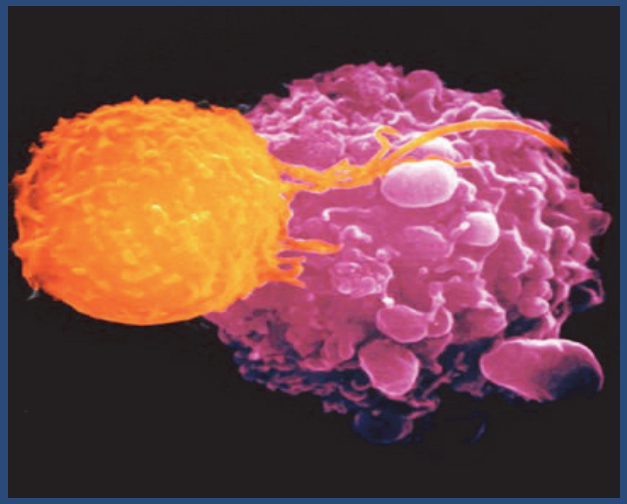
Treatment of Non-Hodgkin Lymphoma with Central Memory Derived CD19-Specific CAR-Transduced T Cells



Leslie Popplewell[1], Christine Brown[1], Xiuli Wang[1], Araceli Naranjo[1], Jamie Wagner[1], Wen-Chung Chang[1], William Bretzlaff[1], Brenda Chang[1], Desiree Daniels[1], Julie Ostberg[1], Renate Starr[1], ChingLam W. Wong[1], Suzette Blanchard[2], Michelle Mott[3], Bernadette Pulone[3], Stanley Riddell[4], Michael Jensen[5], and Stephen Forman[1]

[1]Department of Hematology & Hematopoietic Cell Transplantation, Beckman Research Institute, [2]Department of Information Sciences, and [3]Nursing Department, COHNMC, Duarte, CA;

[4]Program in Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA.

[5]Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA

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Disclosures

- M. Jensen is an inventor of licensed patents and equity holder in ZetaRx, Inc., a licensee of these patents.
- All other authors have no conflicts of interest to disclose.

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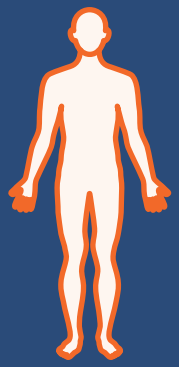
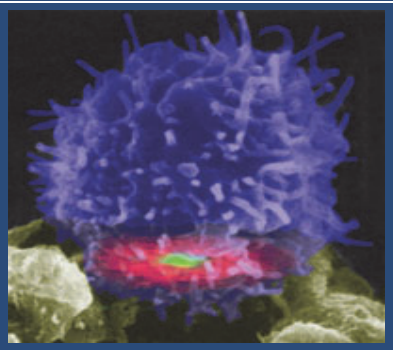
Adoptive T Cell Therapy for Cancer

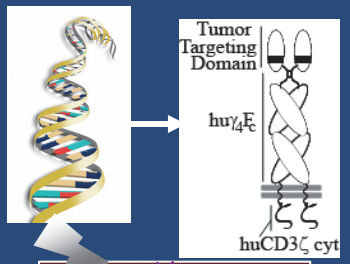


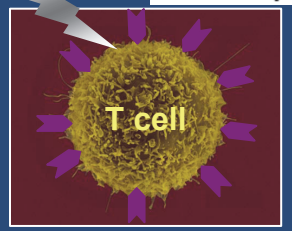
T Cell Donor
(Autologous or
Allogeneic)

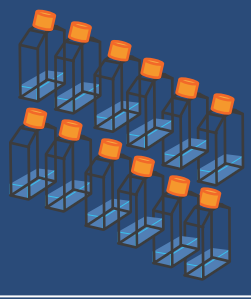
Isolate Cytotoxic
T Lymphocyte



Engineer Cytotoxic T Cells
to Express Tumor Specific
Chimeric Antigen Receptors

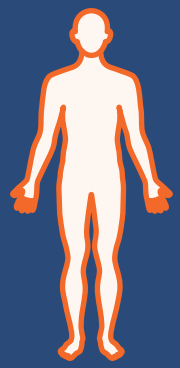






Expand Tumor Specific
T Cells Ex Vivo

Adoptive
T cell Transfer



Patient Recipient

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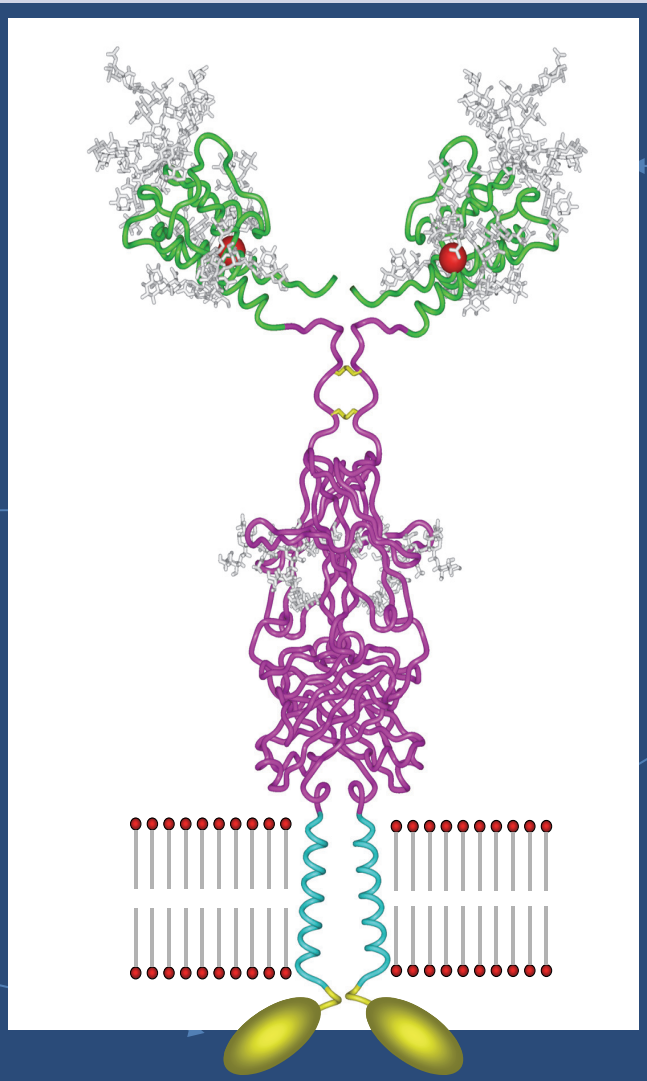
The Chimeric Antigen Receptor (CAR)



CD19-specific scFv

huIgG4 hinge-Fc

$huCD4_{TM}$

huCD3-ζ_{cyto}

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Persistence of Transferred T Cells Correlates with Cancer Regression

Strategies to Improve T Cell Persistence:

- Incorporate lymphodepletion regimens prior to ACT
- Optimize CAR design for improved co-stimulatory signaling
- Reduce transgene immunogenicity
- Engineer T cell subsets with the propensity for long-term persistence (i.e., memory T cells)

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Research article

Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates

Carolina Berger,[1] Michael C. Jensen,[2] Peter M. Lansdorp,[3,4]
Mike Gough,[5] Carole Elliott,[5] and Stanley R. Riddell[1,6]

[1]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [2]Division of Cancer Immunotherapeutics and Tumor Immunology, City of Hope National Medical Center, Duarte, California, USA. [3]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada. [4]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada. [5]University of Washington National Primate Center, Seattle, Washington, USA. [6]Department of Medicine, University of Washington, Seattle, Washington, USA.

J. Clinical Investigation 2008

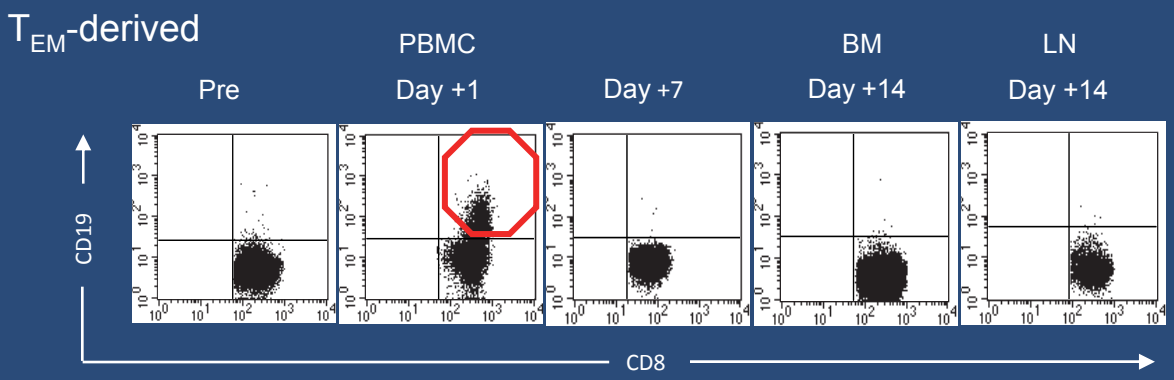
Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice

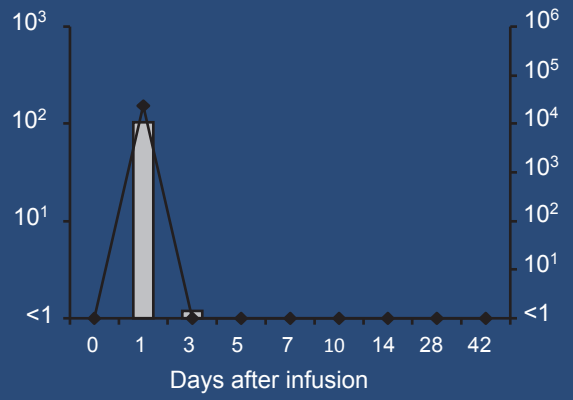
Xiuli Wang,[1] Carolina Berger,[2] ChingLam W. Wong,[1] Stephen J. Forman,[1] *Stanley R. Riddell,[2] and *Michael C. Jensen[1,3]

