

# EVOLUTION OF TRIAL DESIGN USING GENE-MODIFIED T CELLS

*Richard P Junghans, PhD, MD*

Director, Biotherapeutics Development Lab  
Associate Professor of Surgery and Medicine  
Boston University School of Medicine  
Chief, Division of Surgical Research  
Roger Williams Medical Center  
Providence, RI, USA

*No commercial relationships to disclose.*

# History of Biotherapies

HUMORAL

CELLULAR

Lymphoma  
Leukemia  
Melanoma  
Colorectal

Renal Cell  
Melanoma

BIFUNCTIONAL Abs

TUMOR VACCINES

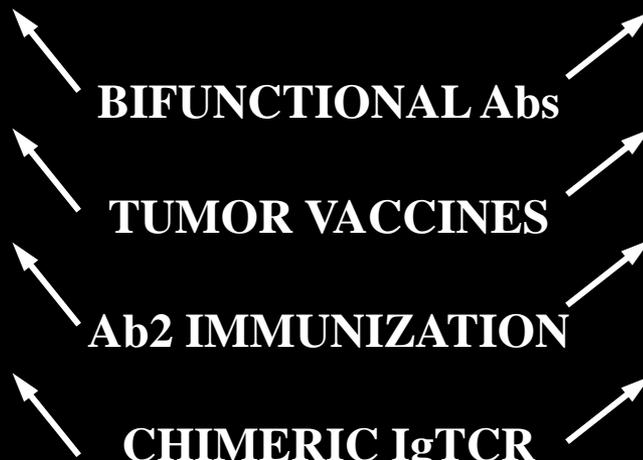
Ab2 IMMUNIZATION

CHIMERIC IgTCR

LAK  
(IL2)  
TIL

SPECIFICITY  
AFFINITY  
ADAPTABILITY

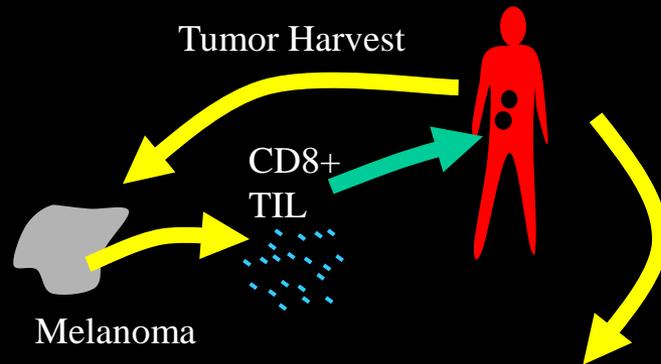
CYTOTOXICITY  
SELF RENEW  
ACCESS



# Cellular therapies

- o LAK therapies
- o IL2 therapies
- o TIL therapies

# TIL -- Melanoma



*But:*

Responses not durable

Only melanoma, limited numbers

Technically challenging, antigen(s)  
unknown

Not reproducible in other studies

# TIL limitations

- o Required acquisition of tumor
- o Technologically challenging
- o Tolerance was dominant in many cases, i.e., not successful in expansion
- o Didn't work very well (5% cured)
- o Confined to melanoma

# T cells attack virus-infected cells

## CYTOTOXIC T-LYMPHOCYTE

A specialized white blood cell responsible for eliminating unwanted body cells (e.g. cancer) kills a cell infected with the influenza virus

# T cells

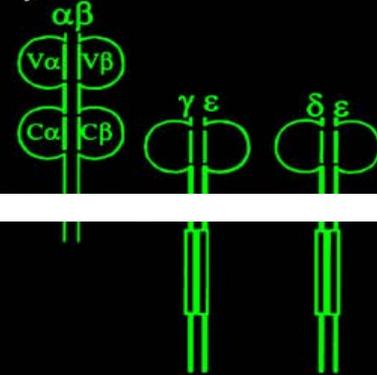
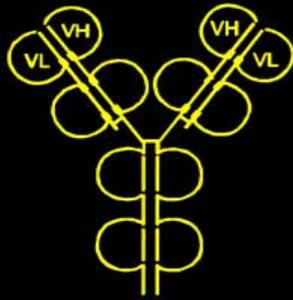
- o Evolved to kill our own cells infected by virus
- o Recognition mediated through variable T cell receptor (TCR)
- o Tolerant to self, including cancer
- o How to overcome this tolerance?
  - I.e., how to “fool” the T cells into “thinking” the tumor has a virus infection????

*“Designer T cells”* →

**Antibody**  
(immunoglobulin)

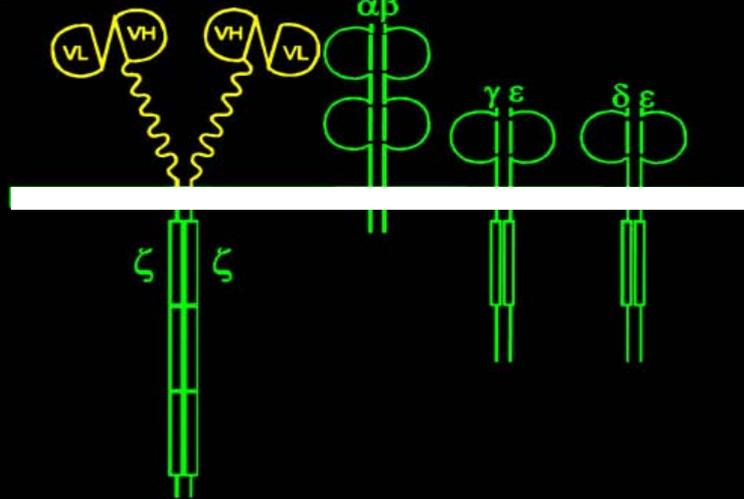
**T cell receptor (TCR)**

Anti-CEA



Normal  
T cells

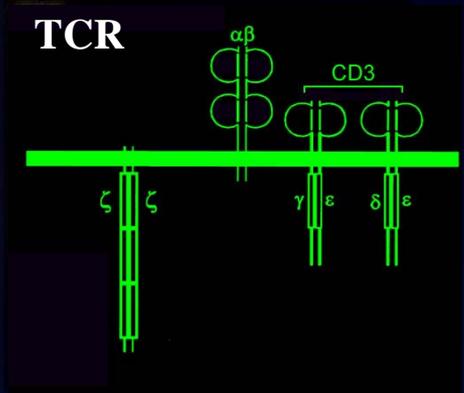
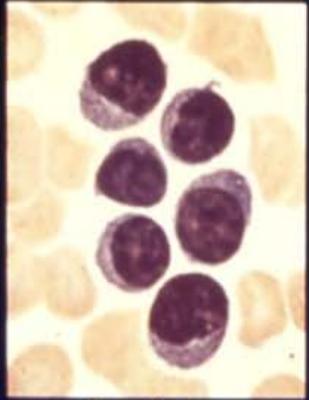
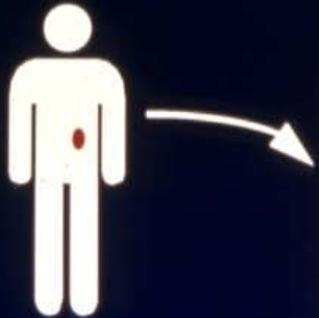
**IgTCR**



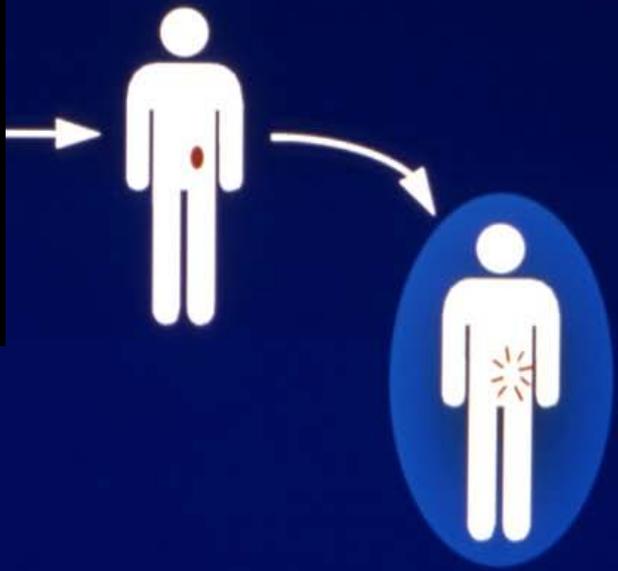
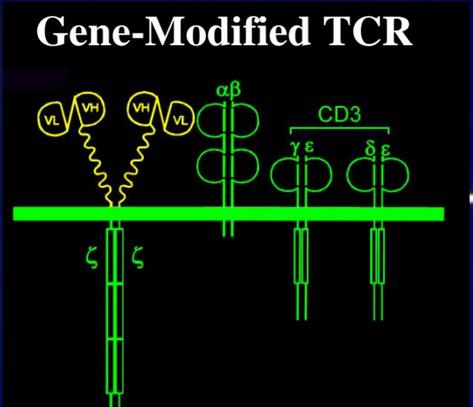
Designer  
T cells

