

# **T Cell Transfer Therapy for Cancer**

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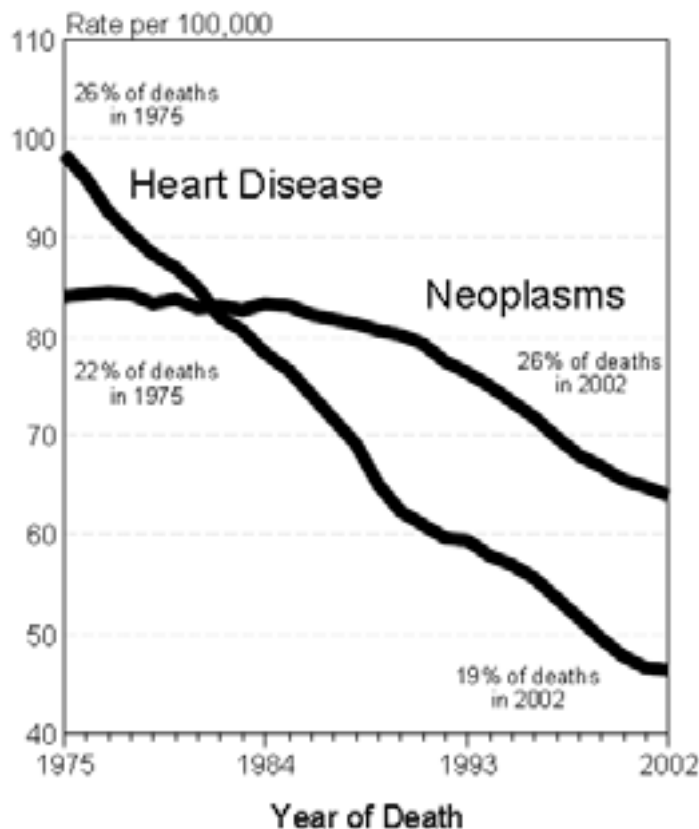


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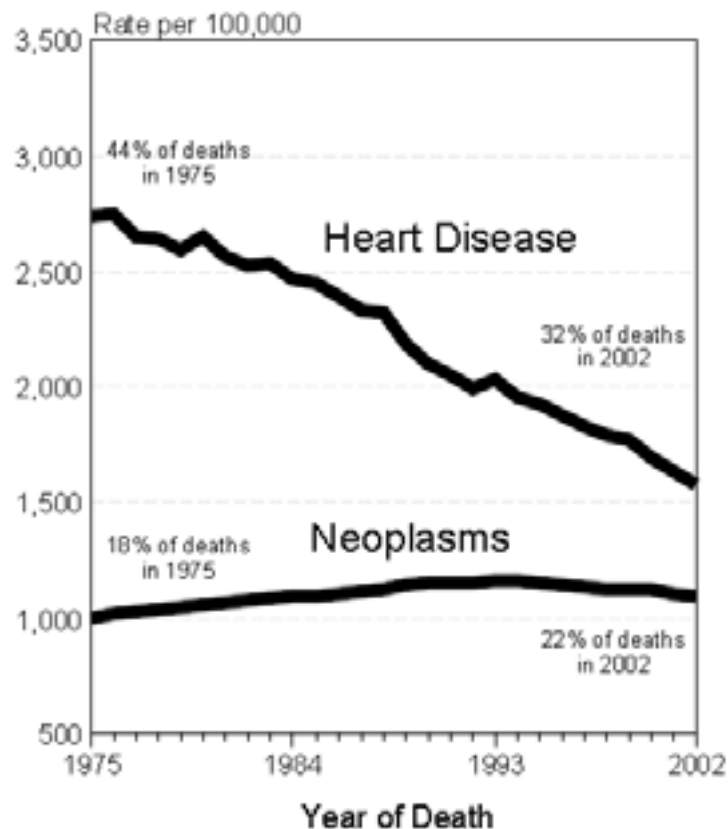
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# Human Costs of Cancer: Improvement Currently Limited to the "Young"

Ages Less Than 65



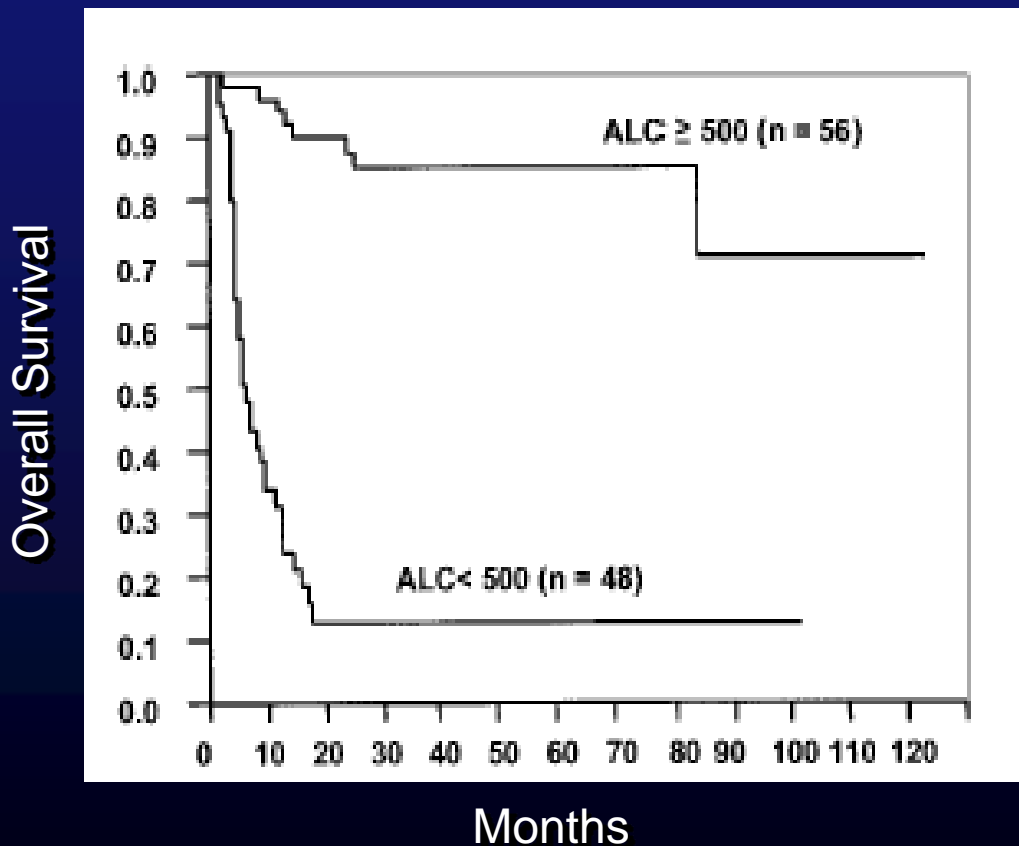
Ages 65 and Over



# Rationale for T Cell Reconstitution

- Dave SS, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med*. 351:2159-69, 2004
- Cox MC, et al. Low absolute lymphocyte count is a poor prognostic factor in diffuse large B cell lymphoma *Brit J Haem* 141; 265-268, 2008
- Joao C, et al. Early lymphocyte recovery after autologous stem cell transplantation predicts superior survival in mantle-cell lymphoma *Bone Marrow Transplantation* 37, 865-871, 2006
- Behl D, et al. Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia* 20:29-34. 2006

# Prognostic Implications of Early Lymphocyte Recovery Post PBSCT for NHL



- NHL after PBSCT (104 patients)
- ALC recovery by day 15

Porrata et al.

*Blood* 2001; 98: 579

# Multiple Myeloma

- Plasma cell neoplasm characterized by monoclonal Ab, osteolytic lesions, anemia, hypercalcemia. 15% of hematologic malignancies.
- Allo BMT: demonstrates GVM effect and cures, but high TRM.
- Autologous HSC effective for tumor reduction but cures are infrequent. Dose of infused lymphocytes in the autograft directly correlates with clinical outcome after autologous PB HSC in myeloma  
(*Leukemia* 2004)

# *Human T Cell Adoptive Immunotherapy (Effectors)*

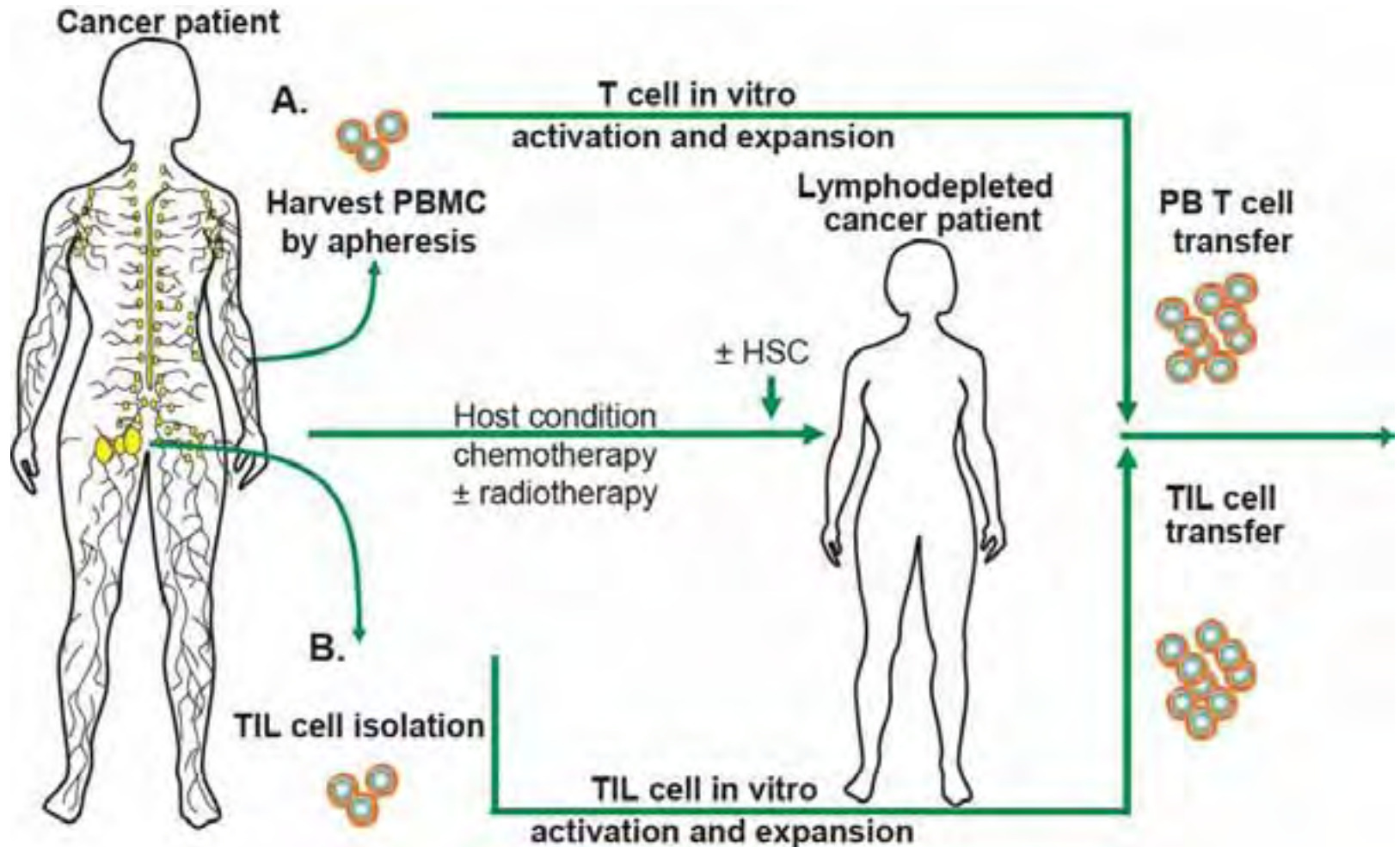
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- **1992: CMV, transfer of human CTL clones from the donor to recipients of allogeneic bone marrow (Riddell et al, *Science* 1992; 257: 238)**
- **2002: Lymphodepletion + TILs + high dose IL-2 for metastatic melanoma (Dudley et al, *Science* 2002; 298: 850)**
- **2002: CD4 effectors in HIV (Levine et al, *Nat Med.* 2002; 8:47 )**
- **2005: Combination vaccination and adoptive transfer of autologous vaccine primed T cells in myeloma (Rapoport et al, *Nature Med* 2005; 11: 1230)**