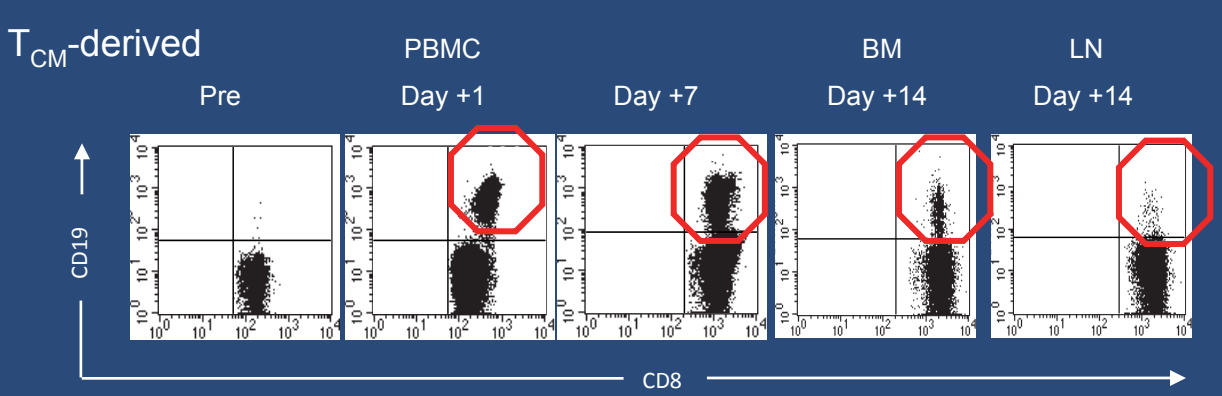
[1]Department of Cancer Immunotherapeutics & Tumor Immunology, Beckman Research Institute, City of Hope National Medical Center, Duarte, CA; [2]Program in Immunology, Fred Hutchinson Cancer Research Center, University of Washington School of Medicine, Seattle, WA; and [3]Center for Immunity and Immunotherapies, Seattle Children's Research Institute, Seattle, WA

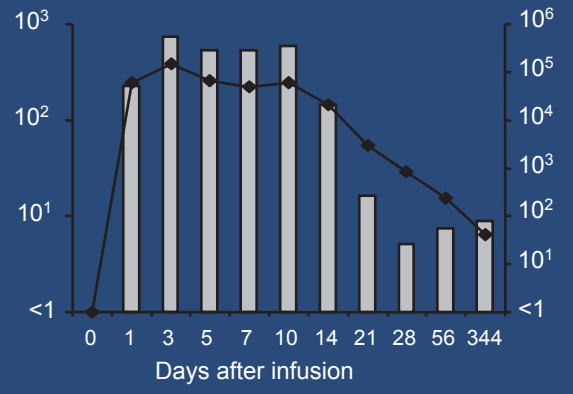
Blood 2010

Distinct Fate of Effector T Cells Derived from T_{CM} vs T_{EM} Following ACT in Non-Human Primate









Berger et al J Clin Invest 2008

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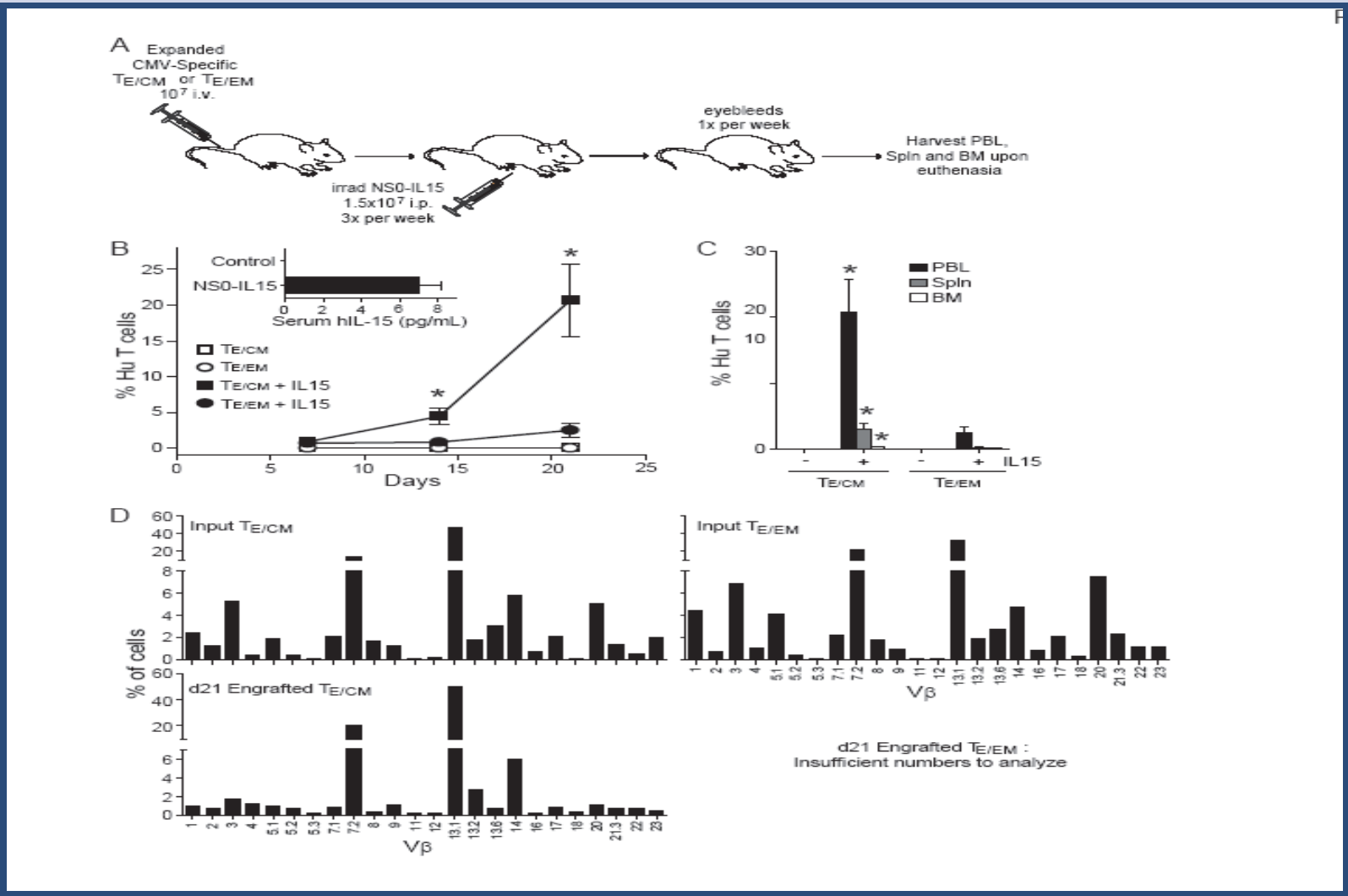
T_{CM} Derived Human Effectors Exhibit Superior Engraftment to T_{EM} Derived Counterparts Following Adoptive Transfer