IgTCR = Immunoglobulin-T cell receptor

CIR = Chimeric immune receptor



# Anti-Cancer T Cell Gene Therapy



# Carcinoembryonic antigen (CEA)

- o Expression
  - High on tumor, low on normal
  - Topological sequestration
- o High clinical relevance:
  - On colorectal, breast, pancreas, lung, others
  - More than 100,000 deaths/yr for CEA+ tumors



# Clinical Retroviral Vector

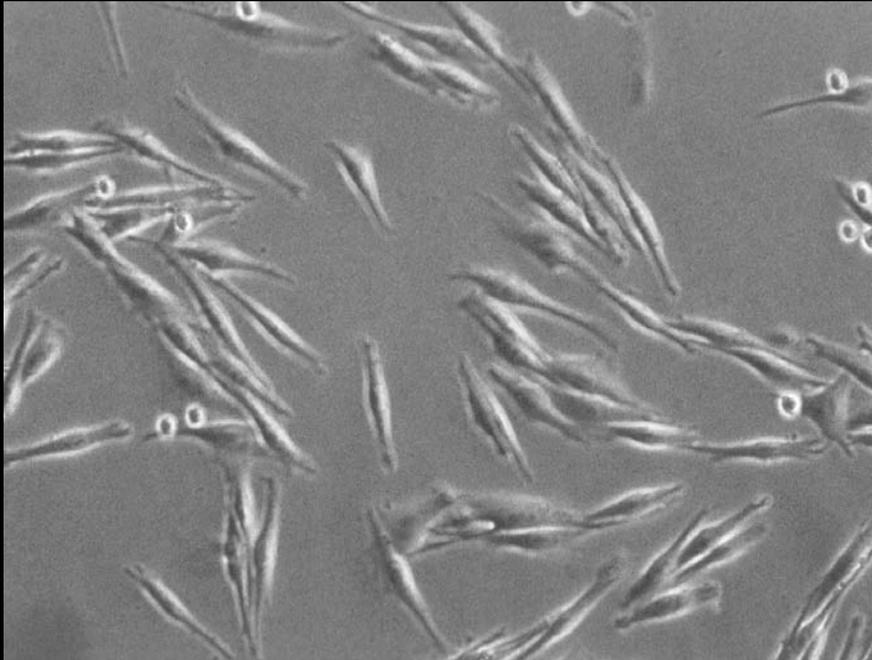


- o Vector = “carries something in”
- o Retroviral vector = virus used to infect T cells
- o Inserts new gene “transgene” into host chromosome
- o Virus dies after it infects once
- o Stable gene expression; T cells permanently modified

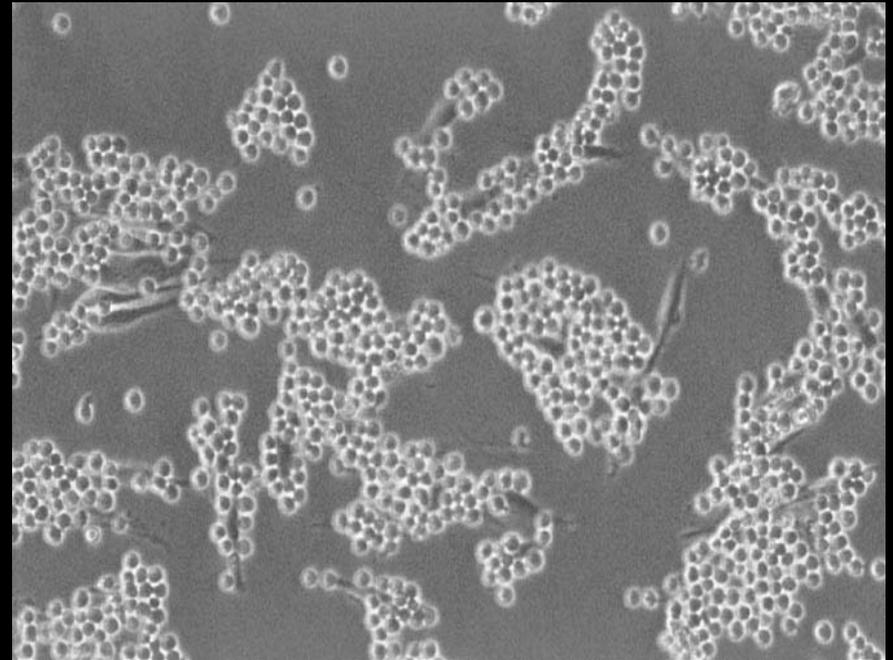
## Binding

# DESIGNER T CELLS BIND TO ANTIGEN+ TUMOR CELLS

*Tumor + Normal T cells*

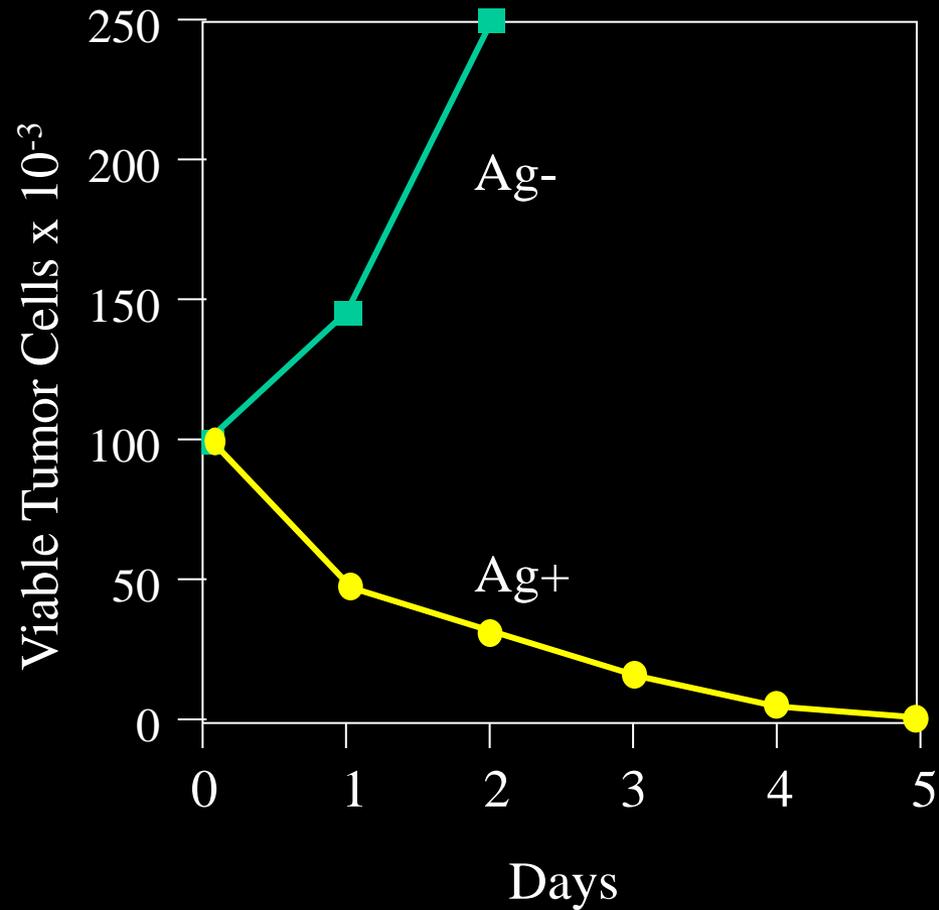


*Tumor + Designer T cells*



## Activation: Cytotoxicity

# DESIGNER CD8 T CELLS KILL Ag+ TUMOR CELLS



Clinical Data: 1<sup>st</sup> Generation

Phase I Study of Anti-CEA Designer T  
Cells in Adenocarcinoma  
("1st generation")