# Hematologic Malignancies: Treatment Related Immunodeficiency

- Cytotoxic therapies cause severe and persistent immunodeficiencies and lymphopenia
- Immune reconstitution after hematopoietic stem cell transplant (HSCT) characterized by:
  - Rapid (1-2 months) recovery of B cells
  - Impaired T lymphocyte recovery (CD4 > CD8)
  - Sustained antibody levels post-HSCT require recovery of competent T cells
- Review of qualitative and quantitative aspects of immune recovery after auto PBSCT:  
Gauillame et al, Blood 92, 1471, 1998

# General Approaches for Adoptive T Cell Therapy

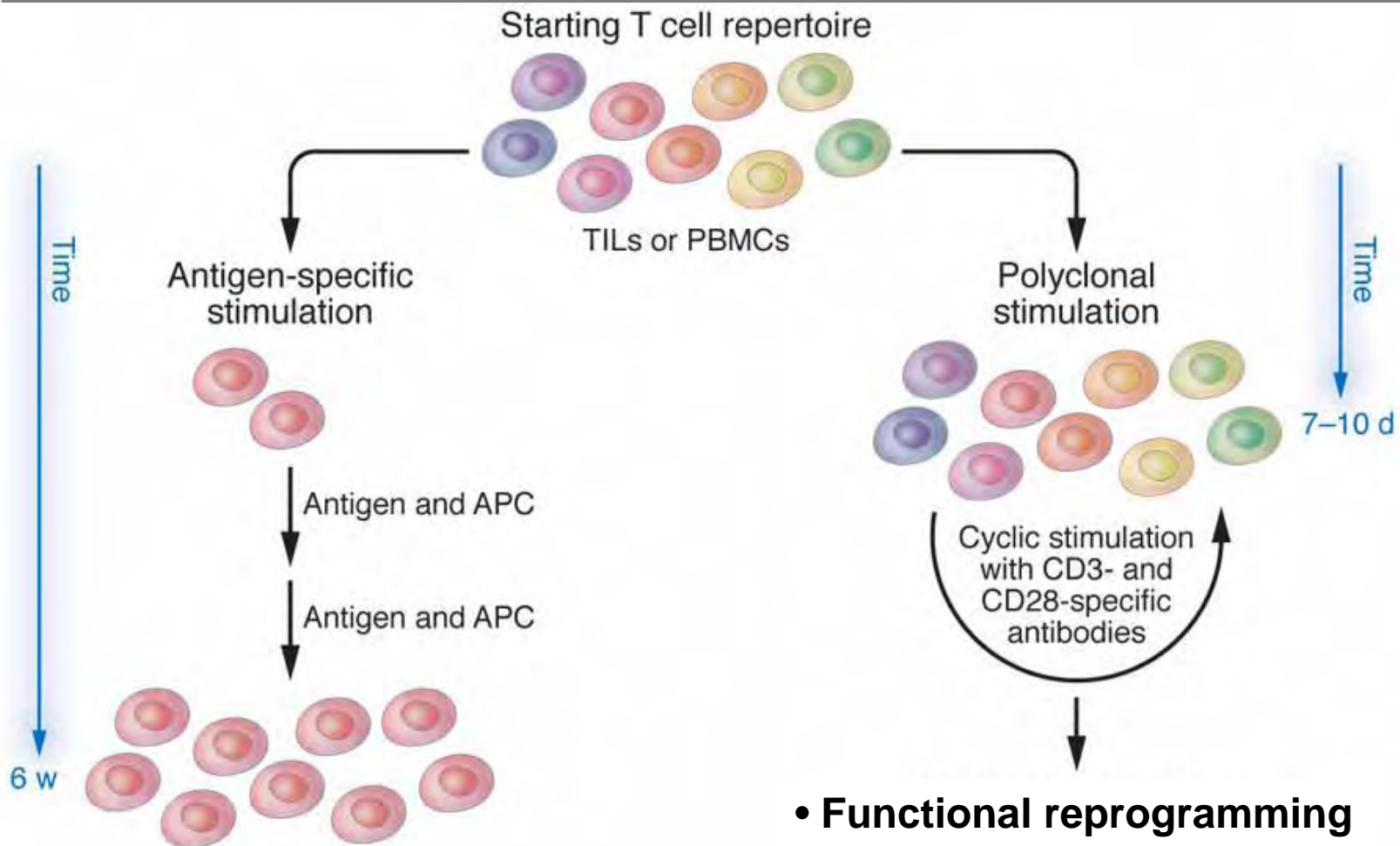


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# Cell Culture Approaches for Adoptive T Cell Therapy



- Functional reprogramming
- T cell selection in the host
- Treg cell depletion
- Genetic modification

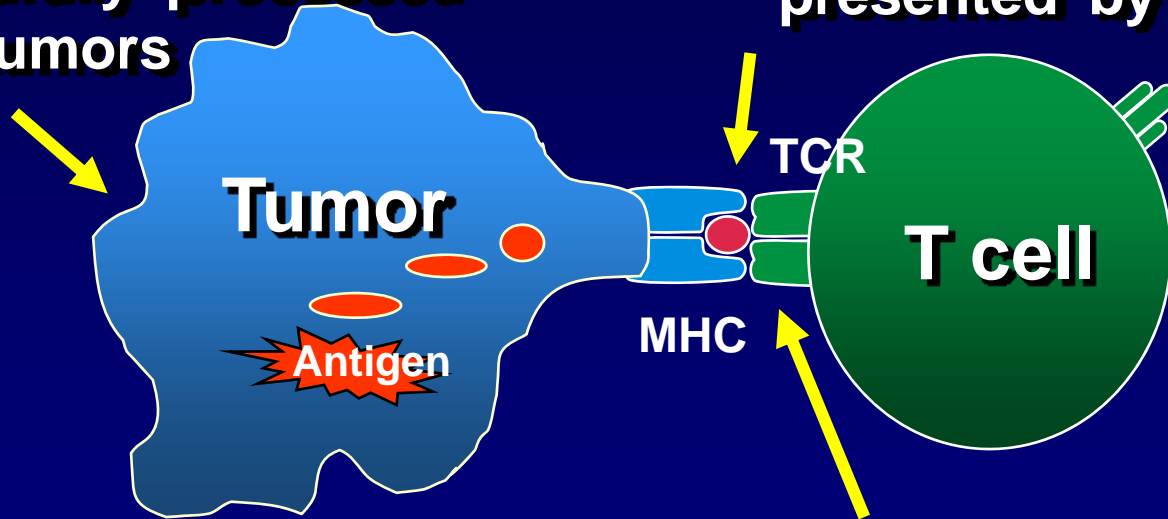
# Underlying hypothesis:

*Exploit T cells to recognize and kill tumors*

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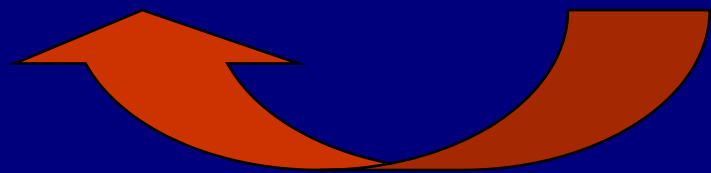
Tumor antigens are naturally 'processed' by tumors