Wang et al Blood 2011

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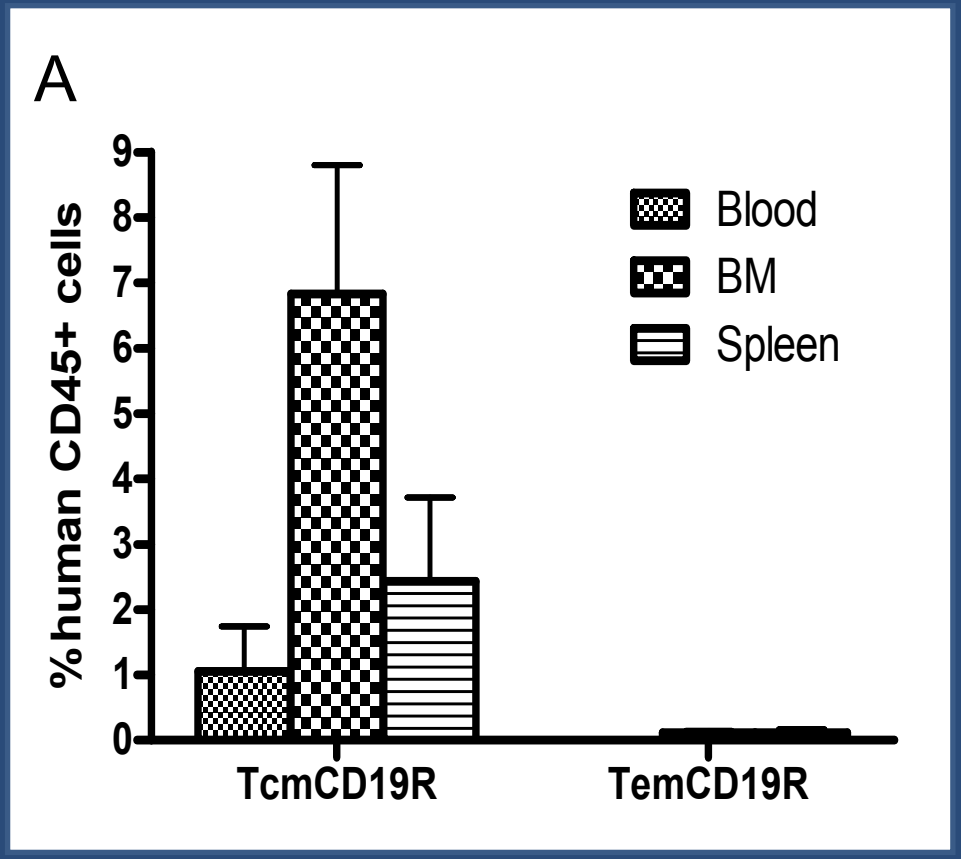
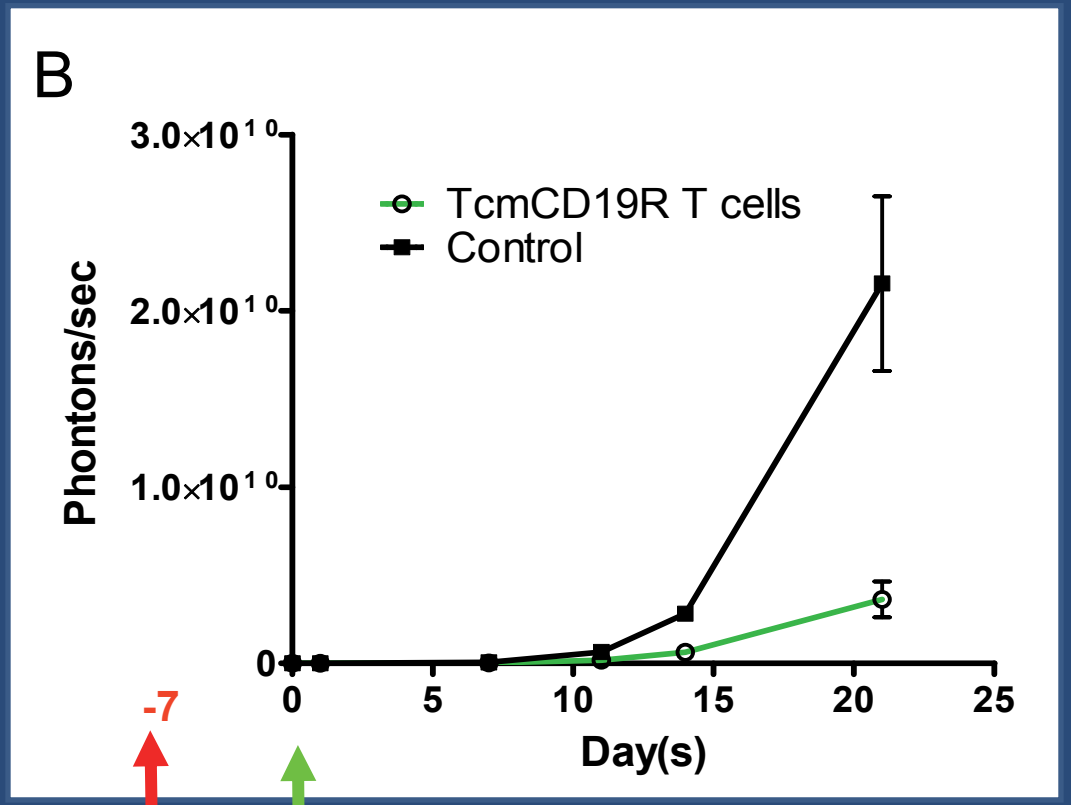
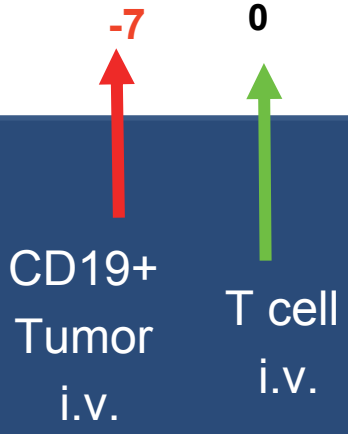
Engraftment Fitness and Anti-Lymphoma Activity of CD19CAR+ T Cells Derived from T_{CM} vs T_{EM}







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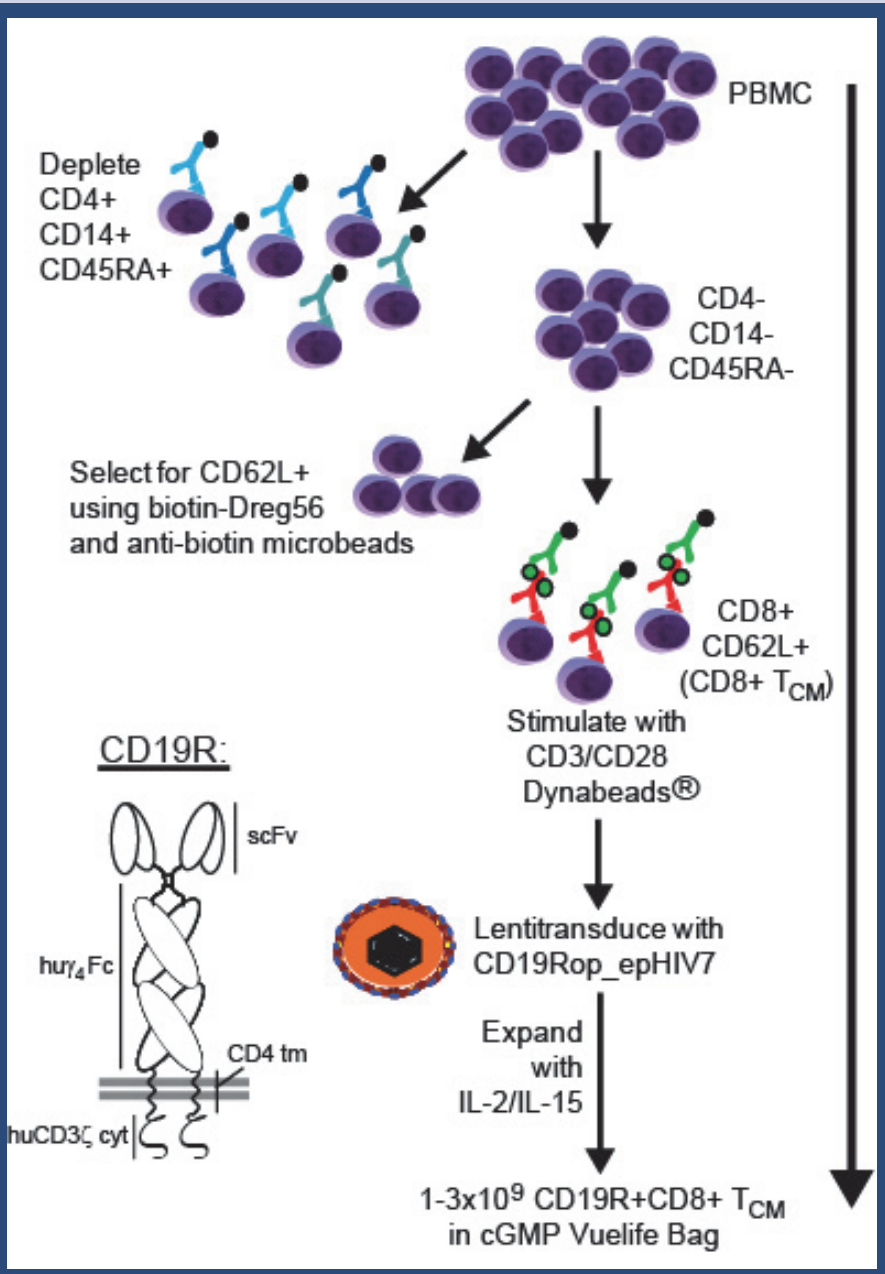
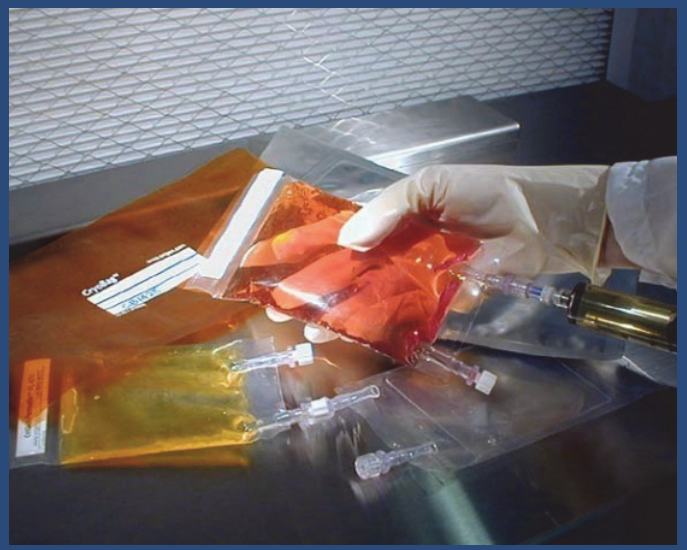
Platform for Manufacturing T_{CM} Derived CD19CAR$^+$ T Cells