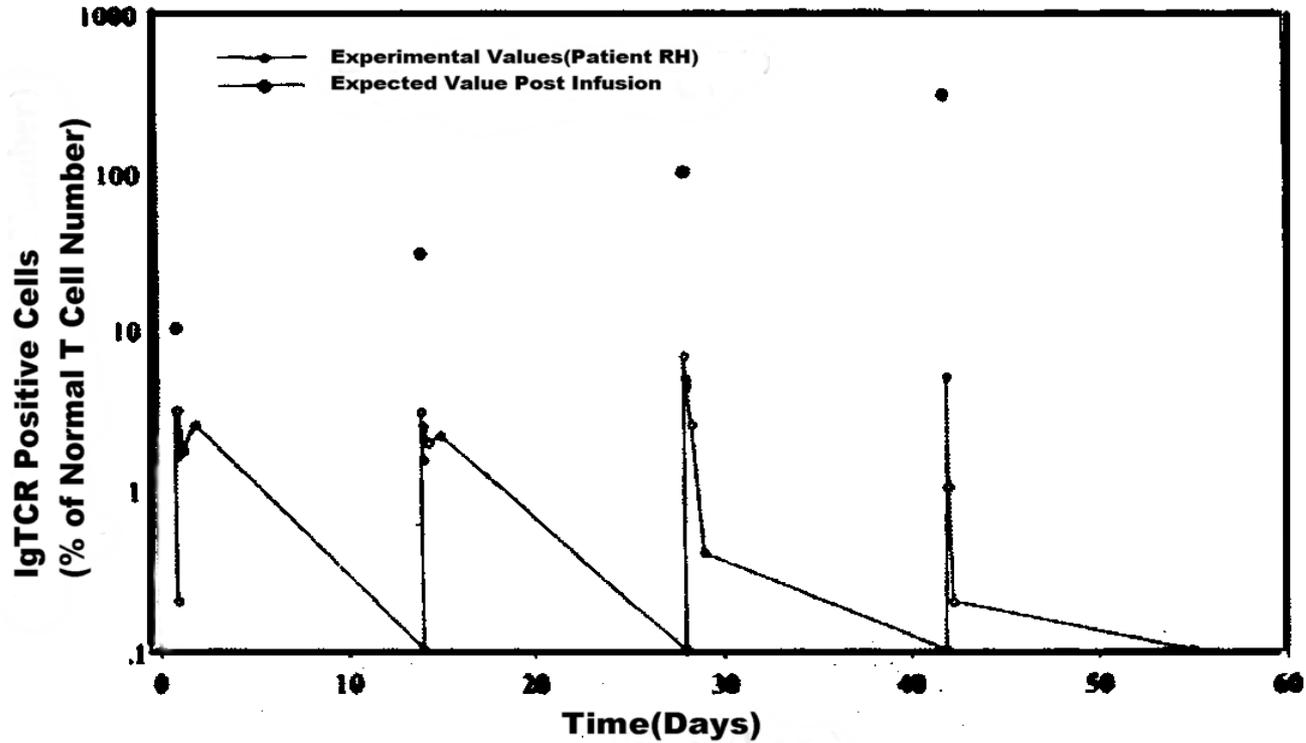
FDA BB IND

7301

# Clinical Summary

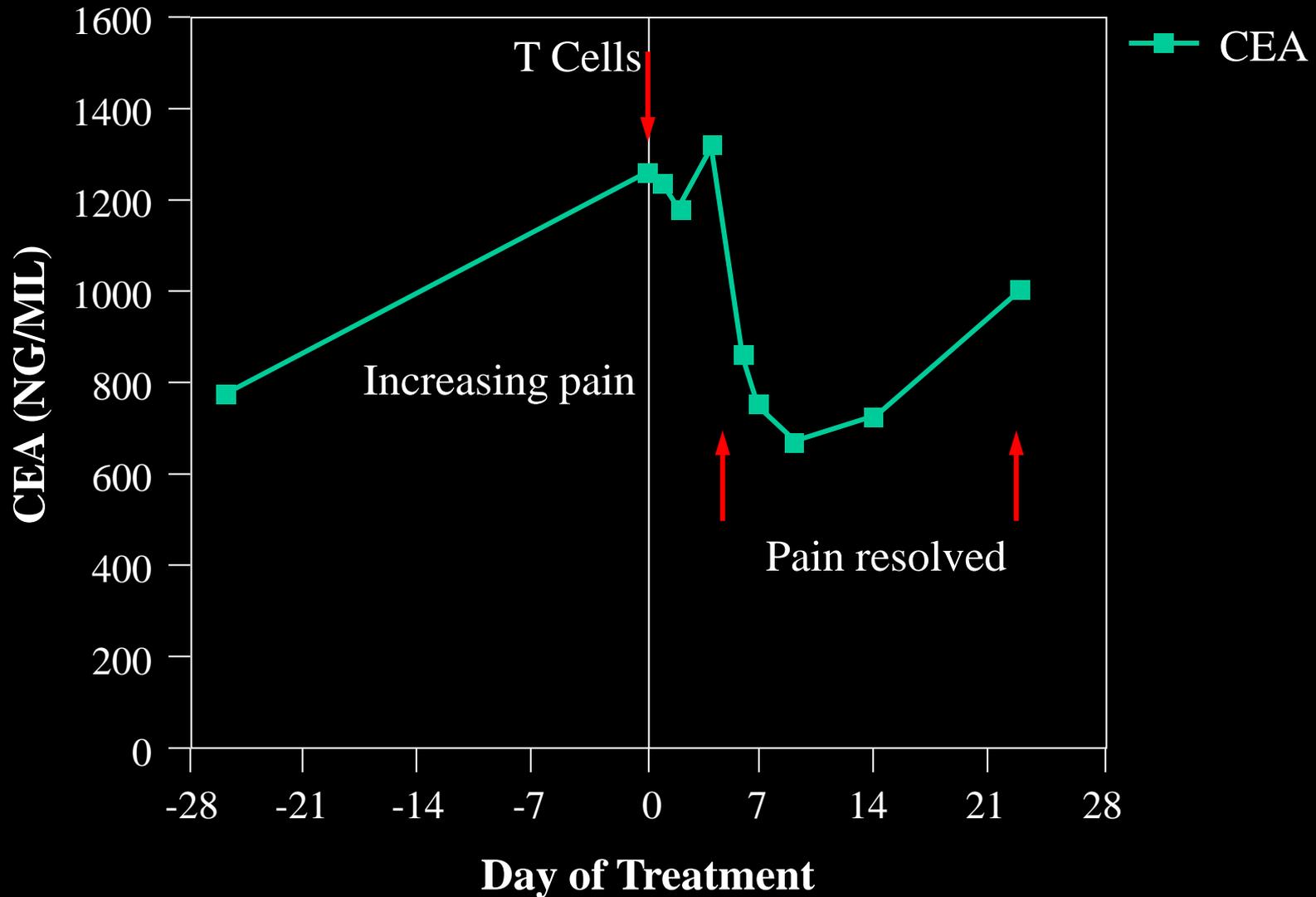
- o Number of doses administered (24)
- o Patients treated (7): 5 colorectal, 2 breast
- o Doses sizes administered
  - $1 \times 10^9$ ,  $3 \times 10^9$ ,  $1 \times 10^{10}$ ,  $3 \times 10^{10}$ ,  $1 \times 10^{11}$  cells