Peptides from antigen are 'presented' by MHC

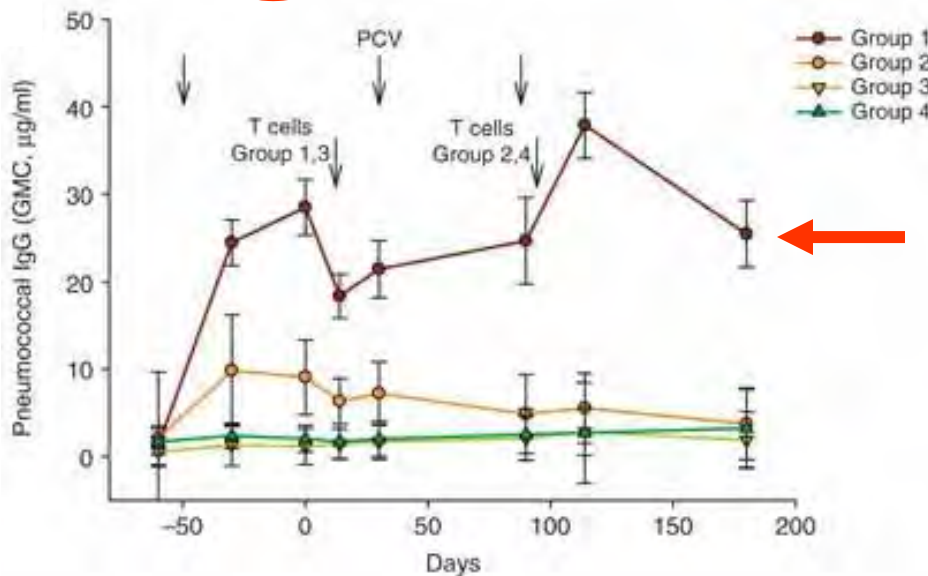
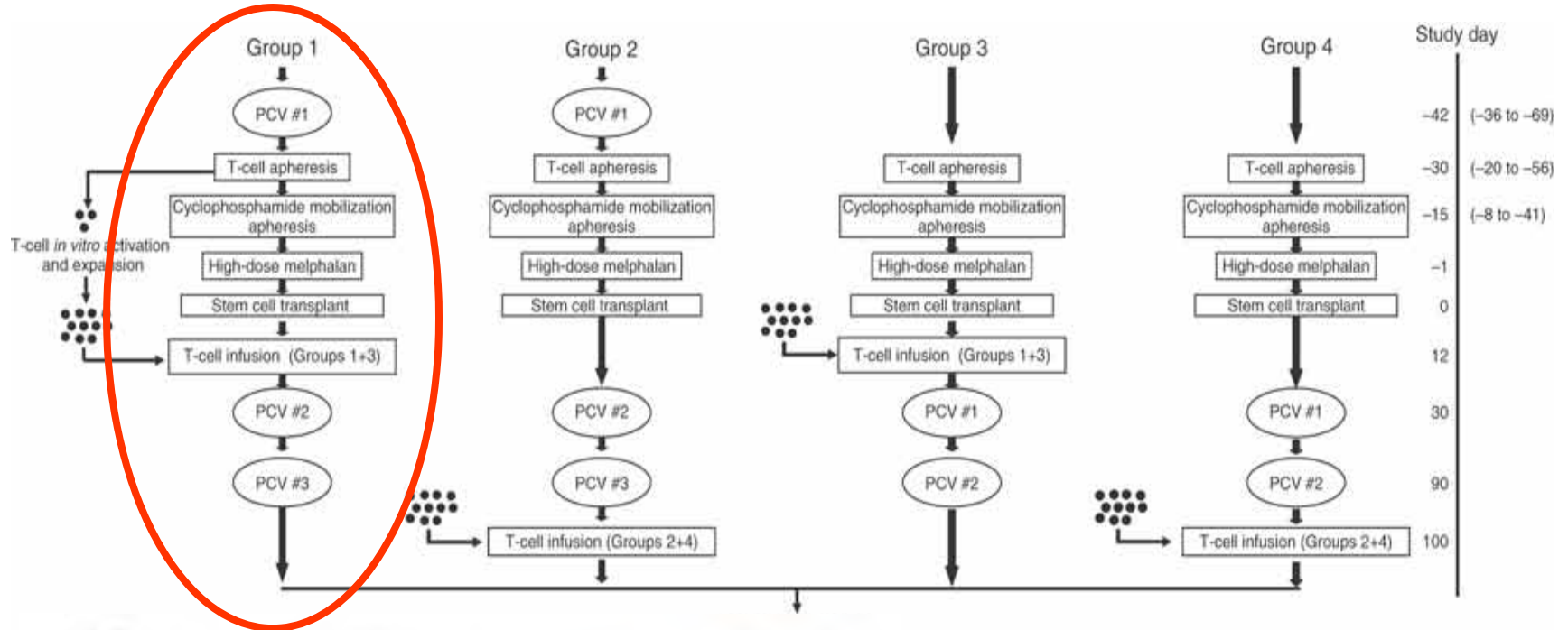


Recognition leads to lysis of tumors

T cell receptor binding to peptide/MHC complex



# First trial:



PCV= pneumococcal vaccine  
N= 54 patients

**Current trial:**  
N=41 so far (goal 55)

**Myeloma  
HLA-A2+  
(Arm A)**



**PCV + hTERT, Survivin, CMV**

IND, Vonderheide

**T Cell Collection**

**Mobilization  
Stem Cell Collection  
High-dose Melphalan  
Stem Cell Transplant**

**T Cell Infusion Day 2**

**hTERT, Survivin, CMV  
+ Plevnar**

**Immune Assessment Studies**

**T Cell In Vitro Activation and  
Expansion to Infuse 10<sup>10</sup> Cells**



IND, June

**Equal number of HLA-A2<sup>neg</sup>  
patients but no peptide  
vaccine (Arm B)**

# **Vaccine Primed T Cell Adoptive Transfers**

## **Interim Summary**

- Peripheral blood based adoptive transfer is scalable and amenable to randomized trials
- Schedule dependent effects of day 2 vs day 12 T cell infusions post melphalan
- Homeostatic expansion of infused T cells, with T cell leukocytosis
- Vaccination: T reg depletion and induction of hTERT/survivin CTLs (tetramer)
- Clinical engraftment syndrome: resembles auto-GVHD in a subset of patients

# Rationale for Adoptive T Cell Immunotherapy with Genetically Engineered T Cells

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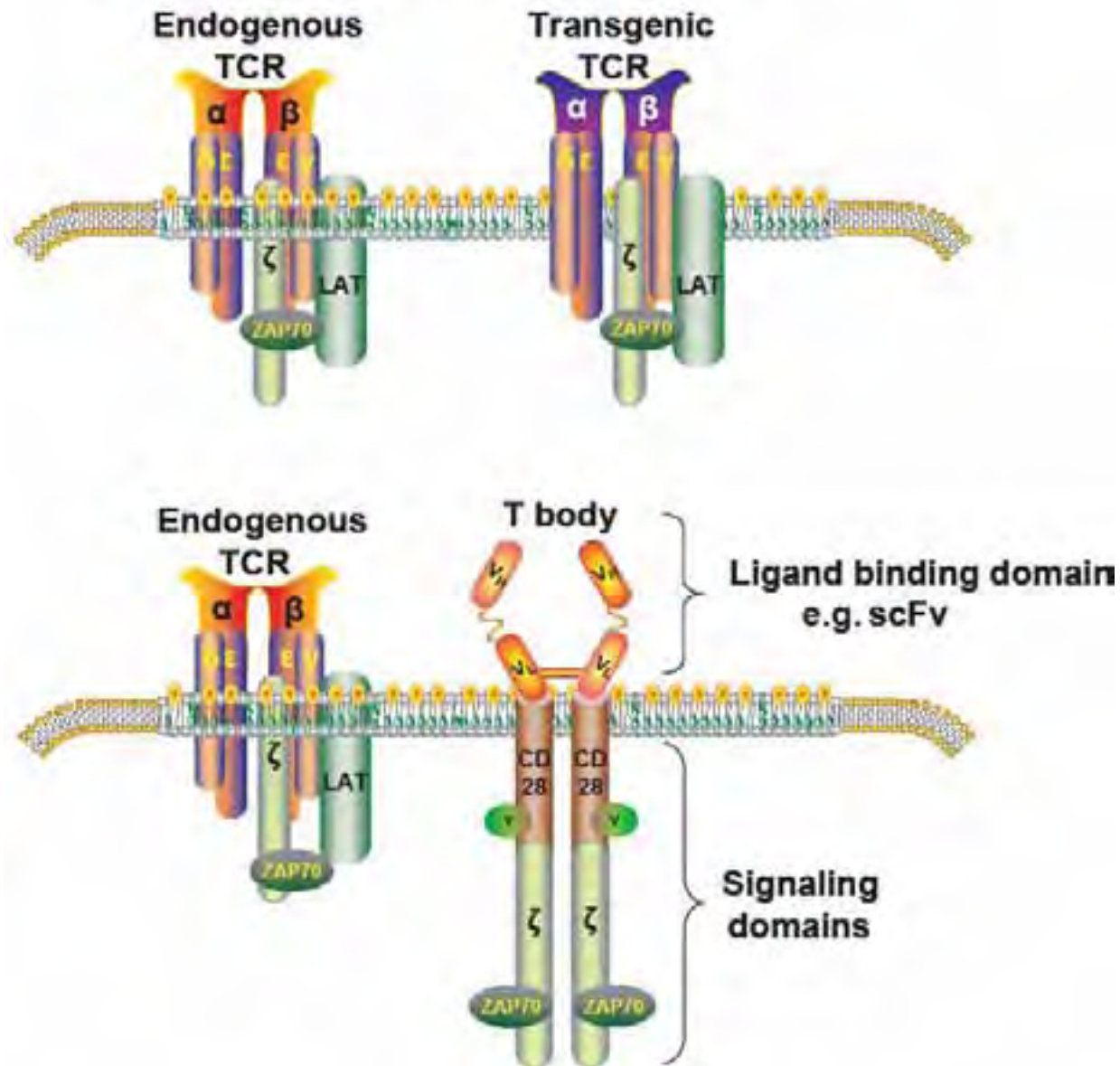
## Natural T Cells

Safety profile established  
T cells have the potential to  
target cancer stem cells  
Anecdotal responses observed  
to immunotherapy  
Repertoire may be inadequate  
or lacking  
Immunosenescence a major  
issue in humans

