Personalized Medicine

Adoptive T cell therapy: Grand Challenges and Opportunities

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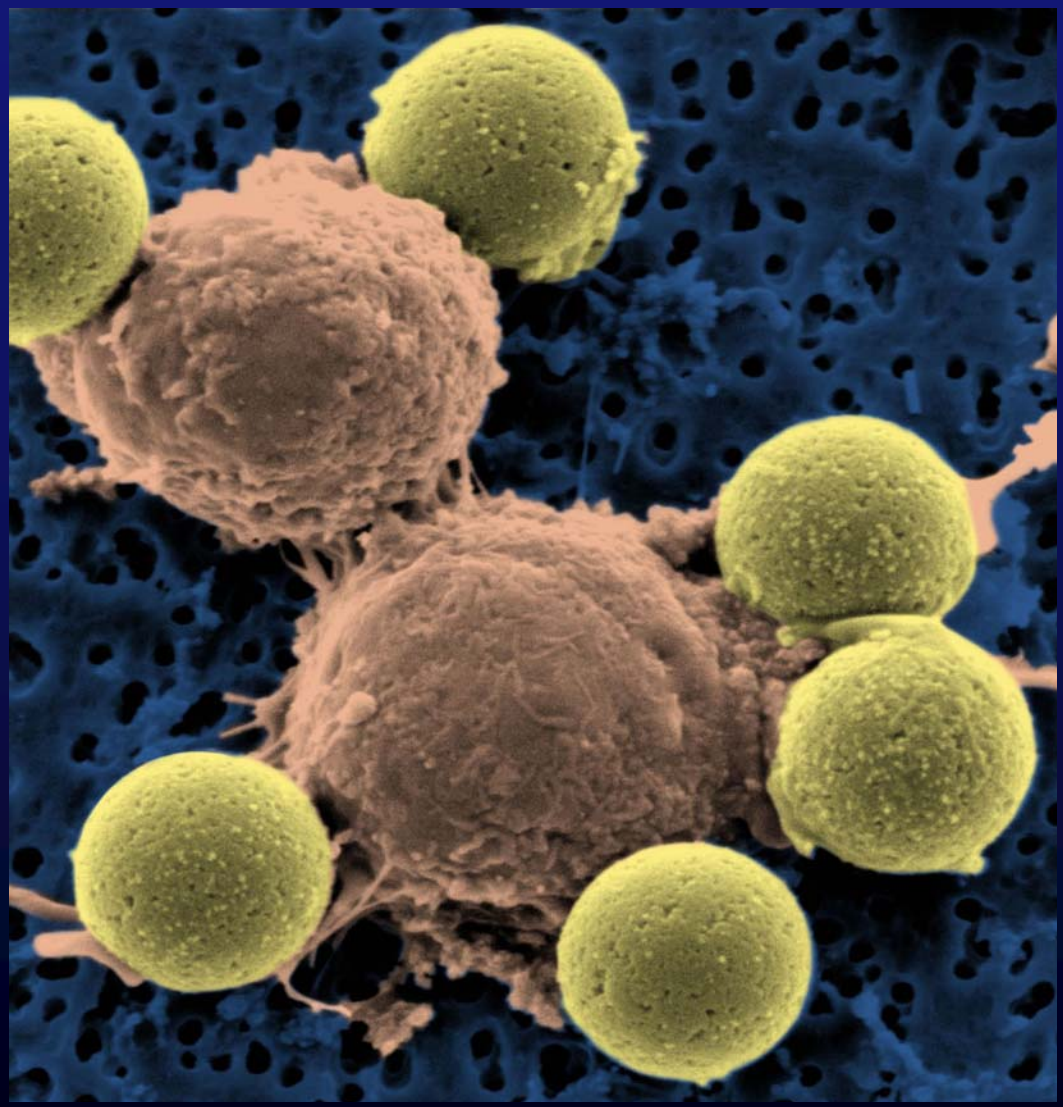
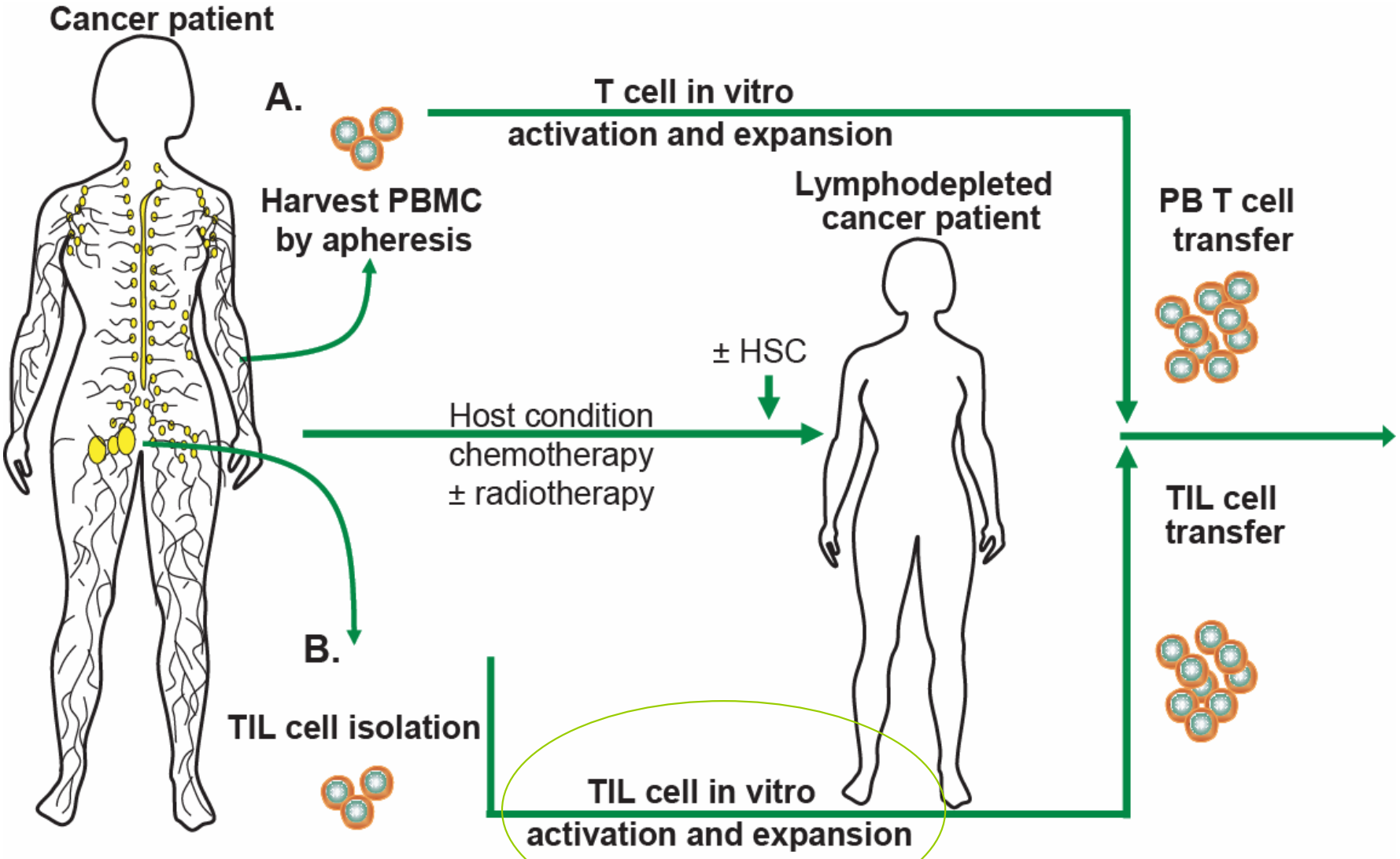
Overview