Day : Bioprocess

Day 1: Leukapheresis

Day 2: CliniMACS Selection of Tcm; Dynabead stimulation



Day 5: Lentiviral Transduction; Initiate Expansion

Day 14-30: Dynabead Removal

Day 26-40: Cryopresservation

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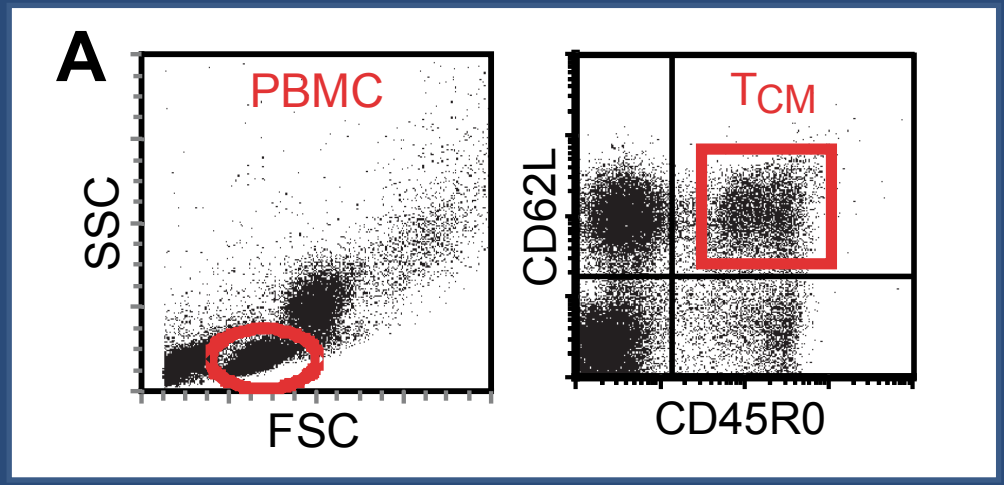
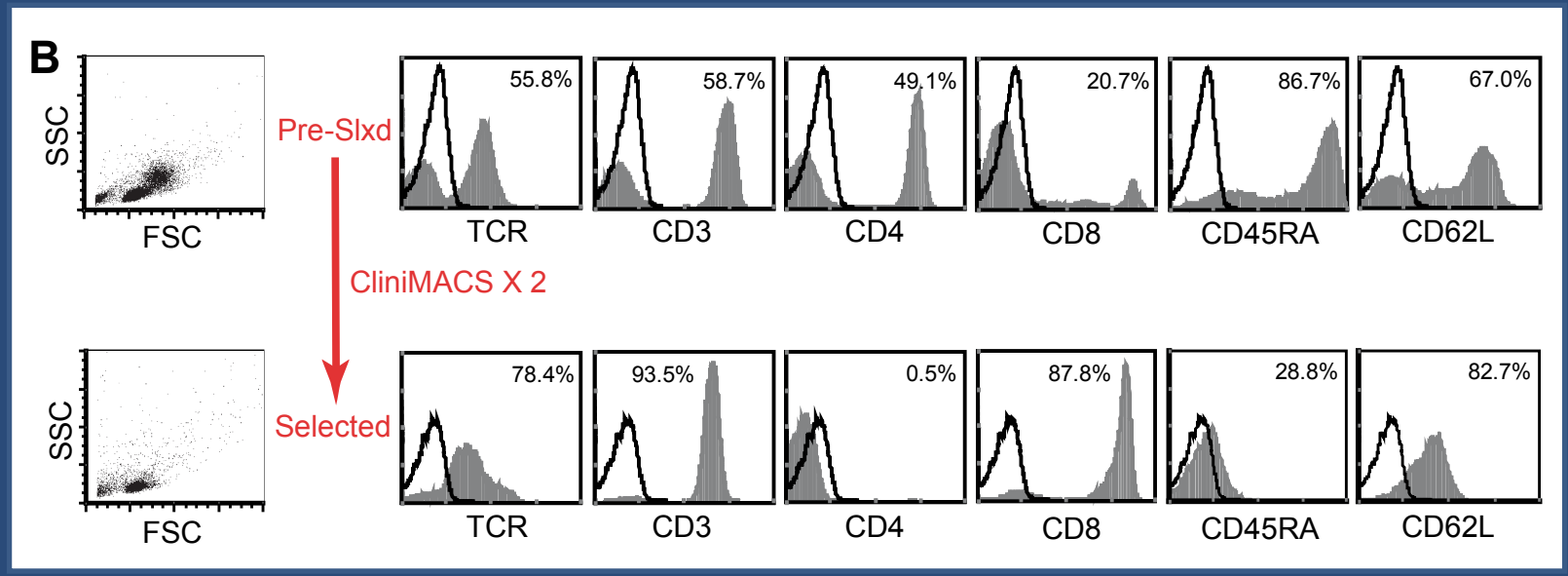
Enrichment of CD8+ T_{CM}

Day 2: CliniMACs

1) CD45RA/CD14/CD4 Depletion

2) CD62L Positive Selection





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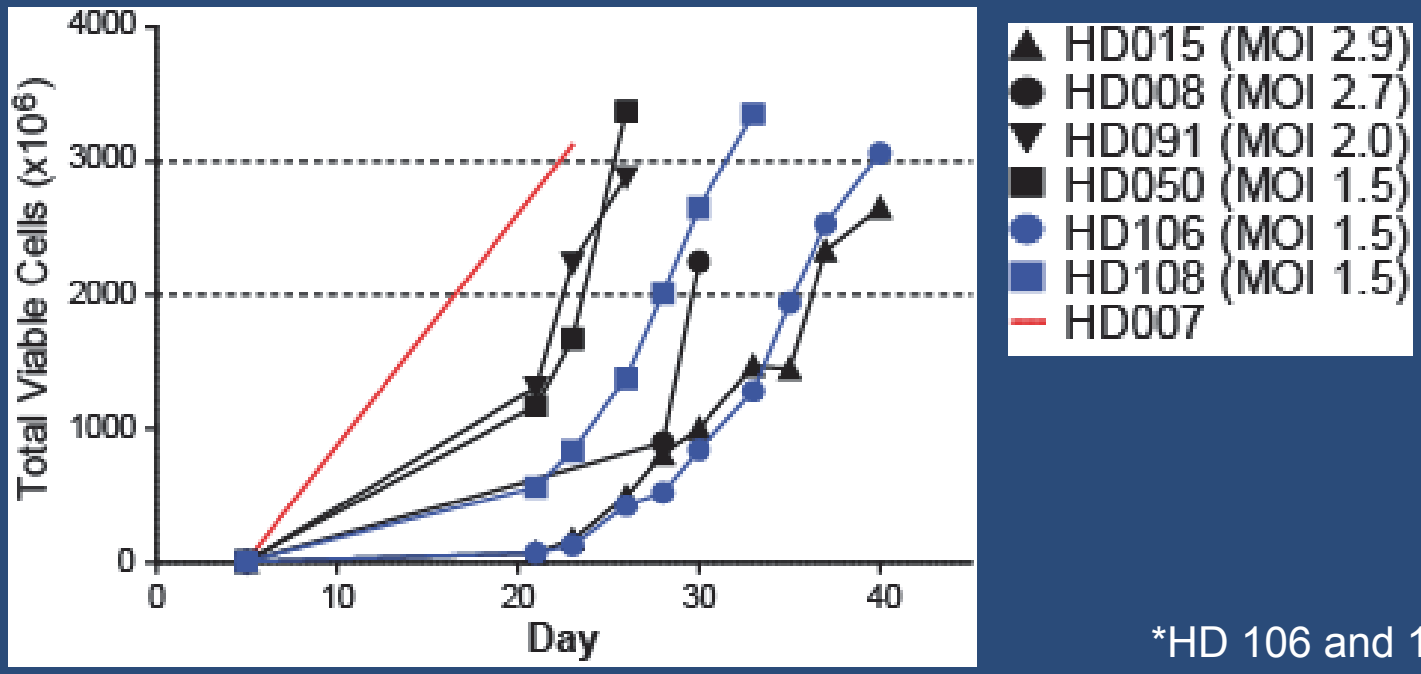
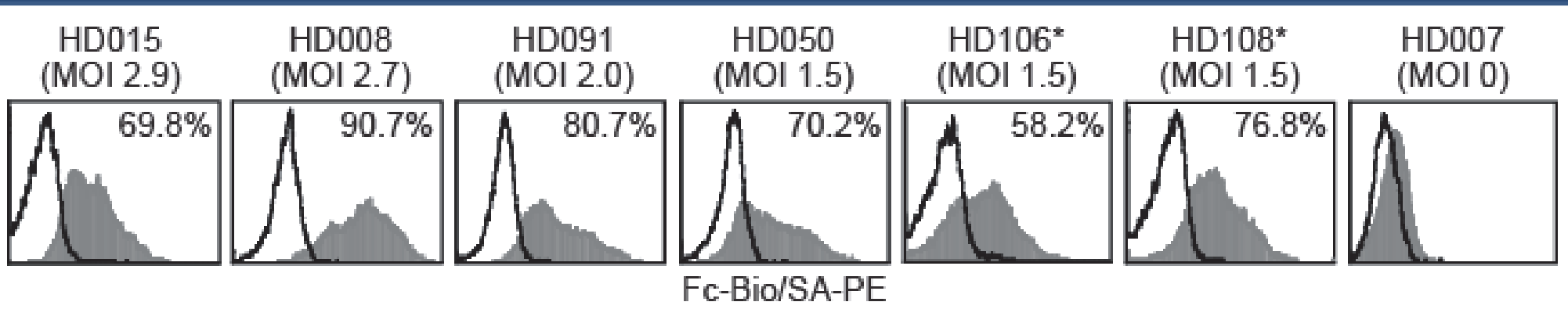
Development of T_{CM} Derived CD19CAR+ Cells (Qualification Runs)