# Pharmacokinetics



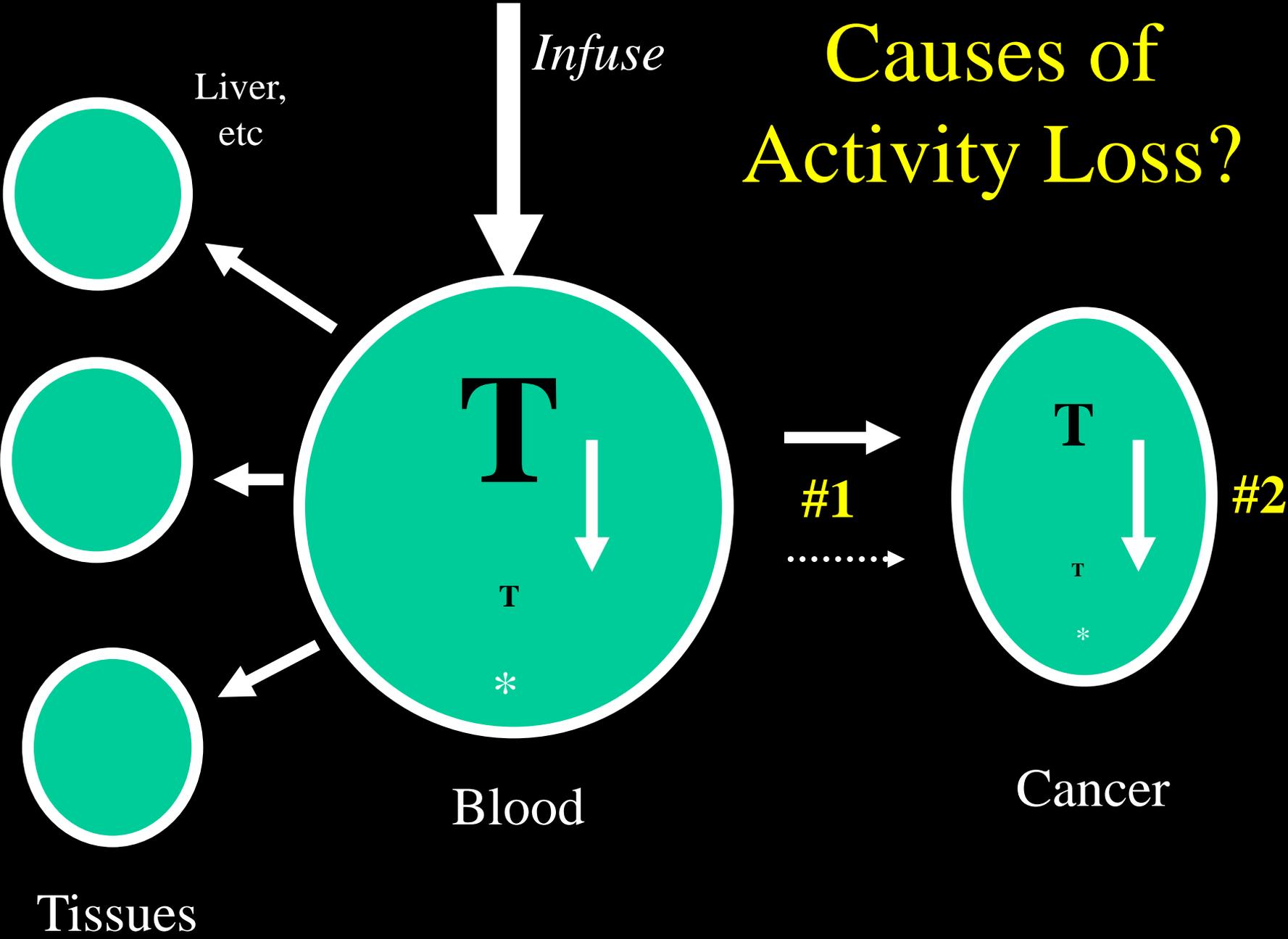
*Rapid Systemic Loss...*

# Response: Proof of Principle



**BUT! Time-Limited in Duration...**

# Causes of Activity Loss?



# Hypothesis

o Tumor eradication would follow if  
*EITHER*

– #1. *T cells persisted systemically*  
(“*bypass co-stimulation*”)

*OR*

– #2. *T cells persisted/expanded intratumorally*  
(“*provide co-stimulation*”)

# Approaches to Overcome AICD/Proliferative Defect

1. Bypass co-stimulation
2. Provide co-stimulation

# Hypothesis 2

## Provide Costimulation

Incorporate Signal 2 into designer T cells  
(2<sup>nd</sup> generation)

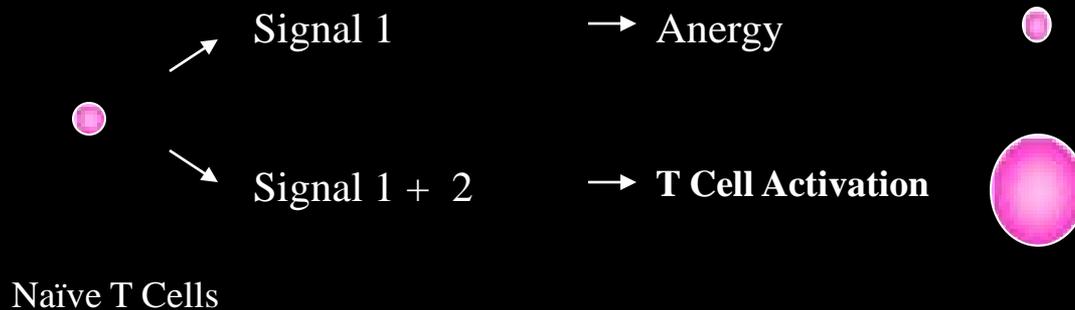
# Immunology 101

Remember!

*“T cells evolved to kill virus-infected cells.”*

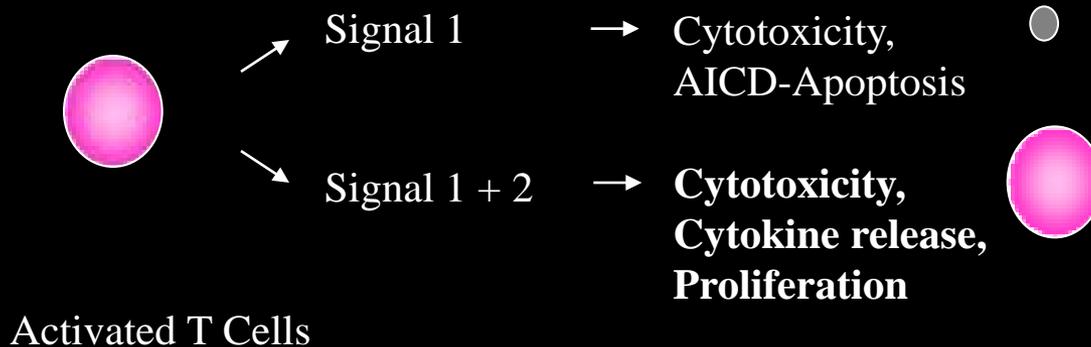
# Costimulation For T-Cell Activation

## Resting T cells



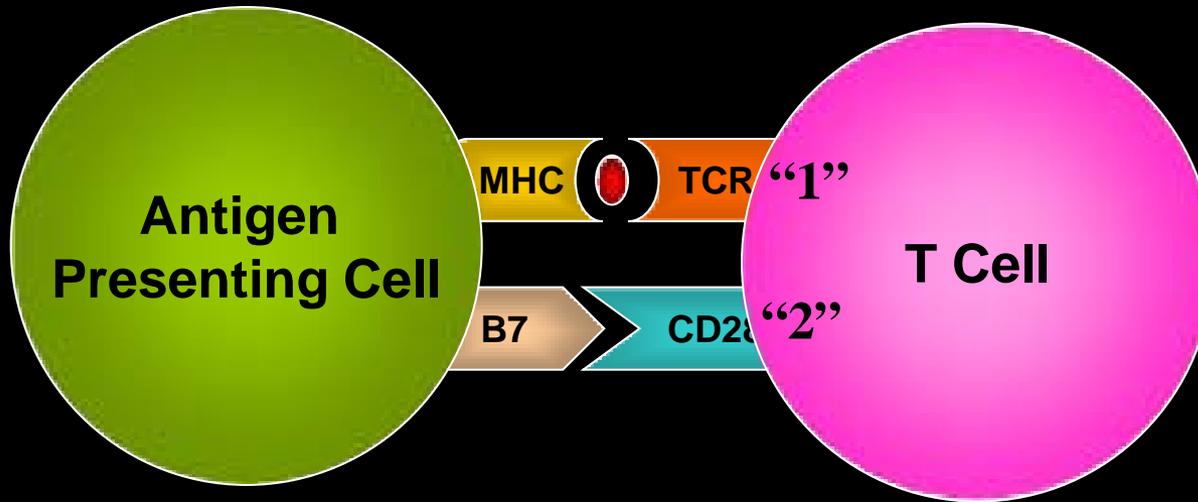
- Gene expression
  - Cytokines (IL-2, 4, IFN- $\gamma$ , etc)
  - Surface molecules (CD25, CD69, CD40L, etc)
- Effector function (T help, Cytotoxicity)
- Proliferation

## Activated T cells



- Apoptosis (AICD – Activation induced cell death)