- Prime-boost strategies
- Lentiviral engineered T cell transfers



General Approaches for Adoptive T Cell Therapy

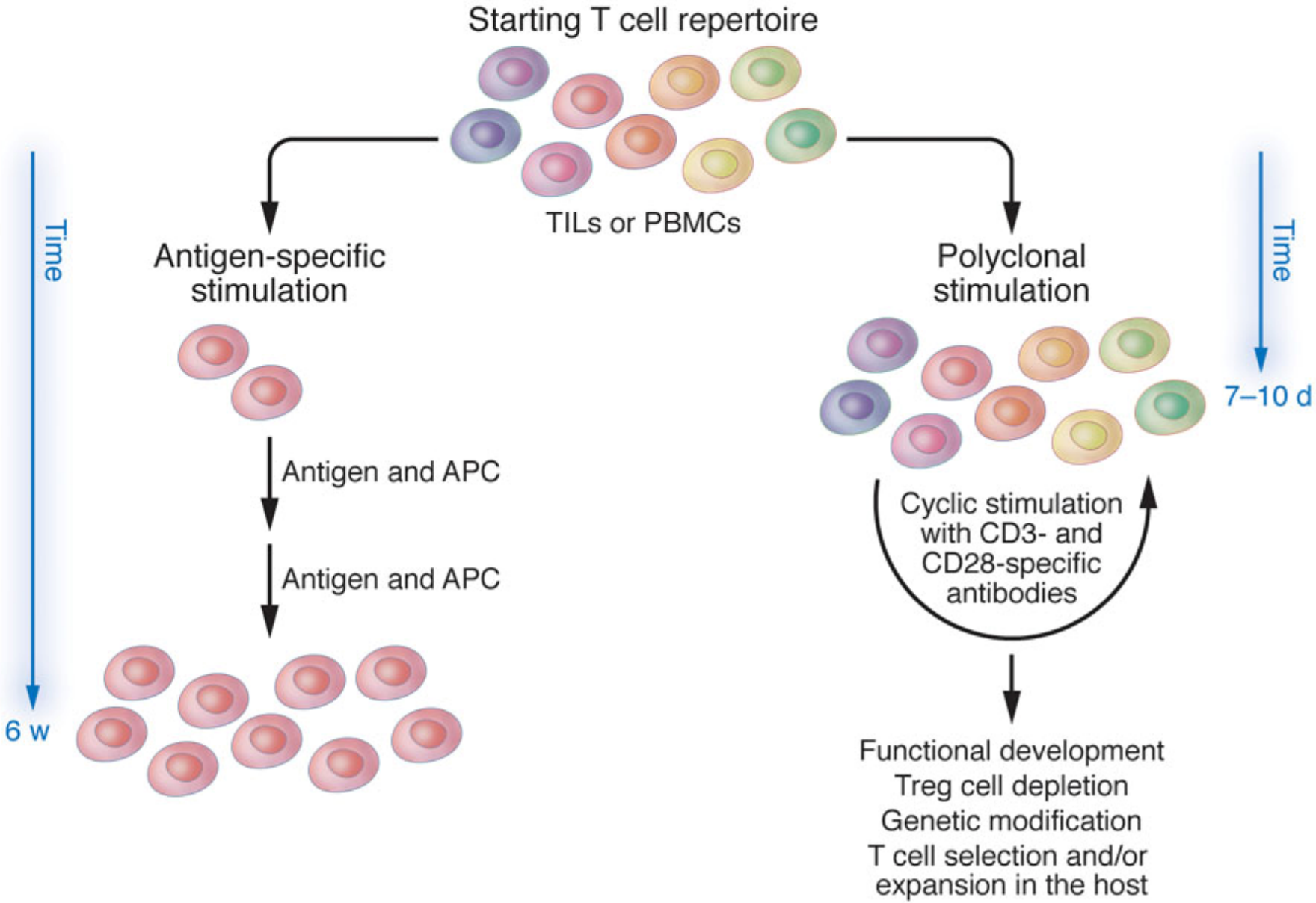


J Clin Invest 2007 117:1466-76

Human T Cell Adoptive Immunotherapy (Effectors)