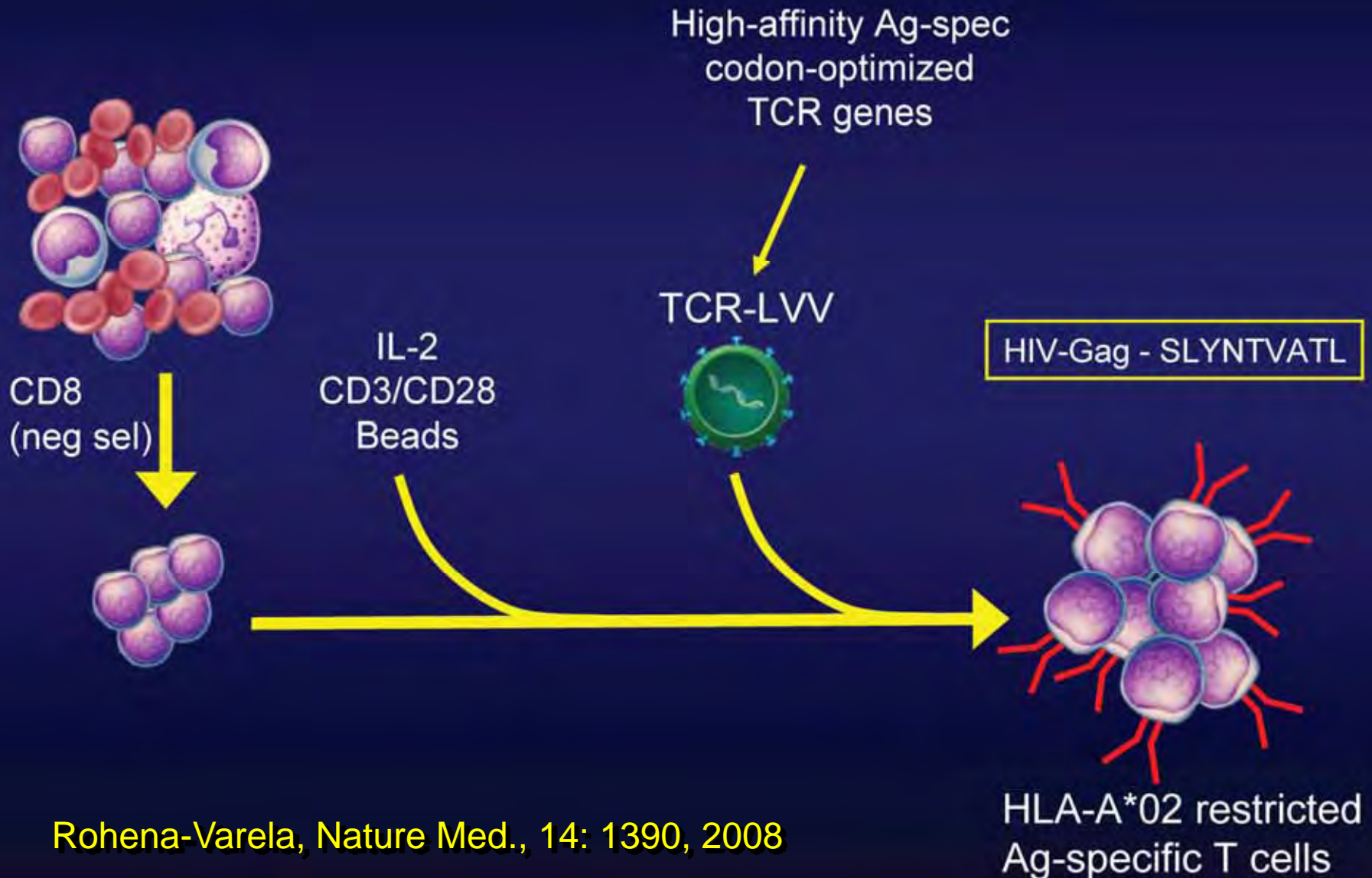
## Gene-Modified T cells

Safety profile scant  
Repertoire limitations can be  
overcome  
Anecdotal responses observed  
in immunotherapy  
Efficient *gene transfer* required  
Efficient *T cell culture* required

# Redirected T Cell Approaches with engineered T cells



# Converting Polyclonal CD8 T Cells to High Avidity CTLs



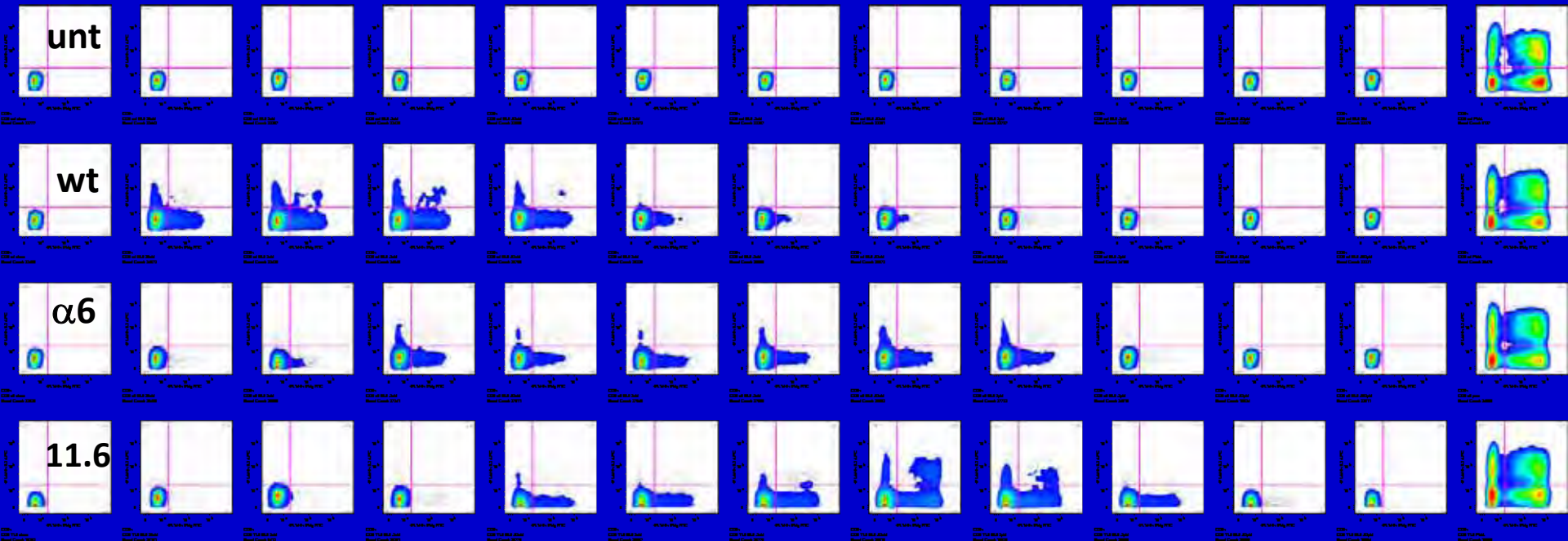
# Creating "Bionic T Cells" with High Affinity TCRs

30  $\mu\text{M}$  3  $\mu\text{M}$  0.3  $\mu\text{M}$  30 nM 3nM 0.3 nM 30 pM 3pM 0.3 pM 30 fM 3fM

SL9 Concentration

I540  
1  $\mu\text{M}$

PMA  
Iono



IL-2

IFN- $\gamma$

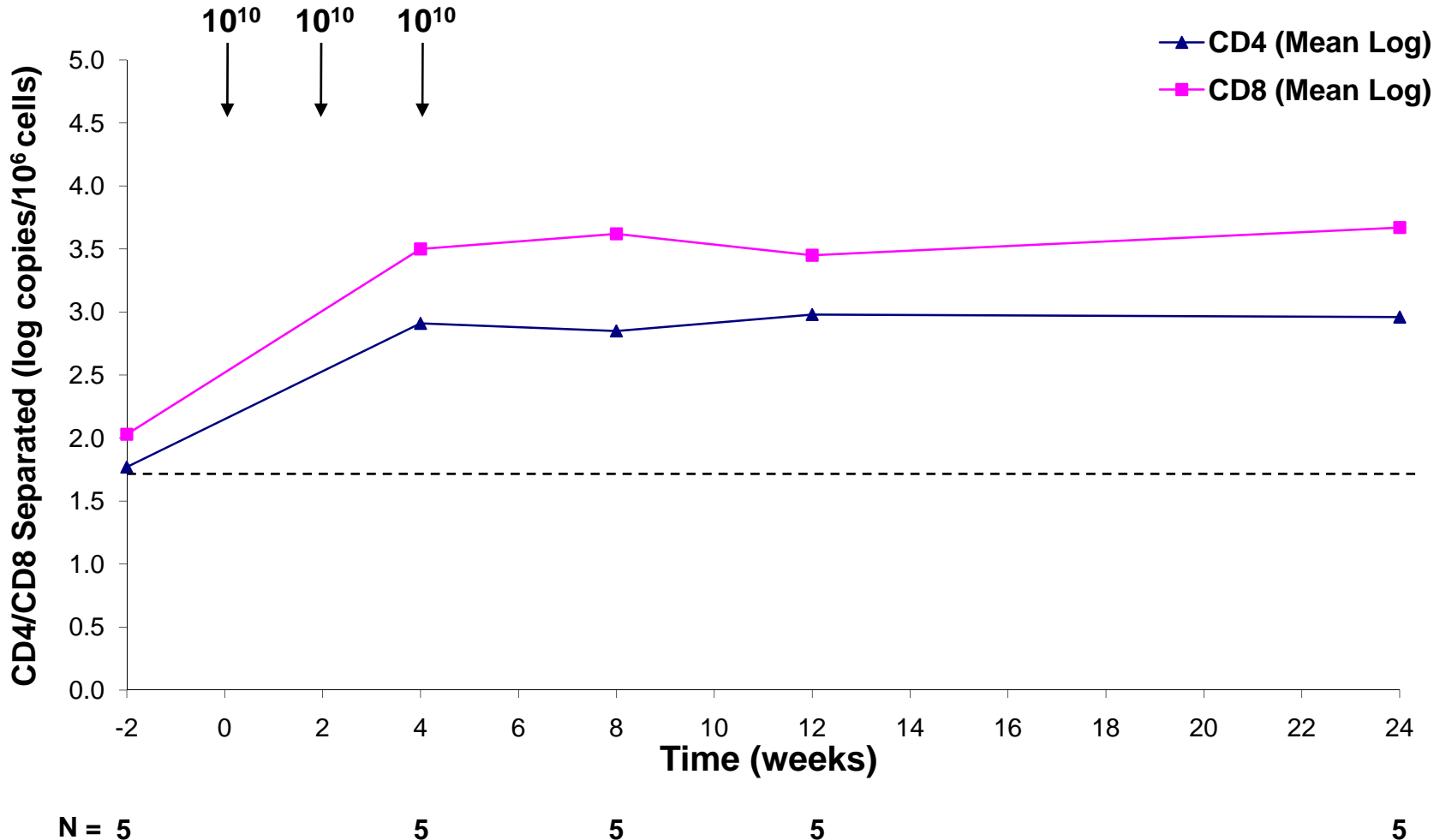
# Issues and Considerations with Engineered TCRs

- **Lentiviral vectors enable efficient "conversion" of polyclonal populations of T cells to clonal T cells with MHC restricted monoclonal repertoire**
- **Conversion is efficient: a single step can produce more effector T cells than months of vaccines**
- **High affinity TCRs enable T cells to be extraordinarily efficient biosensors**
- **Why hasn't nature evolved high affinity TCRs?**
  - **Is nature content to be suboptimal?**
  - **Is this nature's compromise to avoid autoimmunity?**

# Long Term Persistence of Adoptively Transferred Engineered Human T Cells

- In the setting of homeostatic expansion: Muul, et al. 2003. Persistence and expression of the adenosine deaminase gene for 12 years. *Blood* 101:2563-2569, 2003
- Steady state lymphopoiesis in HIV infection: Deeks, S., et al. A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination anti-retroviral therapy. *Mol Ther* 5:788-797, 2002.

# >6 Month Persistence of Adoptively Transferred CD4 $\zeta$ T Cells: Subsets



# scFv Chimeric Receptors: Background

- 1989 Redirected T cell concept pioneered in vitro by Eshhar and colleagues: (Gross et al, PNAS 86: 10024, 1989)
- Despite strong pre-clinical rationale, technical difficulties have prevented clinical translation:
  - Efficient T cell culture systems
  - Efficient gene transfer systems
- 2006 First clinical experience in cancer: Kershaw et al. *Clin Cancer Res.* 12: 6106–6115.