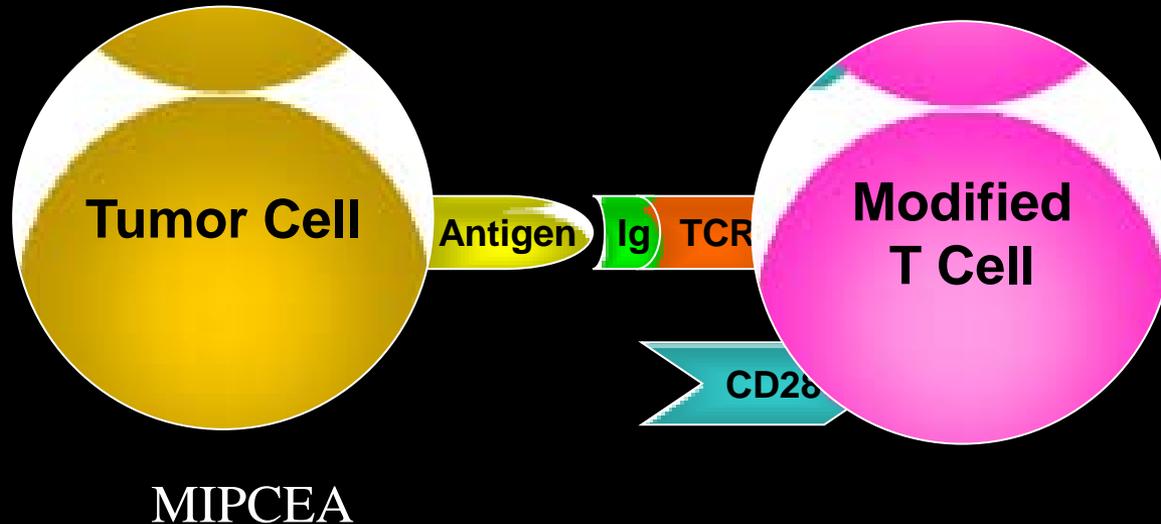
# T Cell Activation



- o Gene expression
  - Cytokines (IL-2, 4, IFN- $\gamma$ , etc)
  - Surface molecules (CD25, CD40L, etc)
- o Cytotoxicity
- o Proliferation

# Designer T Cells – First Generation

- o **IgTCR** – *chimeric immunoglobulin – T cell receptor*



**Advantage:** IgTCR provides Signal 1: adequate T cell cytotoxicity.

**Disadvantage:** Lacking Signal 2, undergoes Activation-Induced Cell Death (AICD) after killing target cells. **[HYPOTHESIS]**

# Signals

## o Signal 1:

- Activated: T cells kill tumor >> and die by AICD
- Resting: anergy

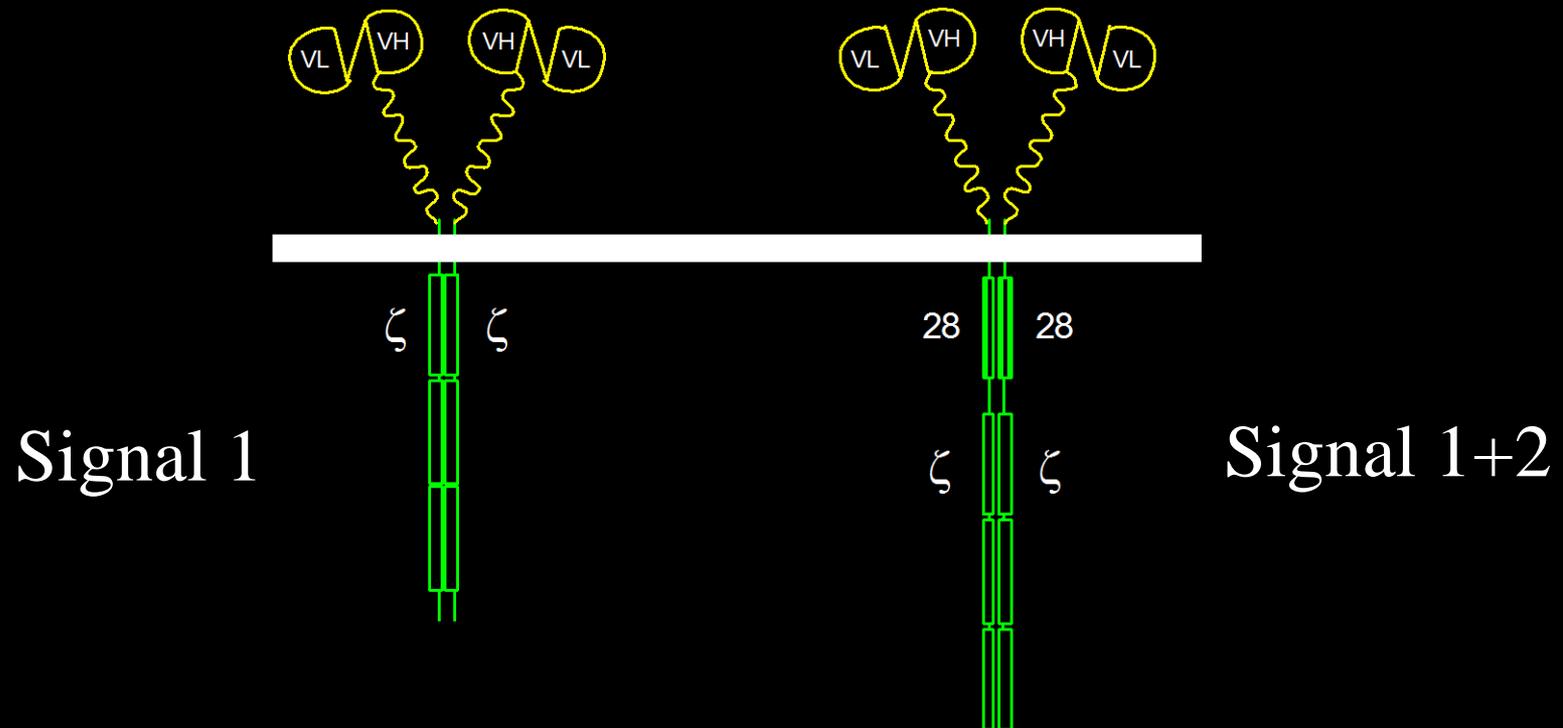
## o Signal 1+2:

- Activated: T cells kill tumor >> and proliferate
- Resting: activation, see above...

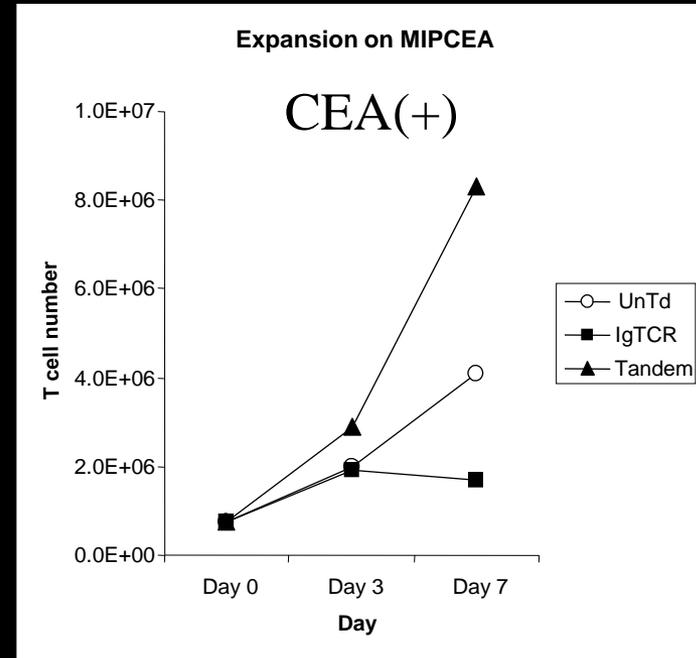
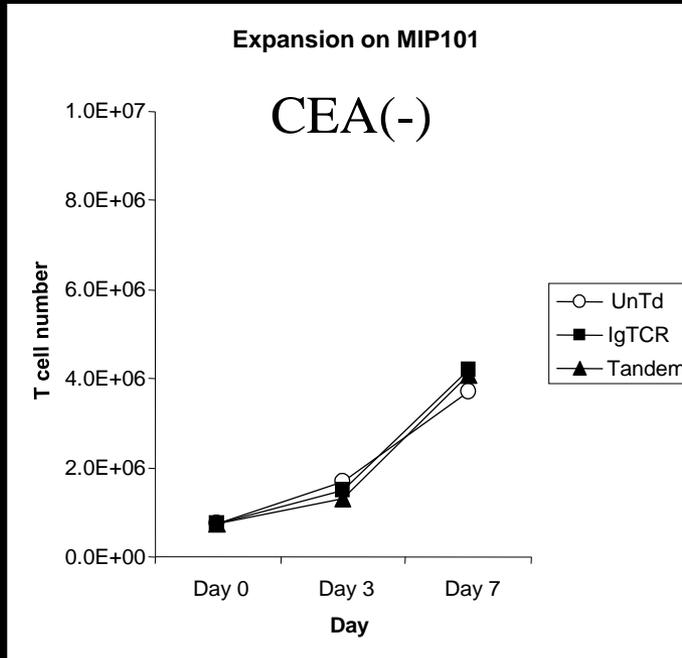
# 1<sup>st</sup> and 2<sup>nd</sup> Gen Constructs