Wang et al J Immunother 2012



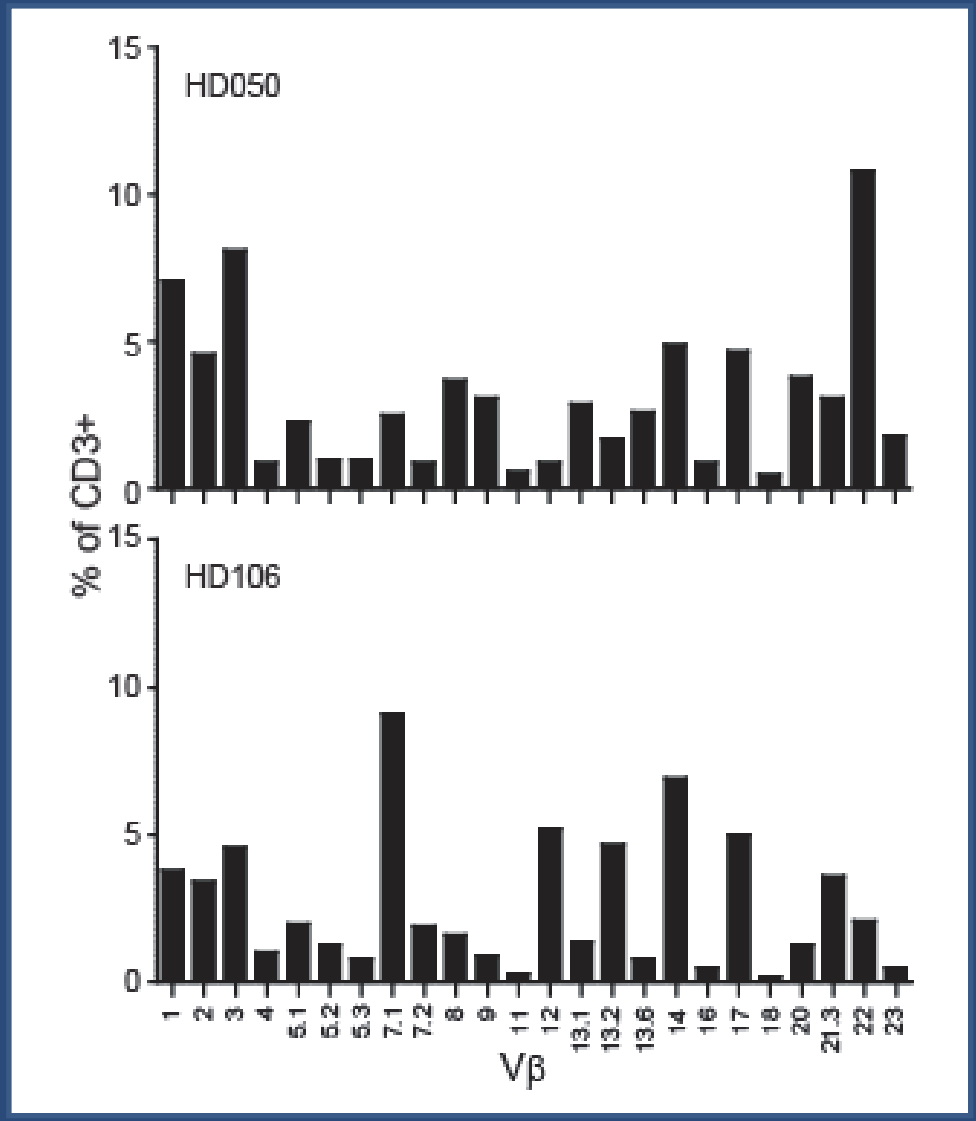
*HD 106 and 108 were from Lymphoma donors



Broad TCR Vβ Repertoire of T_{CM} Derived CD19CAR+ Cells

Representative Healthy Donor:

Representative Lymphoma Patient:



Wang et al
J Immunother 2012

Freshly thawed qualification run T cell products were stained with the IOTest® Beta Mark TCR Vβ Repertoire Kit.

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IRB 09174/BB-IND 14645

- Relapsed B Cell Lymphoma: Induction failure, recurrence of large cell, mantle cell lymphoma
- Poor prognosis with transplant
- Infuse cells on day +2 after transplant
- Lymphodepletion, homeostatic expansion
- Engraft cells as a component of the reconstituted immune system

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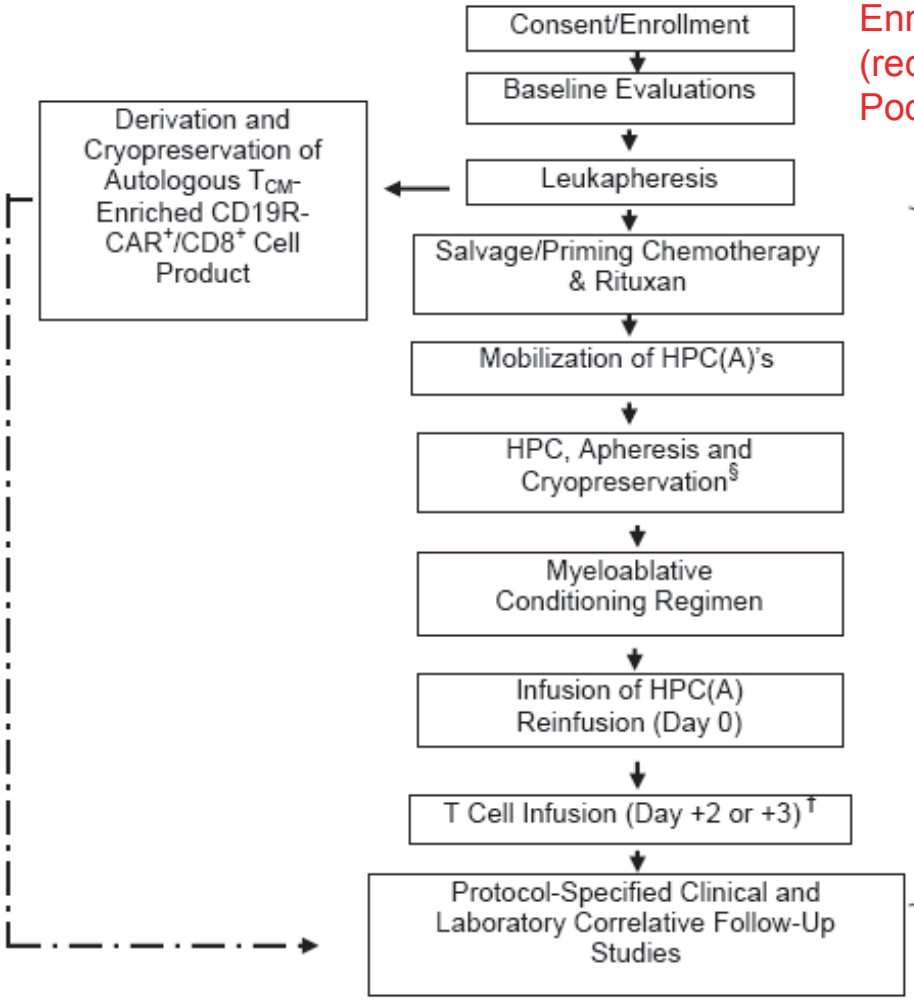
IRB 09174/BB-IND 14645

Phase I/II study of cellular immunotherapy using central memory-enriched CD8+ T cells lentivirally transduced to express a CD19CAR following HSCT for patients with high risk intermediate grade B lineage NHL.



Enrollment: Relapsed B Cell Lymphoma (recurrent large cell & mantle cell lymphoma); Poor prognosis with auto-transplant.

Salvage Therapy, Hematopoietic Progenitor Cell, Apheresis (HPC(A)) Mobilization and Collection, Myeloablative Preparative Regimen, HPC Rescue, and Supportive Care per COH Hem/BMT Program Standard Practice SOP's

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

Dose Schedule			
Starting Dose Dose 0	Dose 1	Dose 2	Dose 3
50M	**100M**	**500M**	**1000M**

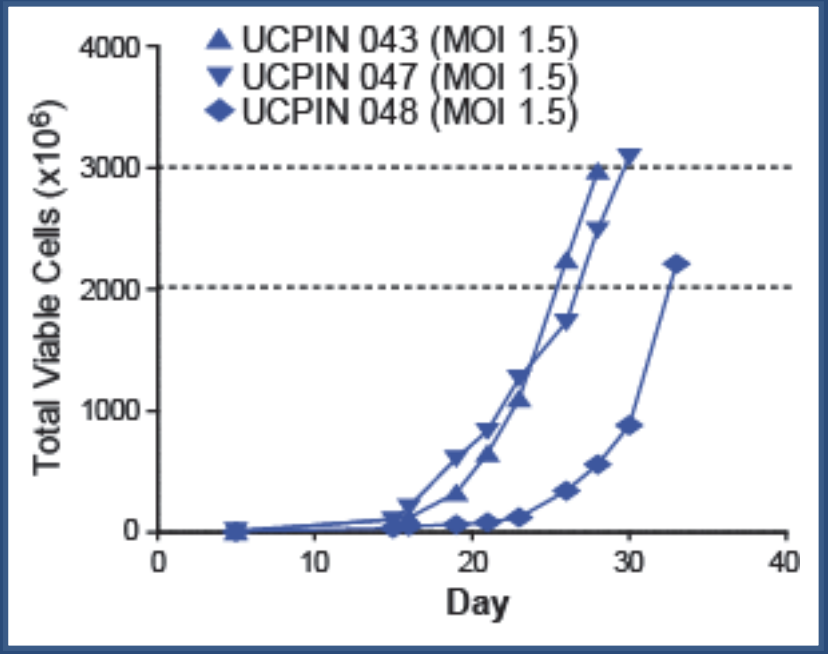
IRB 09174/BB-IND 14645: Patient Accrual

FDA limit to one patient at a time in groups of 3 for each cell dose escalation; Starting dose = 5×10^7