# A Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer

Michael H. Kershaw<sup>1,3,4</sup>, Jennifer A. Westwood<sup>1,3</sup>, Linda L. Parker<sup>1</sup>, Gang Wang<sup>1,5</sup>, Zelig Eshhar<sup>6</sup>, Sharon A. Mavroukakis<sup>1</sup>, Donald E. White<sup>1</sup>, John R. Wunderlich<sup>1</sup>, Silvana Canevari<sup>7</sup>, Linda Rogers-Freezer<sup>1</sup>, Clara C. Chen<sup>2</sup>, James C. Yang<sup>1</sup>, Steven A. Rosenberg<sup>1</sup>, and Patrick Hwu<sup>1,5</sup>

## Abstract

**Purpose**— A phase I study was conducted to assess the safety of adoptive immunotherapy using gene-modified autologous T cells for the treatment of metastatic ovarian cancer.

**Results**— Five patients in cohort 1 experienced some grade 3 to 4 treatment-related toxicity that was probably due to interleukin-2 administration, which could be managed using standard measures. Patients in cohort 2 experienced relatively mild side effects with grade 1 to 2 symptoms. No reduction in tumor burden was seen in any patient. Tracking <sup>111</sup>In-labeled adoptively transferred T cells in cohort 1 revealed a lack of specific localization of T cells to tumor except in one patient where some signal was detected in a peritoneal deposit. PCR analysis showed that gene-modified T cells were present in the circulation in large numbers for the first 2 days after transfer, but these quickly declined to be barely detectable 1 month later in most patients. An inhibitory factor developed in the serum of three of six patients tested over the period of treatment, which significantly reduced the ability of gene-modified T cells to respond against FR<sup>+</sup> tumor cells.

➤ **The central issue with chimeric receptors in cancer is limited in vivo persistence**

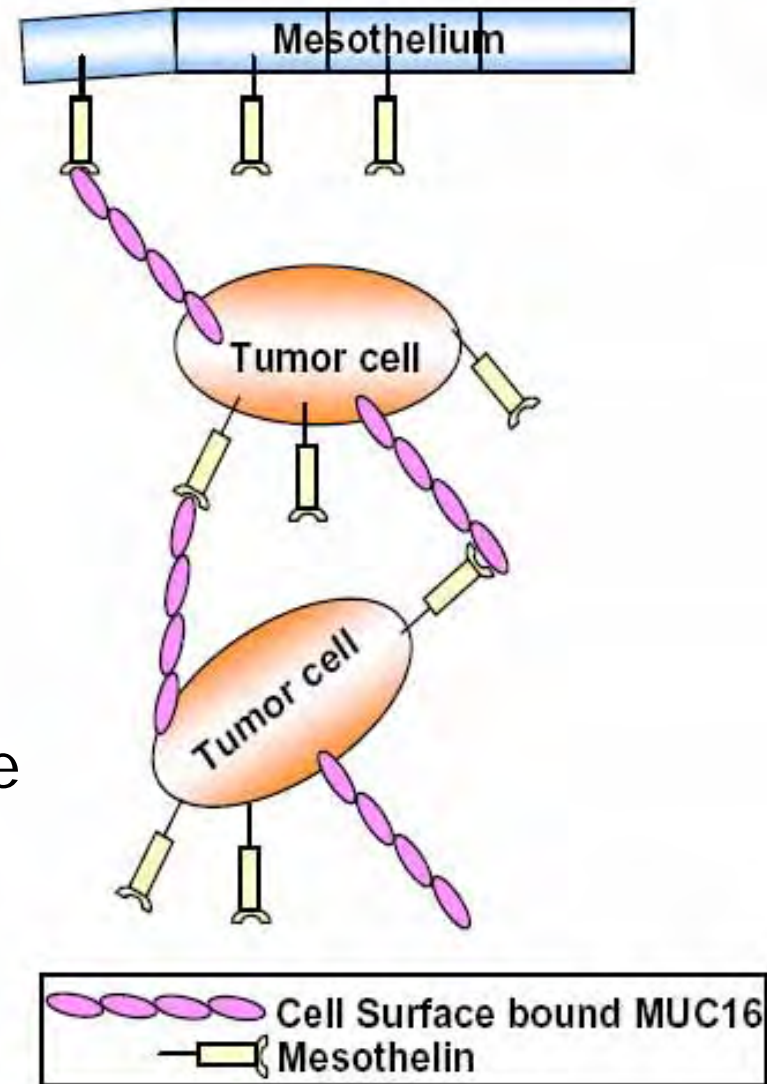
# Mesothelin as a Target for Adoptive Transfer Therapy

- 40kDa gpi linked surface glycoprotein that is widely expressed in ovarian, mesothelioma and pancreatic cancers
- Restricted expression in normal cells

## Mesothelin cell biology

*meso*<sup>-/-</sup> mice are healthy and fertile

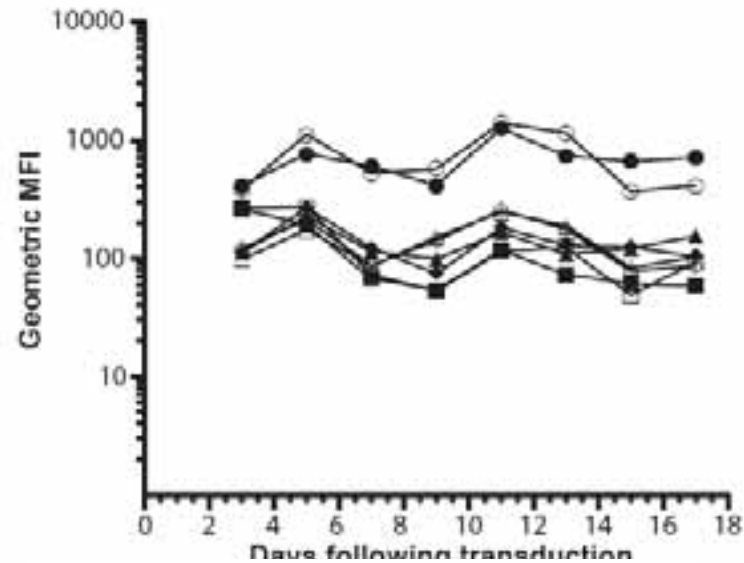
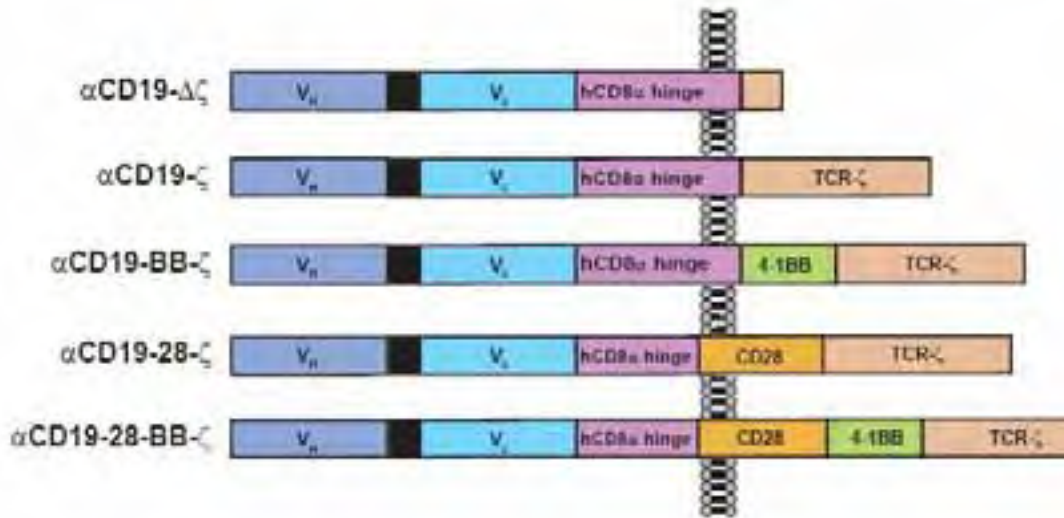
- possible role in adhesion and metastasis
- binds CA125 (*muc16*): large protein expressed by OvCa and normal mesothelial cells



