollment
NCT02208362	Relapsed/Refractory GBM Arm 1: resectable Arm 2: Non-resectable	Autologous IL13(EQ)BBζ/CD19t + T_{CM}	2 to 50x10^6 CAR T cells	12/arm



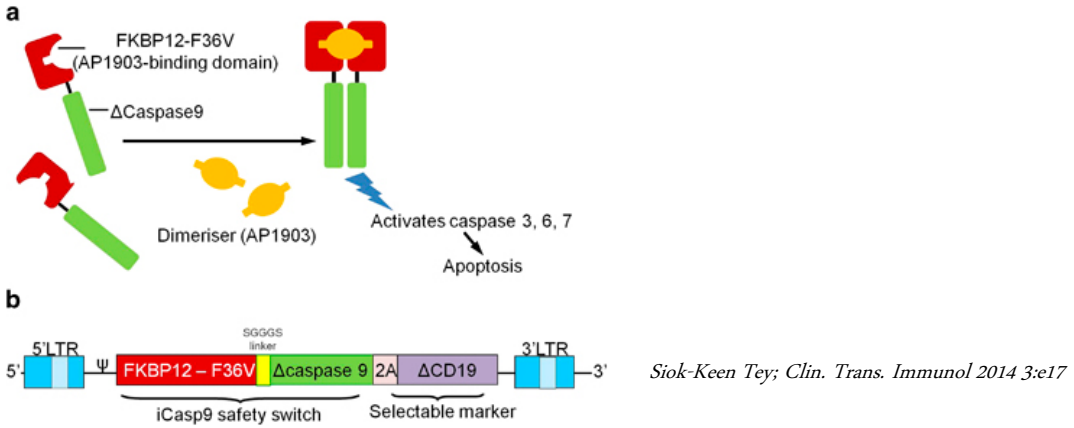
Additional Approaches for Expanding CAR T cells with Stem Cell/Memory Phenotypes

- **Alternative cytokine cocktails**
  - IL-2 promotes T cell differentiation
  - IL-7 & IL-15 promote homeostatic expansion of T_N and T_{MEM} subsets.
  - IL-21 inhibits differentiation of CD8 T cells
- **Inhibition of glycolysis**
  - High levels of glycolysis drive T cells towards a terminally differentiated effector state. Inhibiting glycolysis with 2-deoxyglucose (2-DG) favors long-lived memory T cells that mediate enhanced antitumor responses following adoptive transfer. (Sukumar et al. 2013. JCI. 123:4479; 2014. OncoImmunology. 3:e27573)
- **Induction of Wnt/β-catenin signaling**
  - WNT3A or inhibitors of GSK3β inhibit differentiation of T_N into T_E, while promoting generation of self-renewing T_{MEM}. (Gattinoni et al. Nature Med. 2009 15:808)
- **Inhibition of AKT signaling**
  - Inhibition of Akt promotes generation of T_{SCM} (van der Waart et al. Blood. 2014 124:3490)

CAR T Cell Persistence May Increase Toxicities

Strategies to Control CAR T cell Persistence

- **Transient expression** of CARs using RNA electroporation limits CAR expression and activity can be controlled by a repetitive dosing regimen.
  Zhao et al. 2010. Cancer Res. 70:9053; Maus et al. 2013. Cancer Immunol Res. 1:26.
- **Co-expression of suicide genes** to enable elimination of transferred cells.
  - For example, dimerization of iCasp9 via small molecule induces apoptosis, thereby rapidly eliminating therapeutic CAR T cells. *Di Stasi et al. 2011. N Engl J Med. 365:1673*



Siok-Keen Tey; Clin. Trans. Immunol 2014 3:e17

- **Tagging CAR T cells** for immunologic depletion with therapeutic antibodies. *Wang et al. 2011. Blood. 118:1255*

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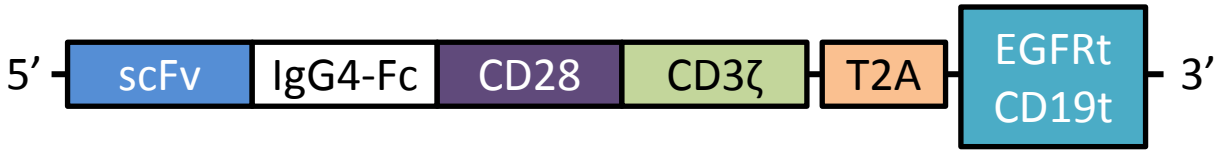
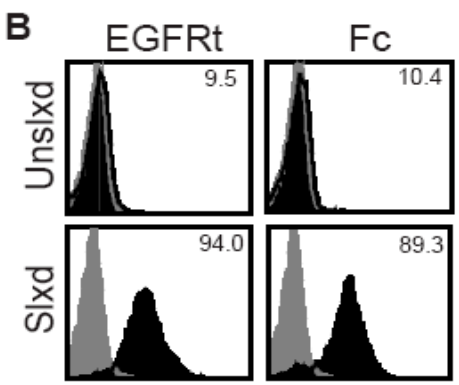
Regulating CAR T cell Persistence with Combined Expression of Cell Surface Antigens

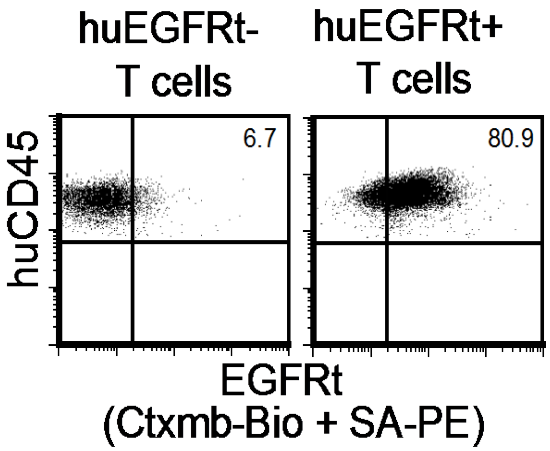


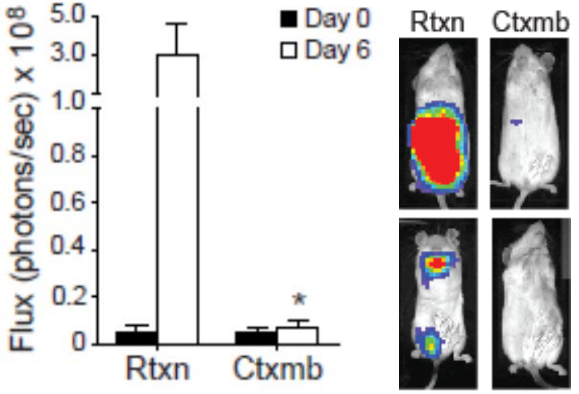
Selection

Detection

Ablation







Wang et al.. Blood. 2011.118:1255

Questions for Thought

- **What is the best T cell population for CAR T Cell Therapy?**
  - CAR T cell products with less differentiated memory phenotypes correlate with improved clinical responses.
  - CD4 & CD8 ratios may also impact T cell potency and persistence
  - The optimal T cell product composition remains to be clinical defined.

- **Is enrichment of defined memory T cell subsets necessary?**
  - Clinical products derived for unselected PBMC products have mediated remarkable clinical responses for a subset of patients.
  - Preclinical data support the potential benefit of selecting/expanding specific T cell subsets for genetic modification
  - Clinical validation of the benefits of starting with an enriched T_{MEM} population is forthcoming.

- **How does CAR-signaling strength and costimulation influence memory maintenance?**
- **How long do CAR T cells need to persist for durable clinical responses ?**

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