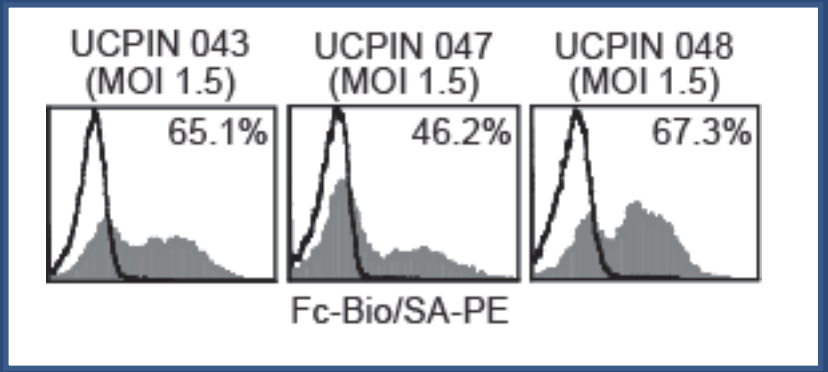
Patient ID	Age Sex	Diagnosis	Prior Therapy	Salvage Prior to Leuk	Leukapheresis Date	Salvage Post Leuk	Disease Status at Time of HSCT	HSCT Date	T Cell Infusion Date
UPN 043	68 yr F	diffuse large B-cell lymphoma, Relapsed10/2011 - mass on right upper back, as well as possibly the uterus	6 cycles R-CHOP including CNS prophylaxis x 2 with high-dose MTX as an inpatient	No	10/20/11	2 cycles R-ICE	2nd Remission	1/16/12	1/18/12
UPN 047	74 yr M	diffuse large B-cell lymphoma, Induction failure 03/2012 – mass in abdomen	6 cycles of R-CHOP	2 cycles of R-ICE	3/29/12	No	Partial Remission	6/5/12	6/7/12
UPN 048	49 yr F	diffuse large B-cell lymphoma, Relapsed 03/2012 – mass in thyroid	6 cycles of R-ICE	No	5/17/12	2 cycles R-ICE	2nd Remission	9/21/12	9/24/12

IRB 09174/BB-IND 14645: First Three Clinical Products

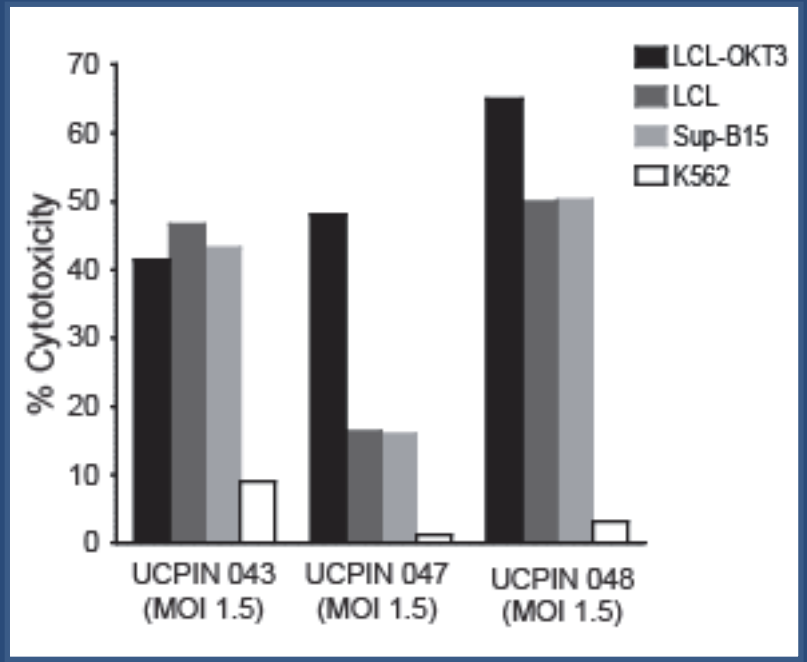
T Cell Product Expansion:



CD19CAR Expression:



CD19-Specific Cytolytic Activity of T Cell Products:



Impact of CD19CAR+ T Cell Infusion on HSCT Engraftment

Patient ID	HSCT Date (Day 0)	T Cell Infusion Date (Day +2/+3)	ANC > 500	Platelets >20K
UPN 043	1/16/12	1/18/12	Day +10	Day +15
UPN 047	6/5/12	6/7/12	Day +12	Day +18
UPN 048	9/21/12	9/24/12	Day +11	Day +16

No Infusional Toxicities

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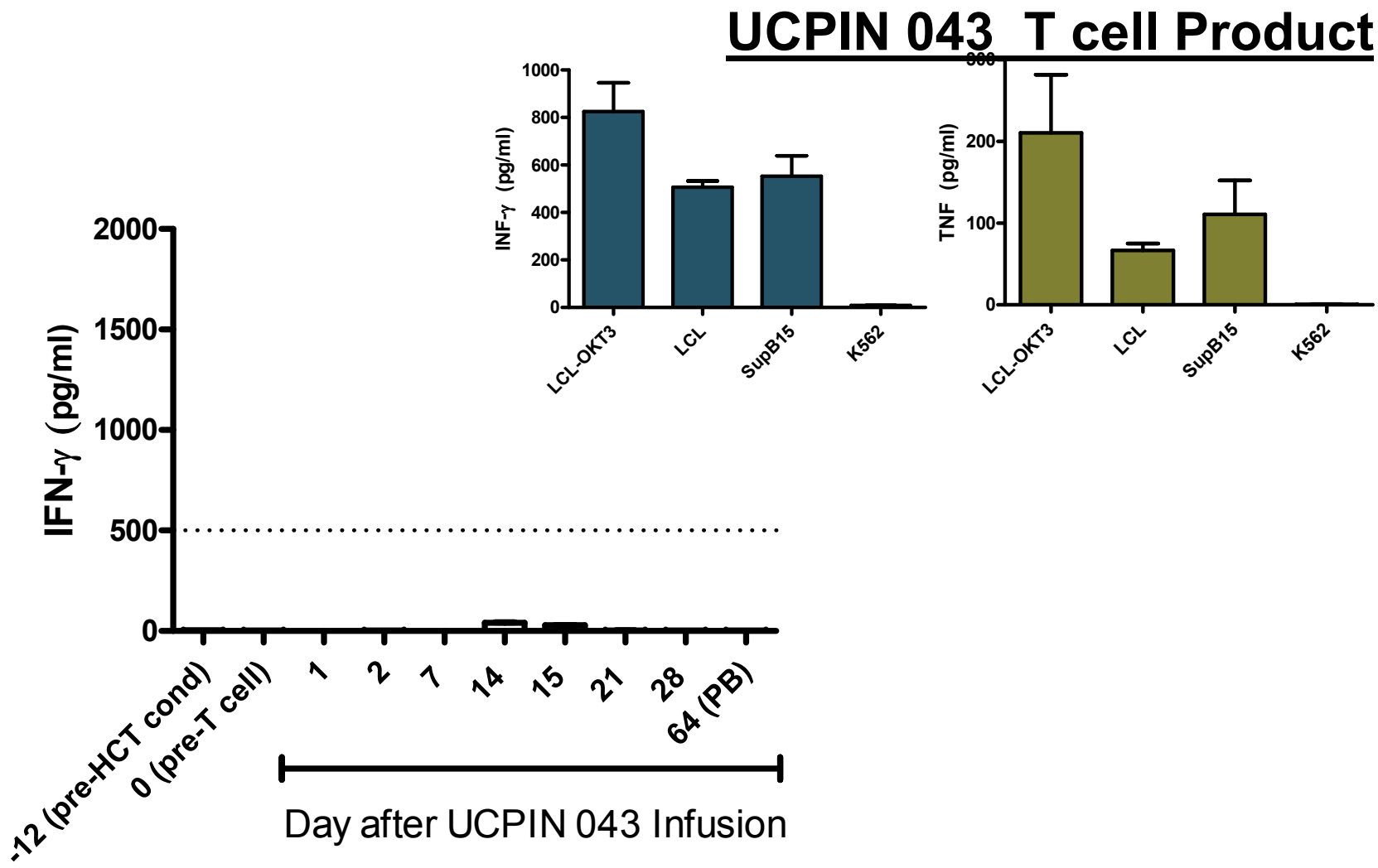
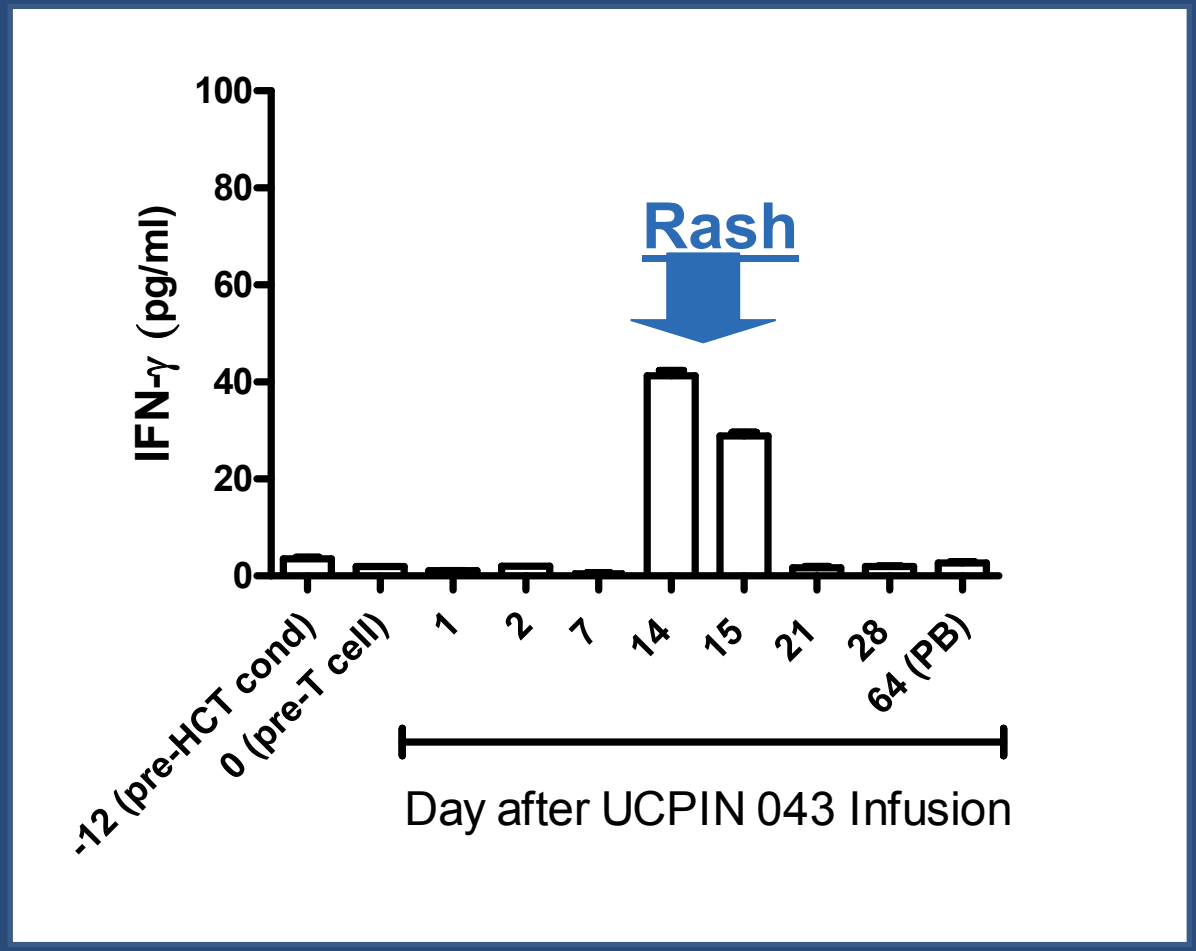
Serum Cytokine Levels Following CD19CAR+ T cell Infusion in UPN 043