IgTCR

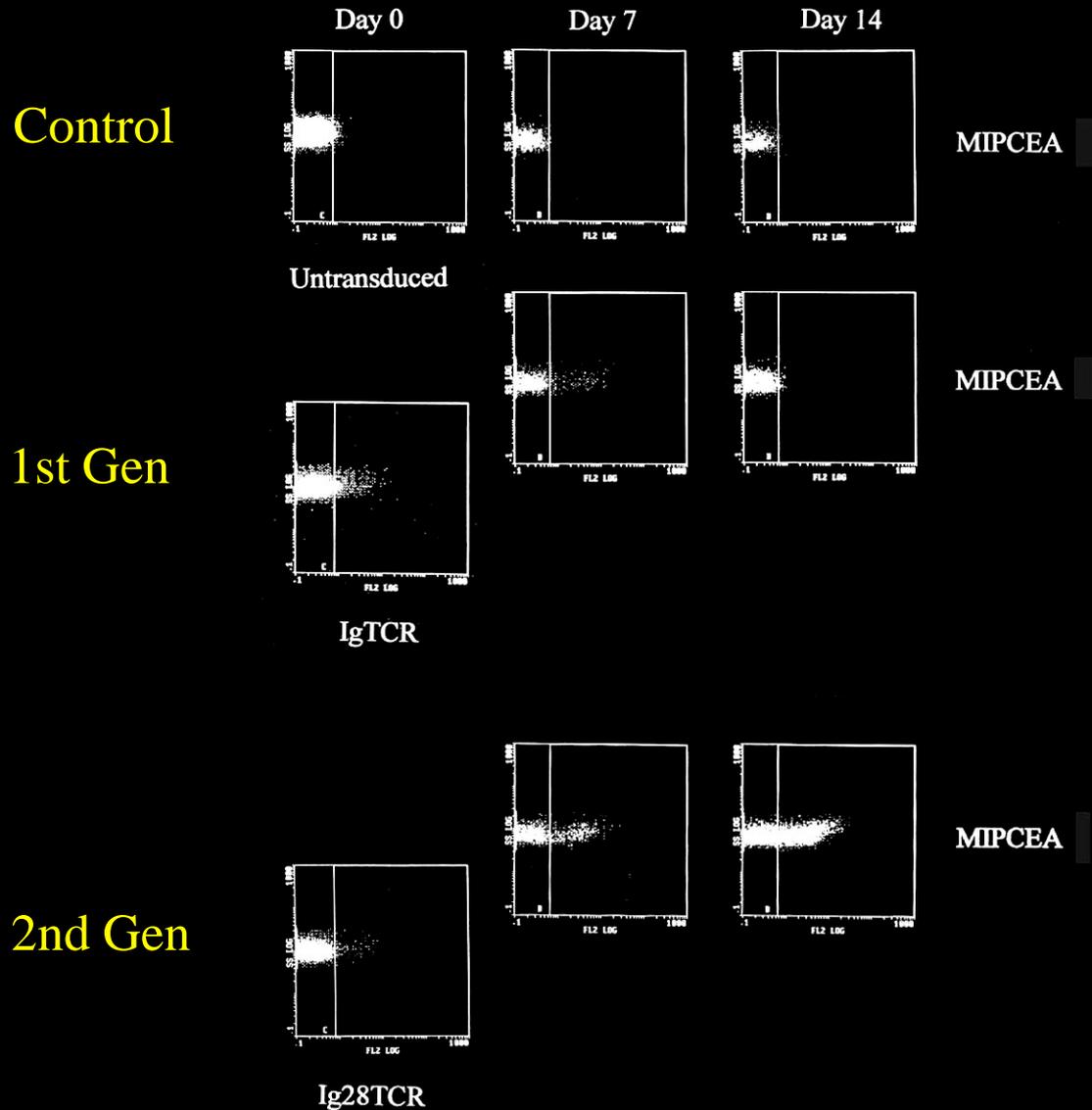
Ig28TCR ("Tandem")



# 2nd Gen T Cell Tumor-Induced Proliferation



# 2<sup>nd</sup> Gen Designer T Cells are Selectively Expanded



Phase Ia/Ib Trial of 2<sup>nd</sup> Generation  
Anti-CEA Designer T Cells in  
Adenocarcinoma

FDA BB IND  
10791

# Hypothesis 1

Bypass co-stimulation:

Auto-Transplant: Engraft designer T cells via lympho-expansive capacities of the body after lympho-depletion treatments