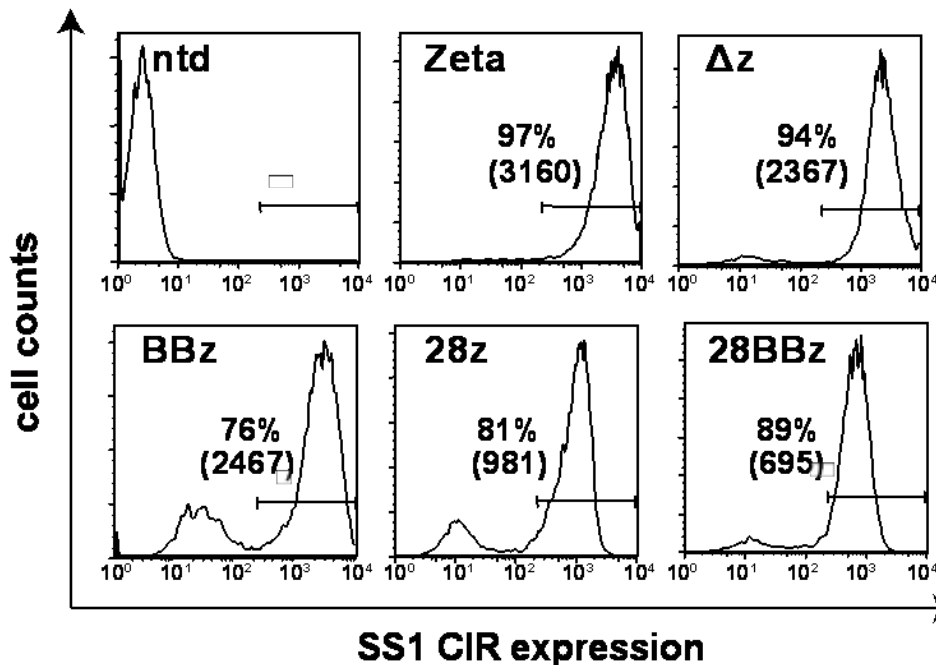
Gubbels *et al*

Mol Cancer 5(1):50

# Lentiviral Redirected T Cells Targeting Mesothelin or CD19

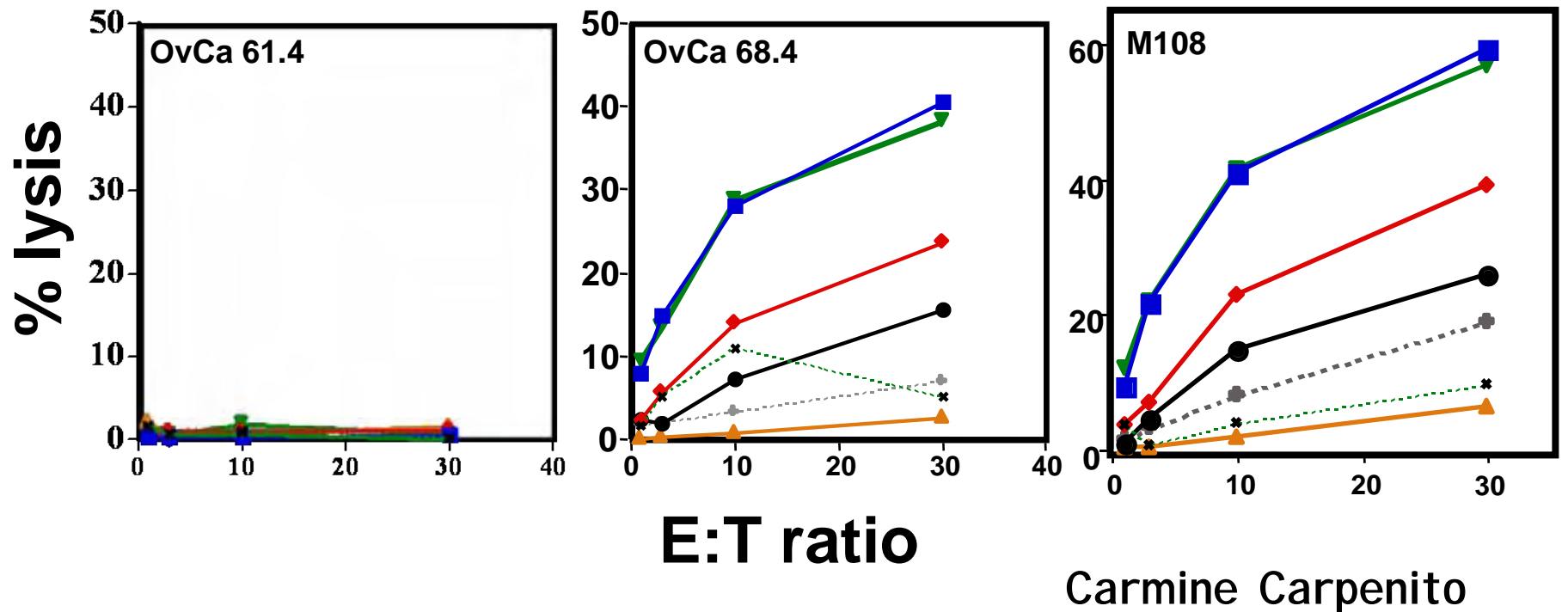
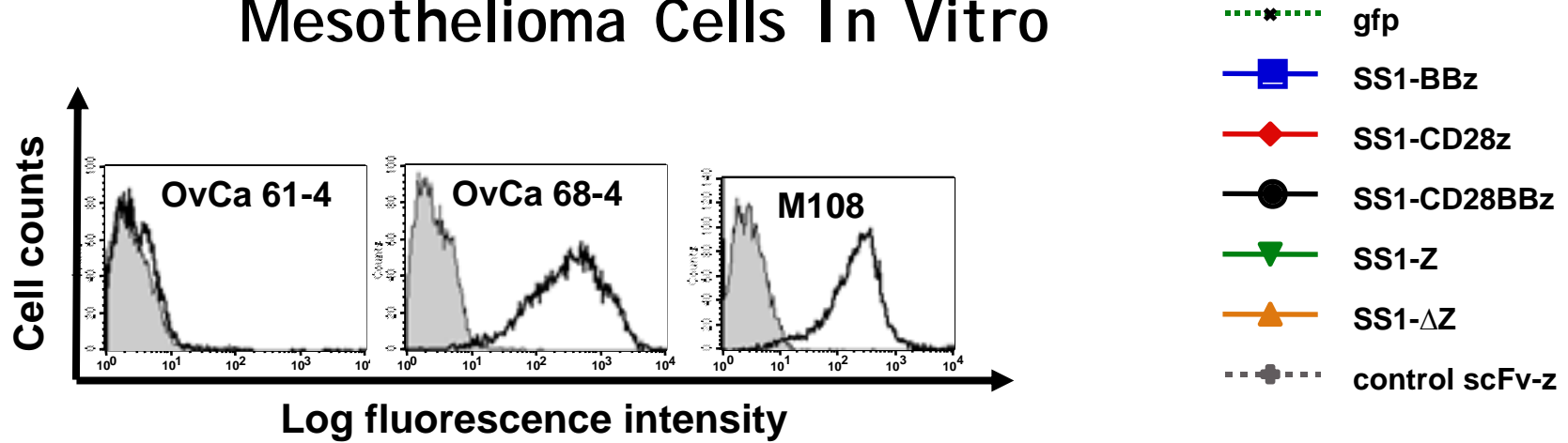


- CMV (CD4)
- EF-1a (CD4)
- ▲ UbiC (CD4)
- ◆ PGK (CD4)
- CMV (CD8)
- EF-1a (CD8)
- △ UbiC (CD8)
- ◇ PGK (CD8)

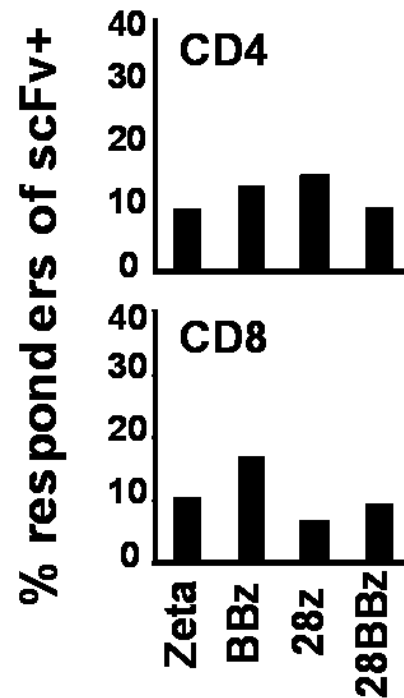


Carmine Carpenito  
Michael Milone

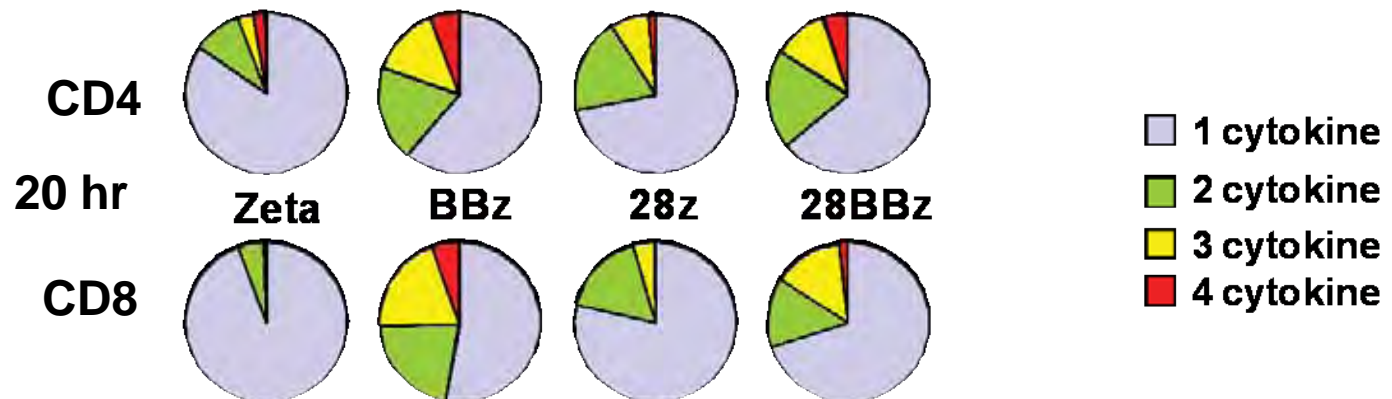
# Mesothelin Redirected T Cells Kill Primary Ovarian and Mesothelioma Cells In Vitro