*** TNF below level of detection for all points***

Serum Cytokine Levels Following CD19CAR+ T cell Infusion in UPN 043



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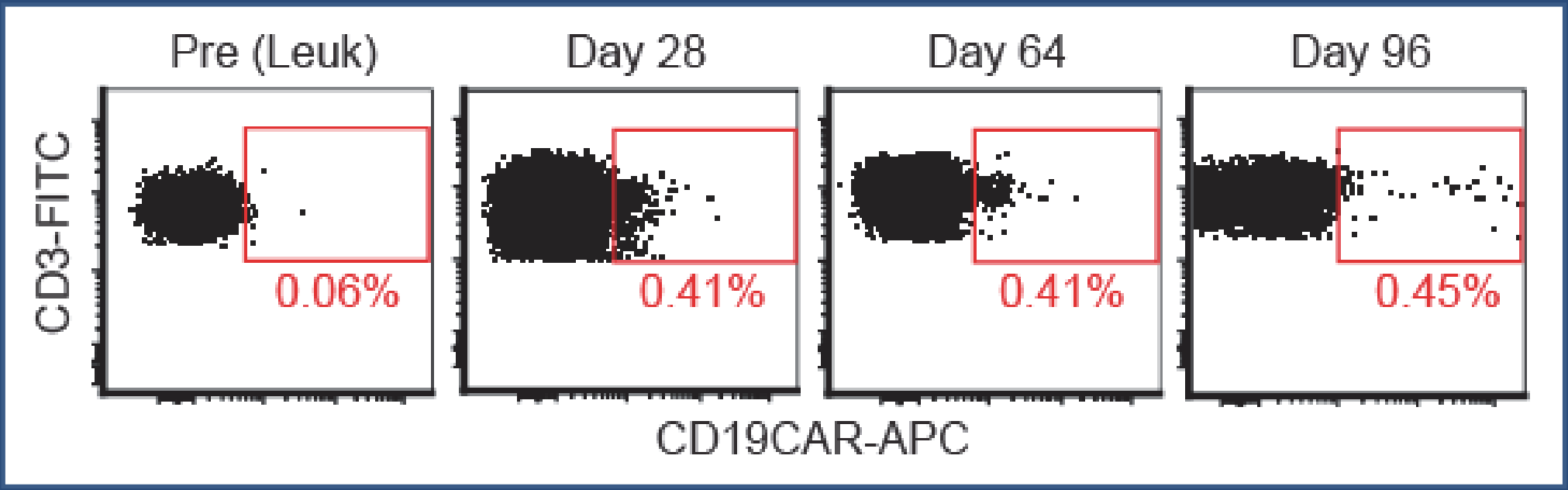
CD19CAR+ T Cell Persistence in Blood (PBMC) of UPN043



** anti-CD19R-CAR antibody kindly provided by Dr. Laurence Cooper, MD Anderson Cancer Center, Houston, TX

Preliminary qPCR data confirms CD19CAR+ T cell persistence:

UPN043 PBMC	gDNA (ng)	WPRE copy#	WPRE Copy# /100ug gDNA
Day 23	200	2.398	1210
Day 28	200	2.534	1267
Day 64	200	1.172	586

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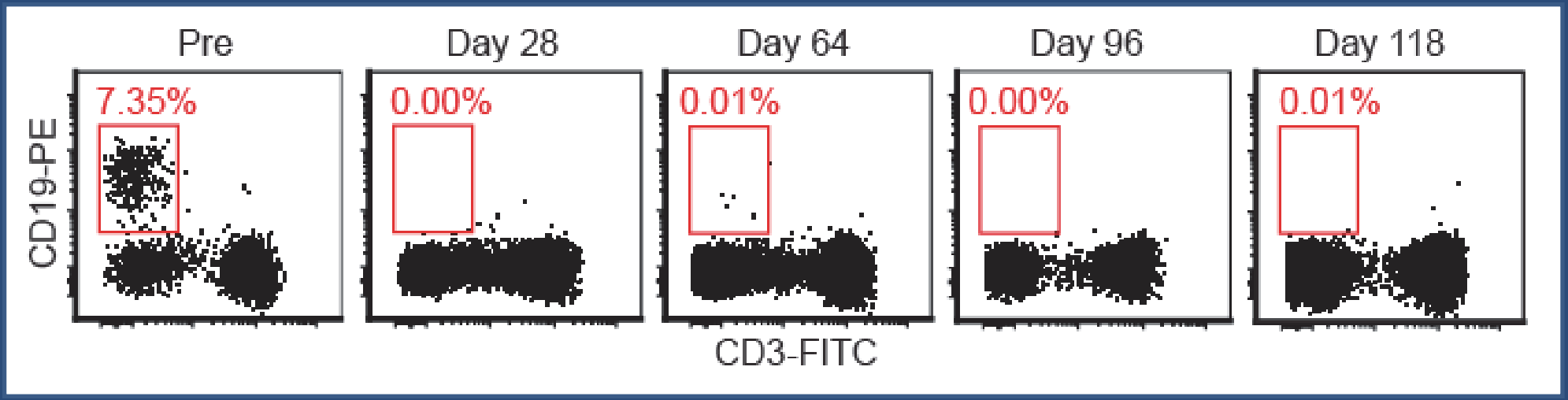
CD19+ B Cell Aplasia in Blood (PBMC) of UPN043



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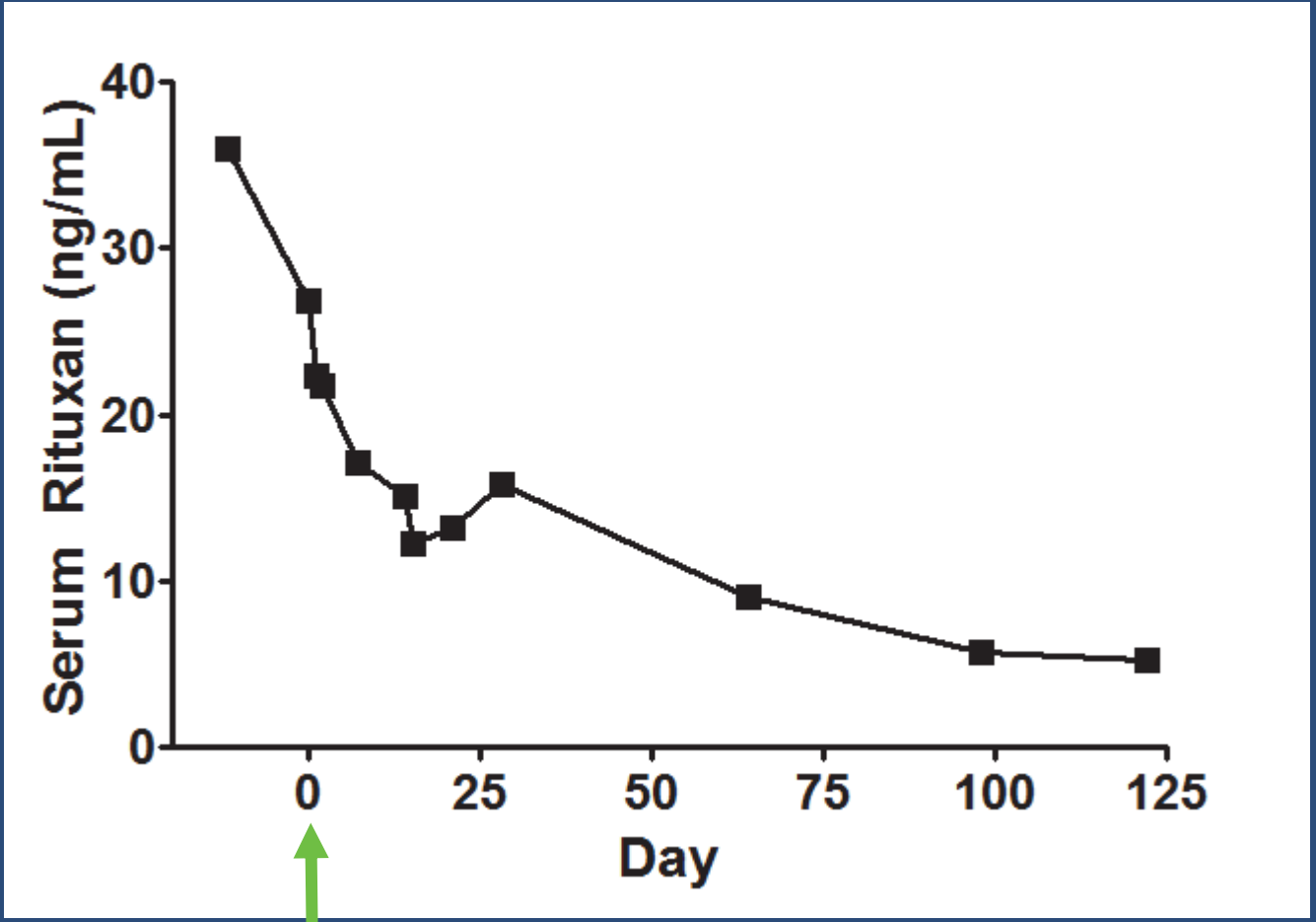
Serum Rituxan Levels in UPN 043