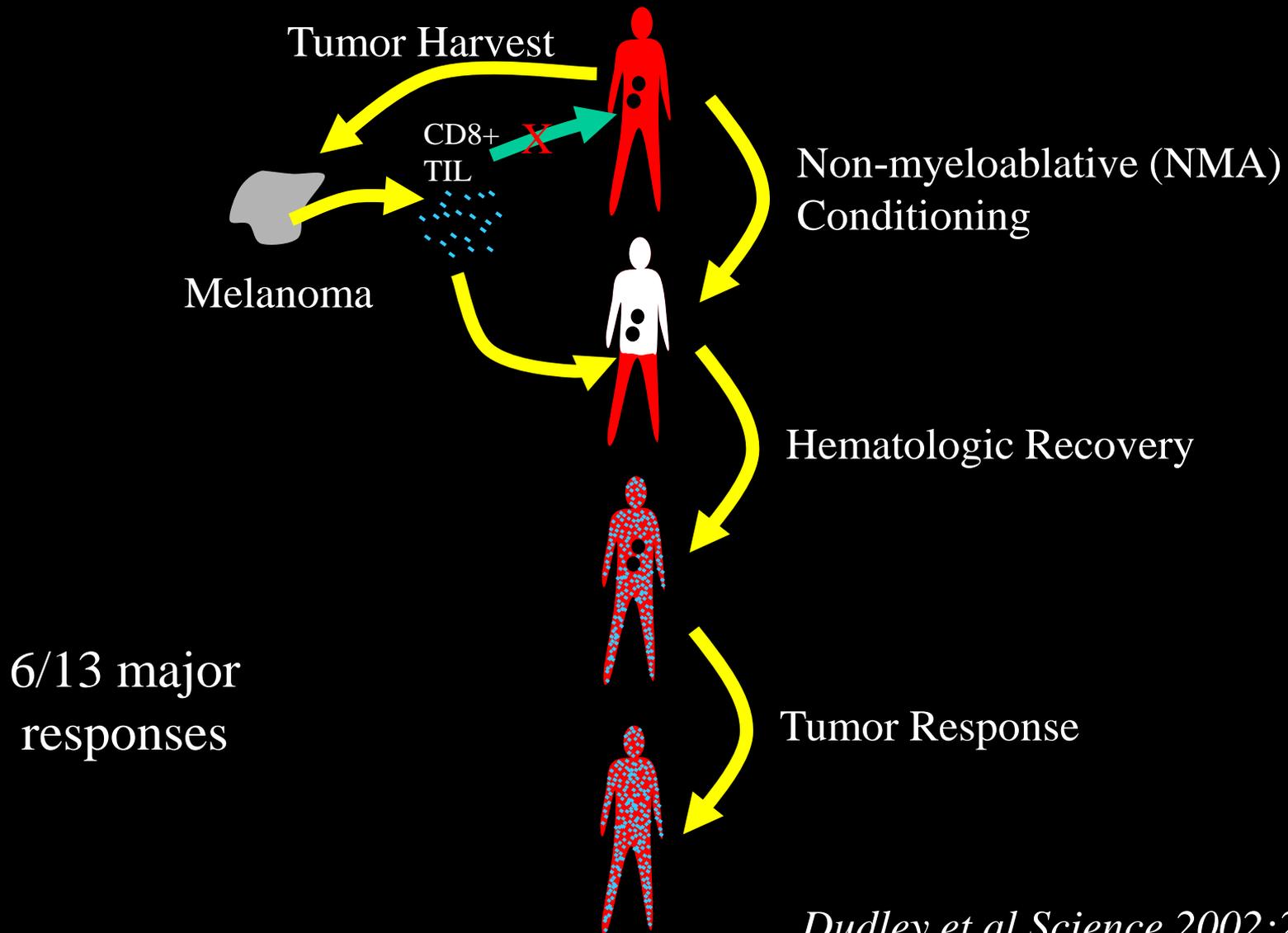
# Engraftment

25 OCTOBER 2002 VOL 298 SCIENCE

## **Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes**

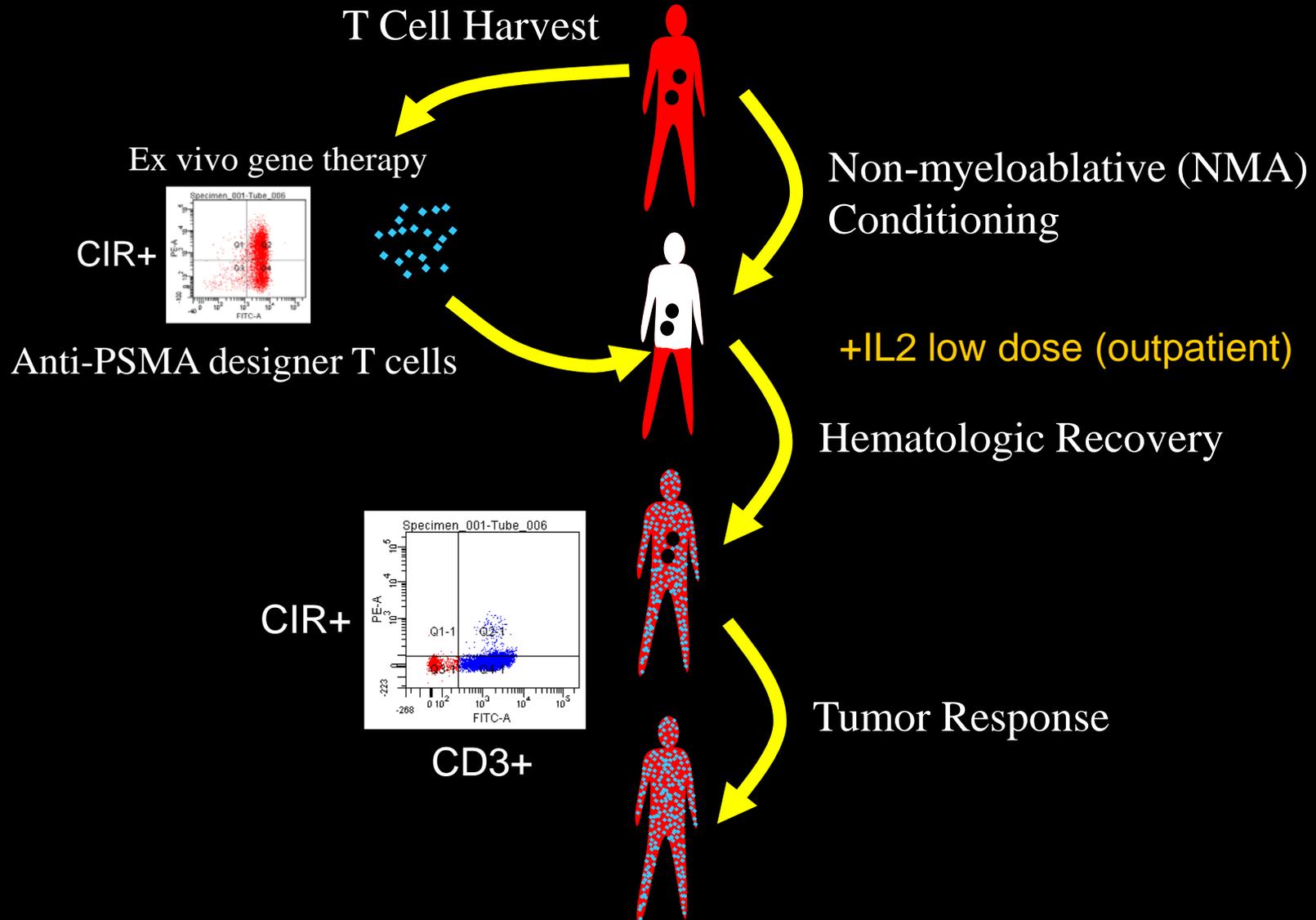
Mark E. Dudley,<sup>1</sup> John R. Wunderlich,<sup>1</sup> Paul F. Robbins,<sup>1</sup>  
James C. Yang,<sup>1</sup> Patrick Hwu,<sup>1</sup> Douglas J. Schwartzentruber,<sup>1</sup>  
Suzanne L. Topalian,<sup>1</sup> Richard Sherry,<sup>1</sup> Nicholas P. Restifo,<sup>1</sup>  
Amy M. Hubicki,<sup>1</sup> Michael R. Robinson,<sup>2</sup> Mark Raffeld,<sup>3</sup>  
Paul Duray,<sup>3</sup> Claudia A. Seipp,<sup>1</sup> Linda Rogers-Freezer,<sup>1</sup>  
Kathleen E. Morton,<sup>1</sup> Sharon A. Mavroukakis,<sup>1</sup> Donald E. White,<sup>1</sup>  
Steven A. Rosenberg<sup>1\*</sup>

# NMA – Melanoma TILs



*Dudley et al Science 2002;298:850*

# Designer T Cell Engraftment



Phase I Study of Autologous  
Transplantation of Anti-PSMA  
Designer T Cells after NMA  
Conditioning in Prostate Cancer