# Redirected T Cells with Costimulatory Domains are Multitasking "Polyfunctional" T Cells

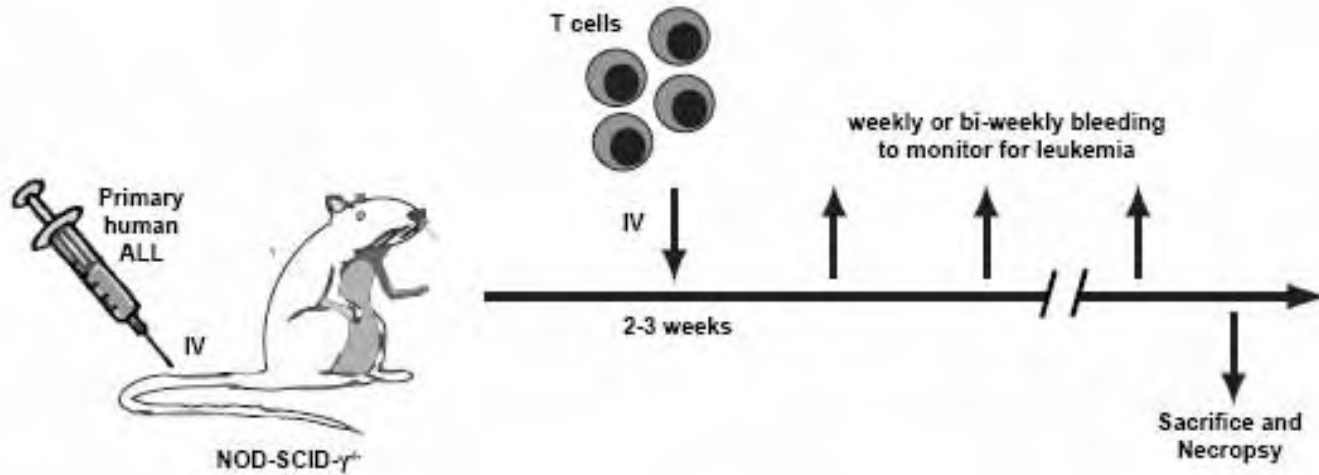


- Betts et al. 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107:4781

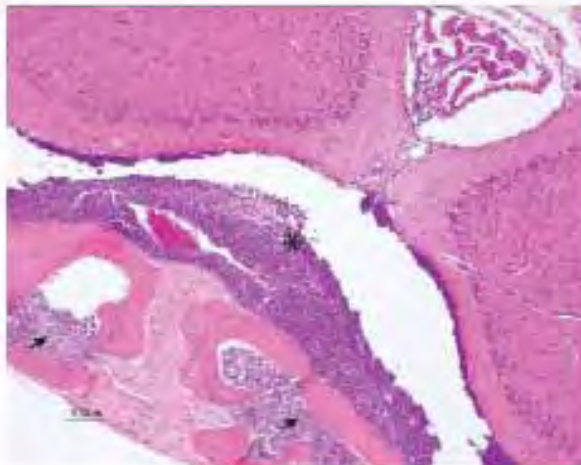


# Development of Rigorous Primary Pre-B ALL Model in Xenografted Mice: day 14 -21 challenge

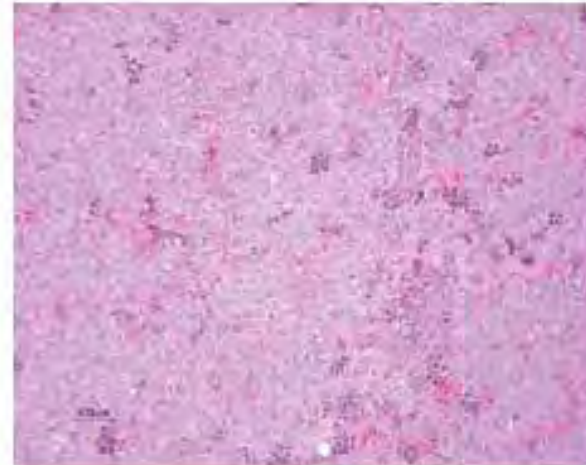
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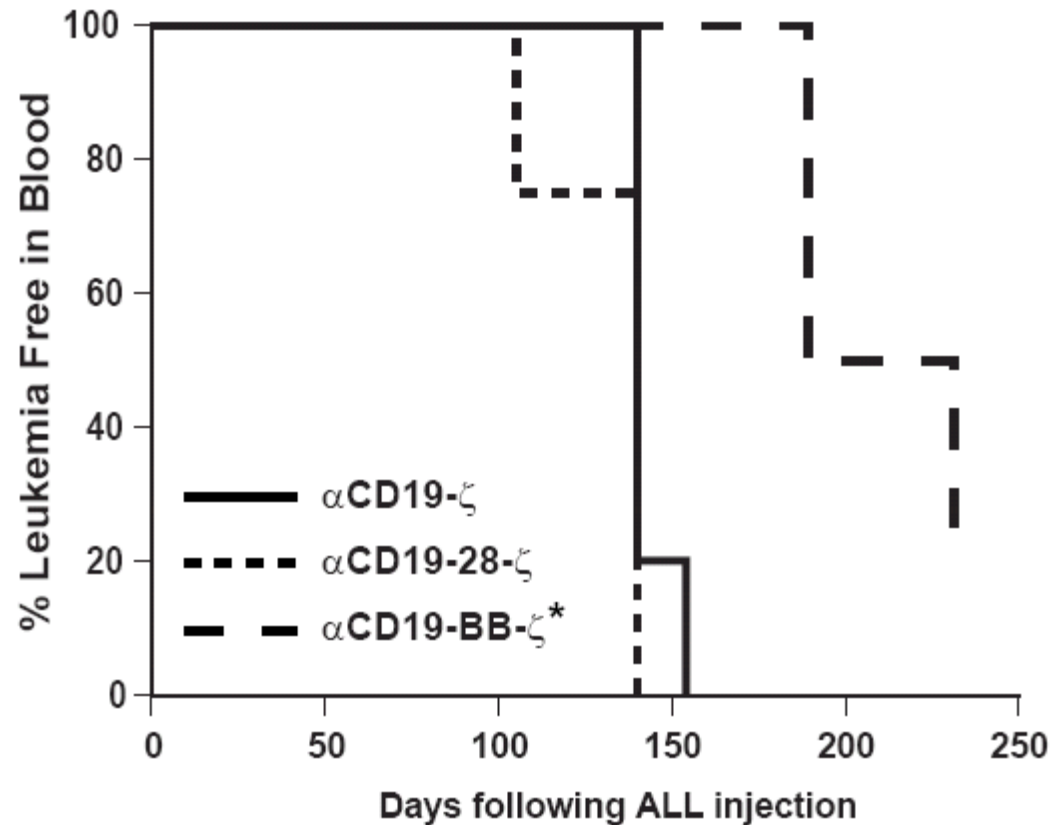
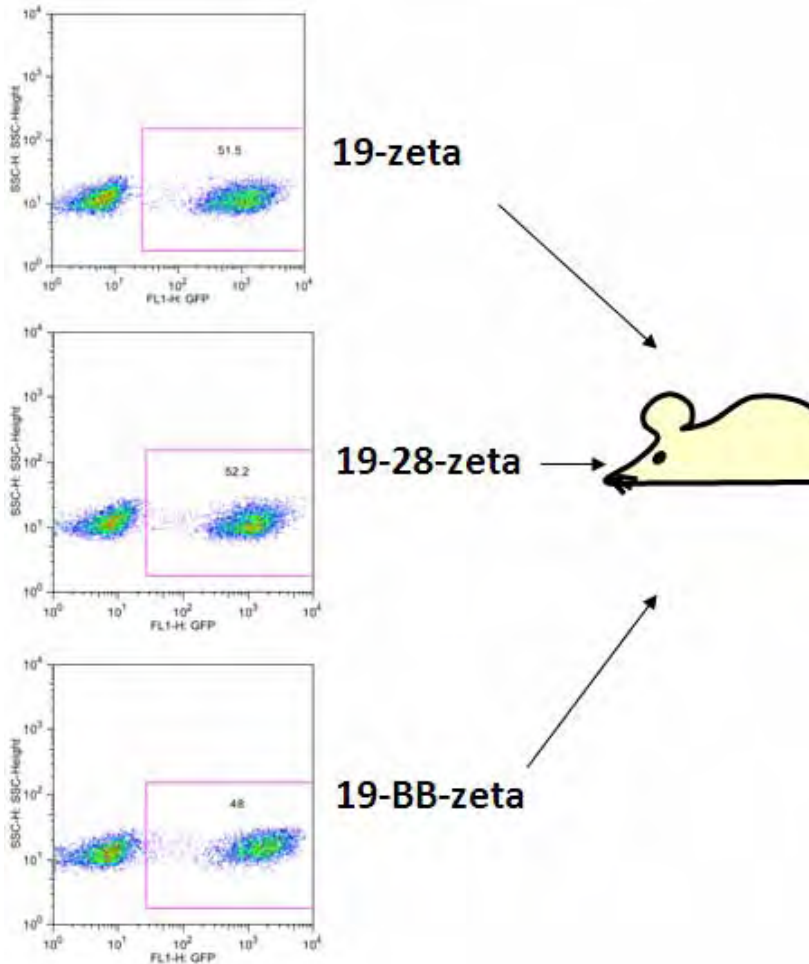
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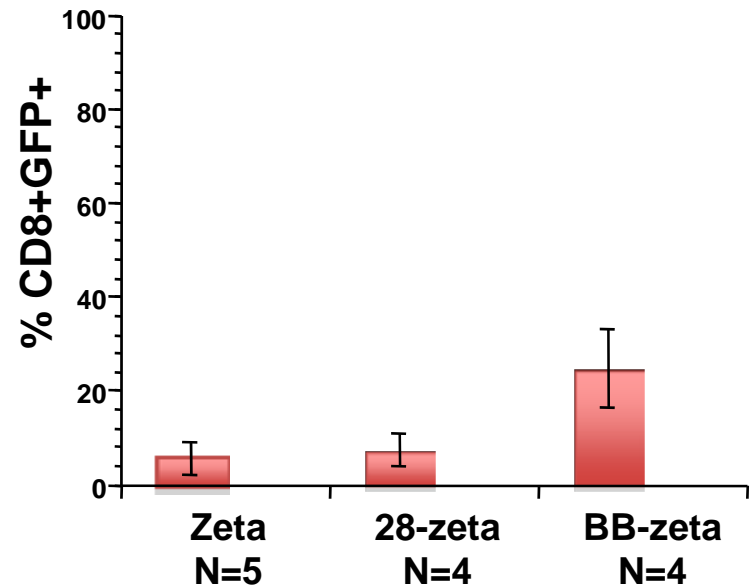
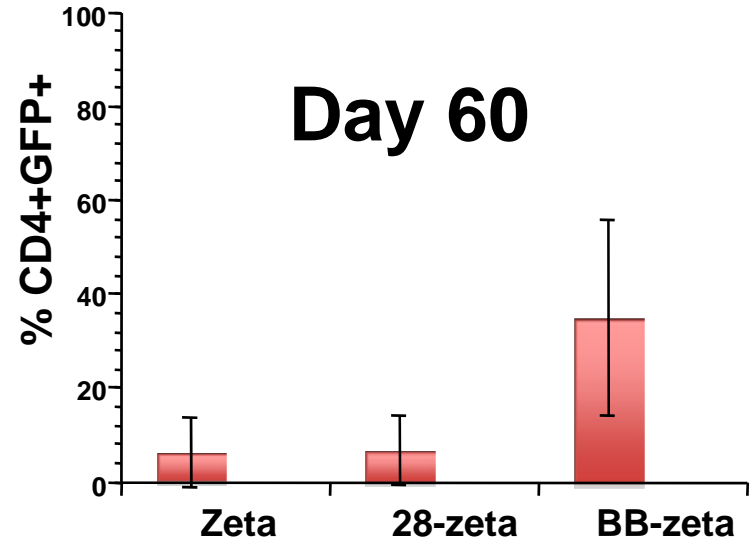
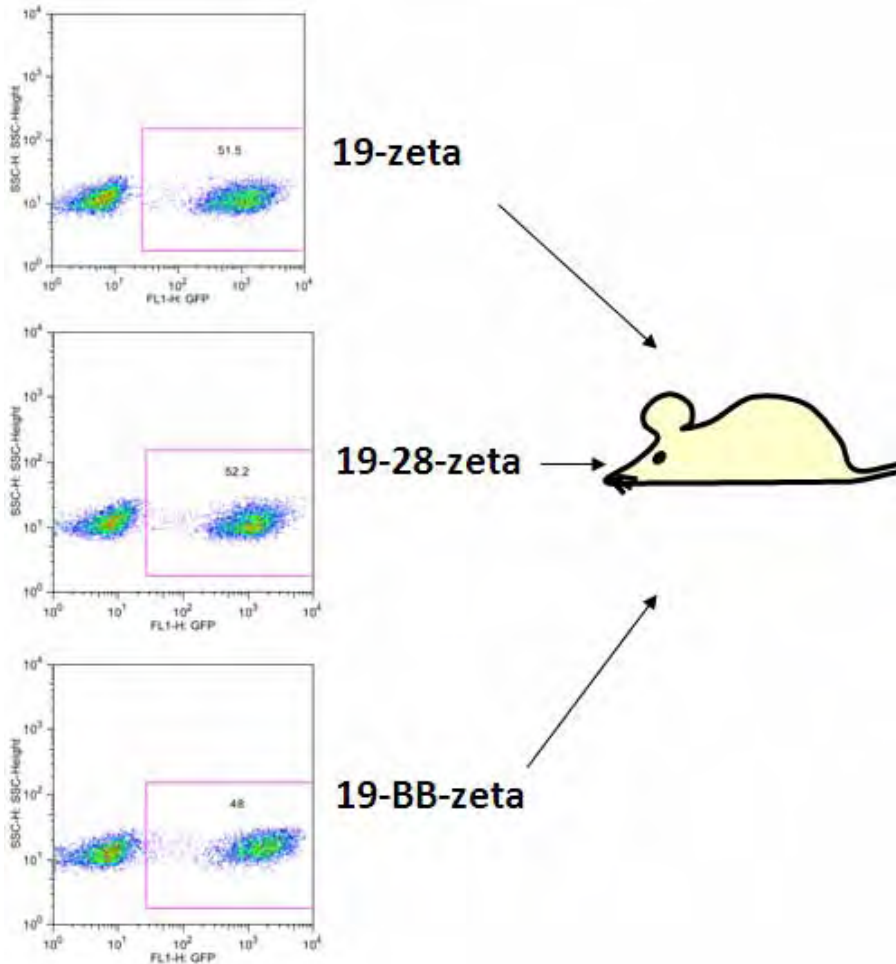
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# Enhanced In vivo persistence of T-body CTLs in leukemia xenografted mice: CD28 vs 4-1BB