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Clinical Research Plan

- Complete 1st cohort at $5x10^7$ CD19CAR+ CD8+ T_{CM}-derived cells
  - Amendment to normalize for CAR+ recently approved by FDA
- Continue dose escalation with $1x10^8$ CD19CAR+ CD8+ T_{CM}-derived cells
- Work with FDA to liberalize accrual design
- Repeat $1x10^8$ dose with CAR+ Bulk T_{CM}-derived cells
  - i.e., remove CD4-depletion step in T_{CM} selection strategy
- Initiate IND with second generation, costimulatory CAR (CD19R:CD28:ζ)

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Acknowledgments

T Cell Therapeutics Research Laboratory

Stephen Forman, MD

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- William Bretzlaff
- Christine Brown, PhD
- Brenda Chang
- Wen-chung Chang
- Desiree Daniels
- Blake Friedman
- Araceli Hamlett
- Lauren Hoffman
- Hao Hong, PhD
- Mahesh Jonnalagadda, PhD
- Vaidehi Mahadev
- Armen Mardiros
- Julie Ostberg, PhD
- Renate Starr
- Leonor Velasco
- Jamie Wagner
- Xiuli Wang, MD PhD
- Lihong Weng, MD
- Winnie Wong

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- Bernie Pulone, RN
- Michelle Mott, RN
- Joel Conrad, MS
- Larry Couture, PhD
- Catherine Matsumoto
- Yasmine Shad
- Suzette Blanchard, PhD
- Simon Lacey, PhD

Michael Jensen, MD
Seattle Children's Research Institute

Stanley Riddell, MD & Carolina Berger, PhD
Fred Hutchinson Cancer Research Center

Support: Lymphoma Research Foundation; NCI Lymphoma SPORE; The Nesvig Lymphoma Research Fund; The Marcus Foundation; The Tim Lindenfelser Lymphoma Research Fund

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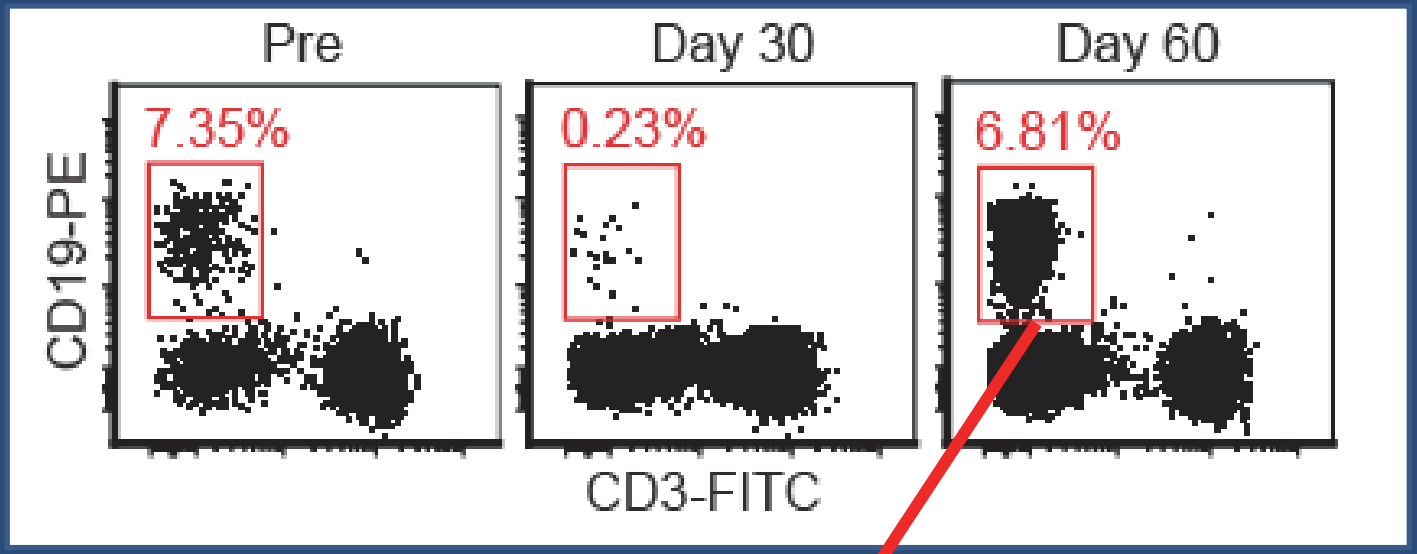
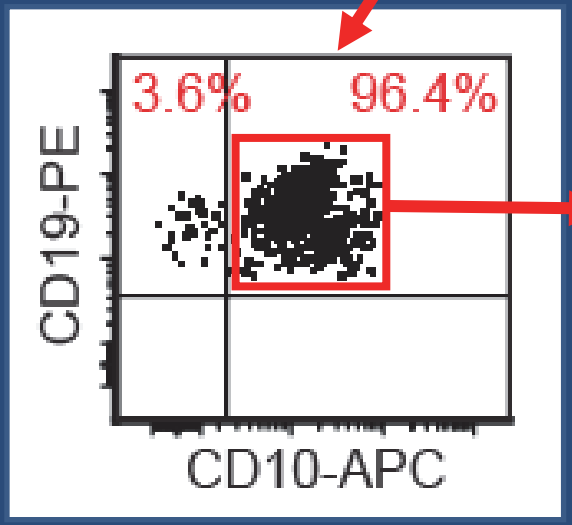
CD19+ B Cells in the Bone Marrow of UPN043





Majority of BM CD19+ cells are CD10+ Pro-B and Pre-B cells

CD19+CD10- cells are mature B cells

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CD19-Targeted Clinical Trials Using CAR T cells

Center[a]	Disease[b]	CAR endodomain[c]	Vector to express CAR[d]	Conditioning regimen	Target[e]	Status[f]	ClinicalTrials.gov NCT no.[g]
MSKCC	CLL-refractory	ζ/28	RV	None vs. cyclophosphamide	CD3-selected	Open 6 treated	NCT00466531
MSKCC	B ALL-relapsed	ζ/28	RV	Cyclophosphamide	CD3-selected	Open 1 treated	NCT01044069
BCM	B NHL and CLL	ζ/28 vs. ζ	RV	None	PBMCs (OKT3 and IL-2)	Open 5 treated: 4 DLBCL 1 B-CLL	NCT00586391
BCM	B NHL and CLL	ζ/28 vs. ζ-EBV	RV	None	PBMCs and EBV CTLs	2 treated	NCT00608270
BCM	B ALL, S/P HSCT	ζ/28	RV	+30 Days after allo-HSCT	Multivirus CTL	Open	NCT00709033
NCI	Lymphoma, CLL	ζ/28	RV	Fludarabine and cyclophosphamide	PBMCs (anti-CD3 + REP)	Open 4 treated	NCT00924326
U Penn	Refractory B leukemia/lymphoma	ζ/41BB vs. ζ	LV	Variable	Auto PBMCs (CD3/CD28 beads)	To open[h]	NCT00891215
U Penn	Relapsed ALL, S/P HSCT	Zζ/41BB	LV	Variable	Allo DLI	To open	
MDACC	B-NHL, S/P autologous HSCT	ζ/CD28	Electroporation/SB plasmids	BEAM-R	Auto PBMCs (± IL-2)	To open	NCT00968760
MDACC	B-lineage malignancy, S/P allogeneic HSCT	ζ/CD28	Electroporation/SB plasmids	Conditioning regimen for HSCT	Allo PBMCs or umbilical cord blood	To open	
COH		ζ	Plasmid	Fludarabine or +28 days S/P HSCT	PBMCs	Closed 3 treated	NCT00182650
COH/FHCRC	Recurrent LCL-MCL, S/P autologous HSCT	ζ	LV	+2 Days after auto-HSCT	Tcm: $CD8^+/CD4^-/CD45RA^-/CD62^+$	To open	NCT01318317

Kohn et al. Mol Therapy. 2011. 19:432