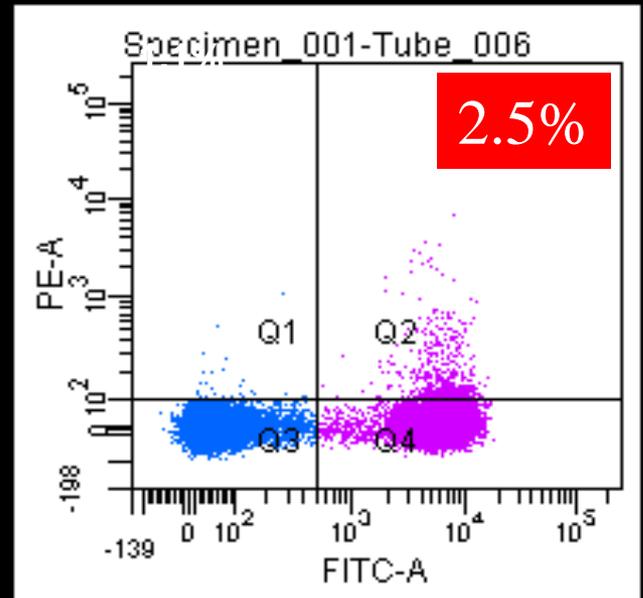
BB IND 12084

# Engraftment

Blood sample #1

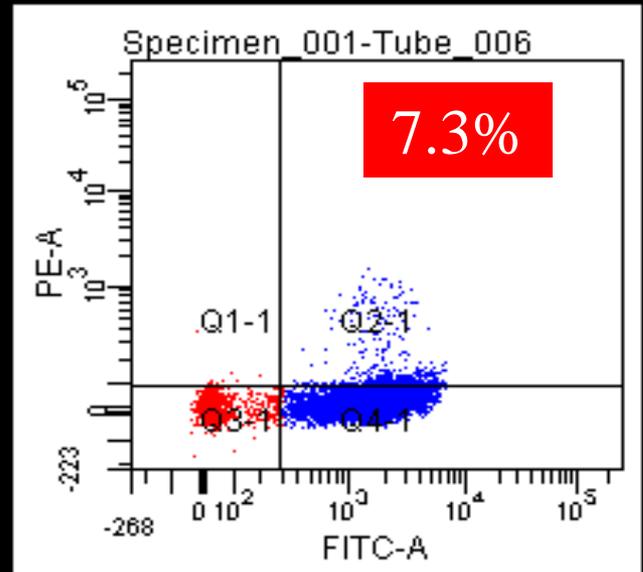
Day +14

CIR+

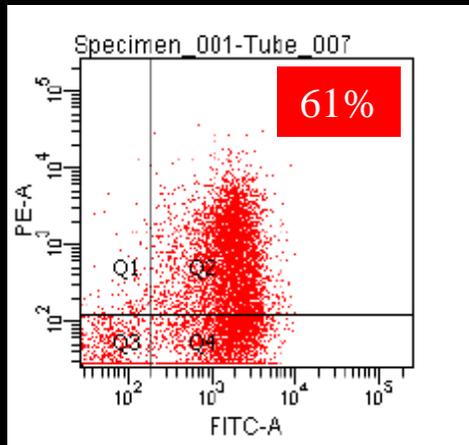


#2

CIR+



Dose



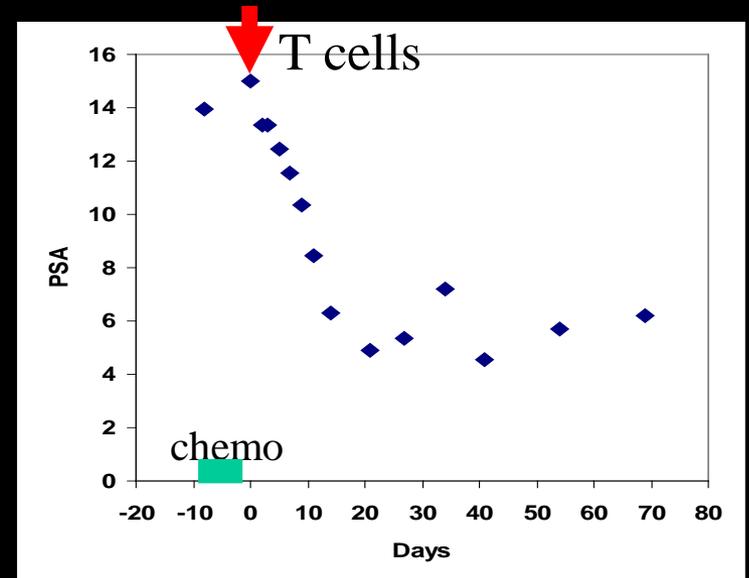
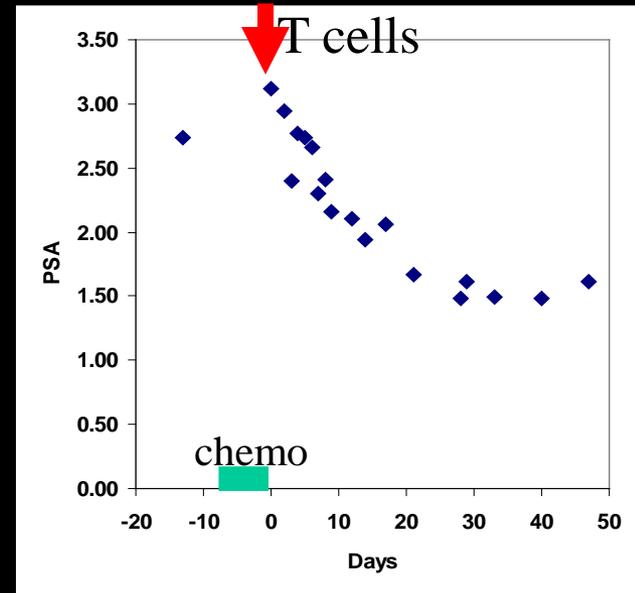
CD3+

# PSA Response

Conditioning d-8 to d-2

T cells infused d0

Low dose IL2 d0 to d28+



# Definitions

## Designer T cell versions

- o 1<sup>st</sup> generation
  - Signal 1 only (TCRs incorporated with or without zeta)
- o 2<sup>nd</sup> generation
  - Incorporates co-stimulation Signal 2
  - Irrespective of number of domains (CD28, OX40, 4-1BB, CD27, etc.); qualitatively same
- o 3<sup>rd</sup> generation ??
  - Something with qualitative difference, e.g., novel chemokine receptor for tumor trafficking

## Administration methods

- o Infusion: intravenous infusion without conditioning
- o Engraftment: infusion after chemo and/or XRT conditioning to expand in lymphopenic environment, more stable persistence

# Features of designer T cell versions

## On Contact with Antigen....

CIR

Resting T cells

Activated T cells

1<sup>st</sup> gen Signal 1

anergy

killing, AICD

2<sup>nd</sup> gen Signal 1+2

activation, killing,  
cytokines, proliferation

super-activation, killing,  
cytokines, proliferation

# Features of T cell administration methods

	<u>Conditioning</u>	<u>In blood</u>	<u>In tumor</u>	<u>Cost (\$K)*</u>
Infusion	None	Transient	Transient: low then none	\$5-10K
Engraftment	Chemo, XRT	Stable	Sustained: low then high	\$60-100K

*\*Costs pertain to the clinical, non-manufacturing component of the patient treatments and monitoring. Manufacturing costs are separate, approximately \$15,000 for a  $10^{11}$  T cell dose, including materials and personnel time, and less for lower doses. Clinical costs do not vary with dose size.*

# Options matrix for designer T cell treatments; strategies

Administration method  
Infuse      Engraft

Designer	1 <sup>st</sup> gen		1	2
T cell				
Version	2 <sup>nd</sup> gen		3	4



STRATEGY		
1	1st gen	infused
2	1st gen	engrafted
3	2nd gen	infused
4	2nd gen	engrafted

# Clinical Trials 2002

*Table 2.* Clinical trials with chimeric receptor redirected T cells

Date initiated	Phase	Disease	Antigen	Structure	Location	Investigator
1995	I	HIV	gp120	CD4- $\zeta$	NIH	Walker
1996	I	Ovarian cancer	FBP	sFv- $\gamma$	NCI	Hwu
1997, 1998	II	HIV	gp120	CD4- $\zeta$	Multi	Hege
1997	I	Adenocarcinoma	TAG72	sFv- $\zeta$	Stanford	Hege
1998	I	Adenocarcinoma	CEA	sFv- $\zeta$	Harvard	Junghans
2000	I	Lymphoma	CD19	sFv- $\zeta$	City of Hope	Jensen
2002 (pending)	I	Renal cell carcinoma	G250	sFv- $\zeta$	den Hoed CC	Bolhuis
2002 (pending)	I	Melanoma	GD3	sFv- $\zeta$	Harvard	Junghans

# Clinical trials 2008

**Table 1** T-Bodies in clinical trials

Tumor	Antigen	Group	Status
Ovarian	FBP	Hwu, Rosenberg, NCI	Performed
Colorectal ca.	TAG-72	McArthur, Cell Genesys	Performed
Colorectal & breast ca.	CEA	Junghans, Harvard	Performed
Renal ca.	Carboxyanhydrase IX	Gratama, Rotterdam	Ongoing
Neuroblastoma	CD171	Jensen Seattle/City of Hope	Ongoing
Glioblastoma	IL-13 Receptor <sup>a</sup>	Jensen, City of Hope	Ongoing
Neuroblastoma	G(D)2	Brenner, Baylor College of Med.	Ongoing
Gastric ca.	CEA (2nd generation)	Junghans, Roger Williams	Recruiting
Prostate ca.	PSMA	Junghans, Roger Williams	Recruiting
Leukemia	CD19	Jensen, City of Hope	Recruiting
Leukemia	CD19	Hawkins, Manchester	Recruiting
Leukemia	CD19	Sadelain, Sloan Kettering	Recruiting
Leukemia	CD19	Brenner, Baylor College of Med.	Approved
Leukemia	CD19	June, Univ. Pennsylvania	Pending
Pancreatic ca.	Mesothelin	June, Univ. Pennsylvania	Pending
Colorectal ca.	CEA	Hawkins, Manchester	In planning
Prostate ca.	PSMA	Sadelain, Sloan Kettering	In planning
Myeloma	Lewis-Y	Kershaw, Melbourne	In planning
Cutaneous lymphoma	CD30	Abken, Cologne	In planning
Lymphoma	CD20	Cooper, MD Anderson	In planning

<sup>a</sup> Updated to April 2007

<sup>b</sup> Redirected by IL-13 ζ CR (not antibody based)

# Designer T cells: “Brave New World” for cancer therapies

- o Not inert chemical or molecule
- o Living cells *of the patient*, miniature “organisms” engineered to seek and destroy
- o Programming the T cells
  - maintain or expand “drug” in presence of tumor
  - “drug” to disappear when all tumor eliminated
- o Reasonable cost; “personalized” but generalizable
- o The Future of immuno-oncology? – Predict FDA approval of *some* designer T cell as standard therapy for breast or other cancer in 5+ years

# The Goal: T cells killing cancer cells

## T-LYMPHOCYTE

These cells recognize and  
kill cancer cells in the  
body and are shown here  
attacking melanoma cells

T cells homing in on target