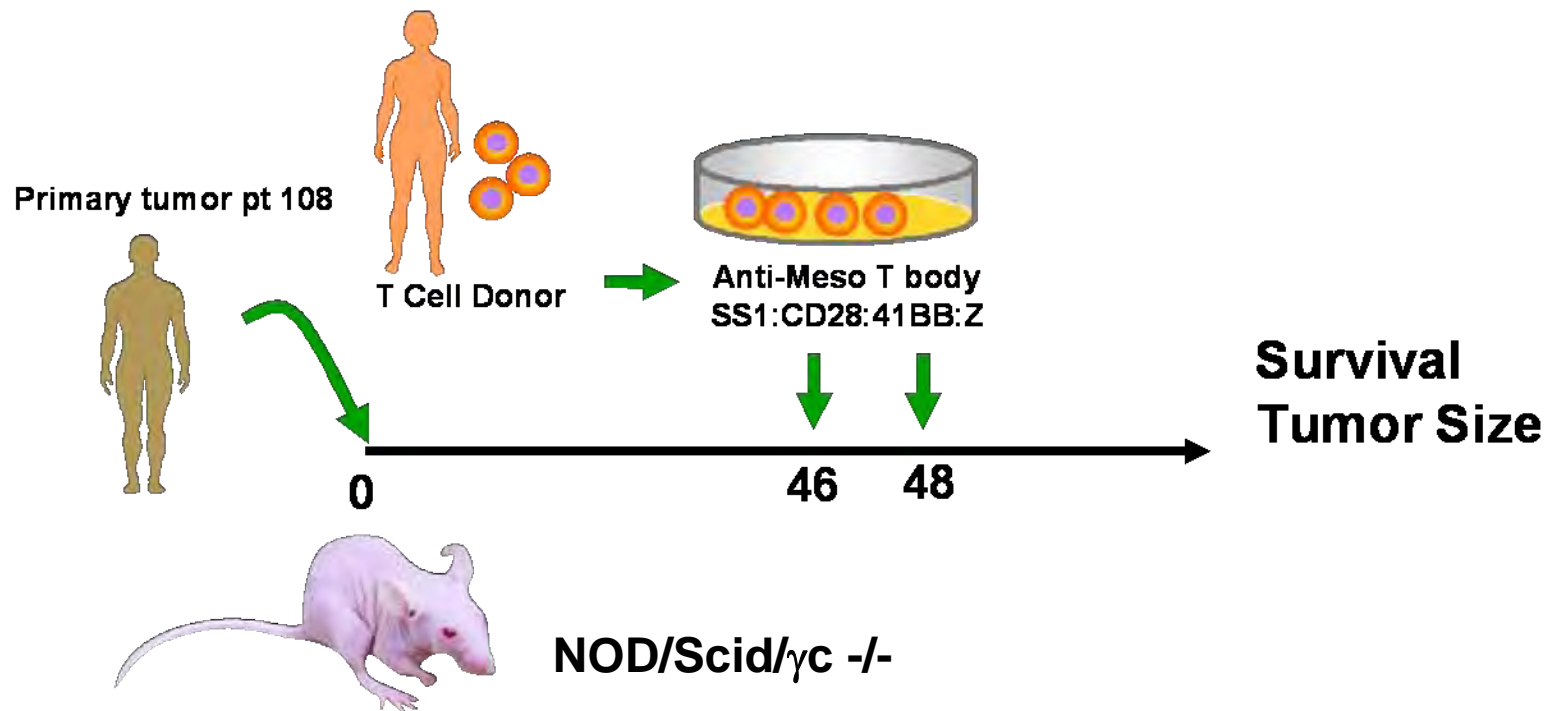
# Enhanced In vivo persistence of T-body CTLs in leukemia xenografted mice: CD28 vs 4-1BB



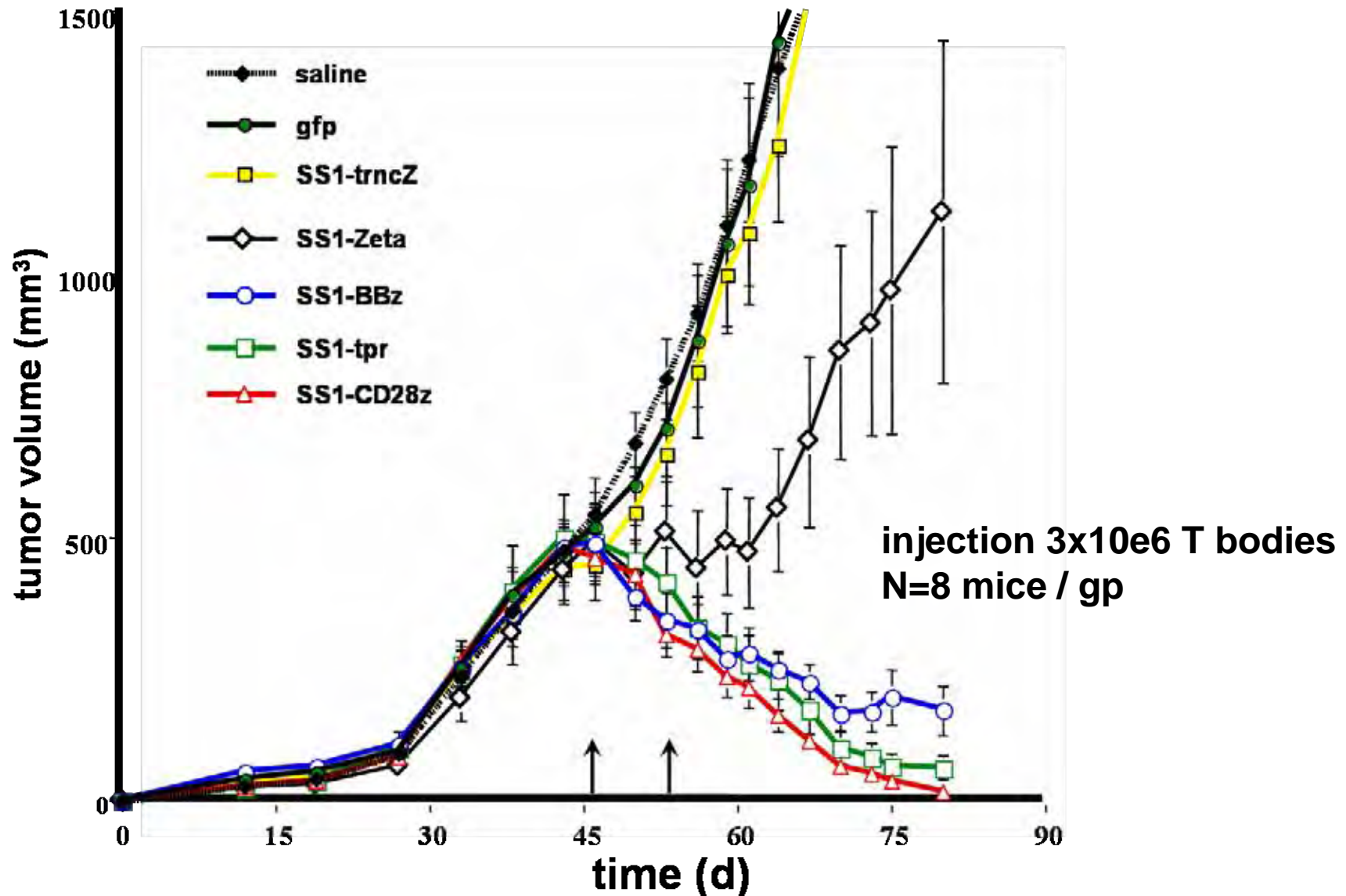
# CD137 (4-1BB) Signaling Can Confer Ag Independent Survival Advantages

- Pulle G, et al. IL-15-dependent induction of 4-1BB promotes antigen-independent CD8 memory T cell survival. *J Immunol* 2006;176:2739-2748)
- Zhu Y et al. CD137 stimulation delivers an antigen-independent growth signal for T lymphocytes with memory phenotype. *Blood* 2007;109:4882-4889.

# Development of Rigorous Pre-clinical Model Mesothelioma Xenografts: Day 45 Challenge



# Eradication of Large Established Primary Mesothelioma Xenografts: Day 45 Challenge



# Combinatorial Signaling Domains

	Zeta only	28:z	41BB:z	28:41BB:z
kill	++	++	++	++
cytokine	+	+++	++	+++
Prolifera- tion	+	+++	+++	+++
In vivo survival	+	+	+++	+++

# Take Home Messages

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- Schedule dependent effects of adoptively transferred T cells
- Adoptive transfer of retroviral and lentiviral transduced Tcm feasible
- Mesothelin redirected T cells eradicate large established tumors in xenografted mice at in vivo ~E:T 1:100
- In vivo T cell persistence and not cytokine secretion correlates with antitumor efficacy: biomarker of ability to survive tumor microenvironment?

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# Collaborators and Acknowledgements

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