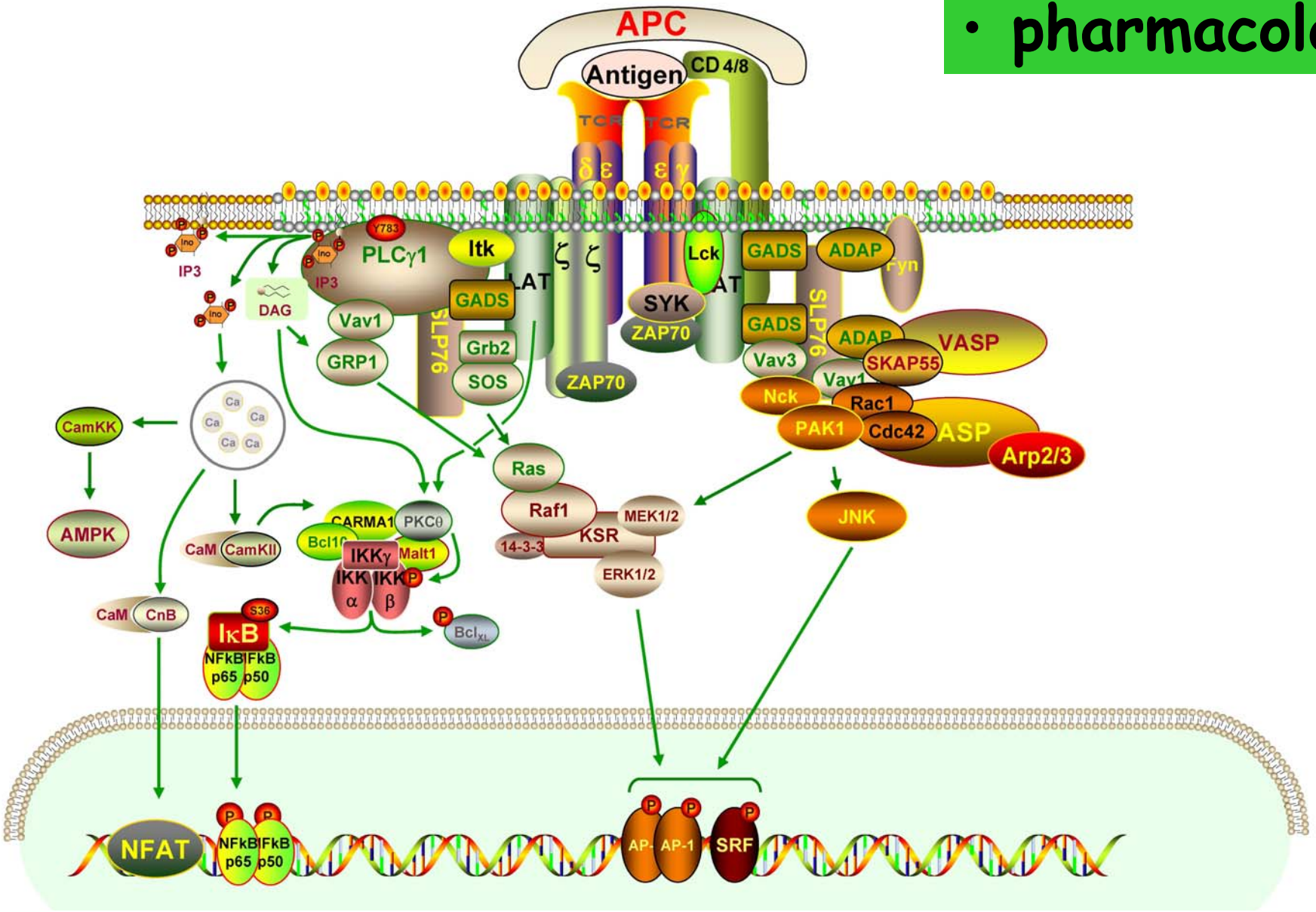
- **1992: CMV, transfer of human CTL clones from the donor to recipients of allogeneic bone marrow (Riddell et al, *Science* 1992; 257: 238)**
- **2002: Lymphodepletion + TILs + high dose IL-2 for metastatic melanoma (Dudley et al, *Science* 2002; 298: 850)**
- **2002: CD4 effectors in HIV (Levine et 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)**

Cell Culture Approaches for Adoptive T Cell Therapy

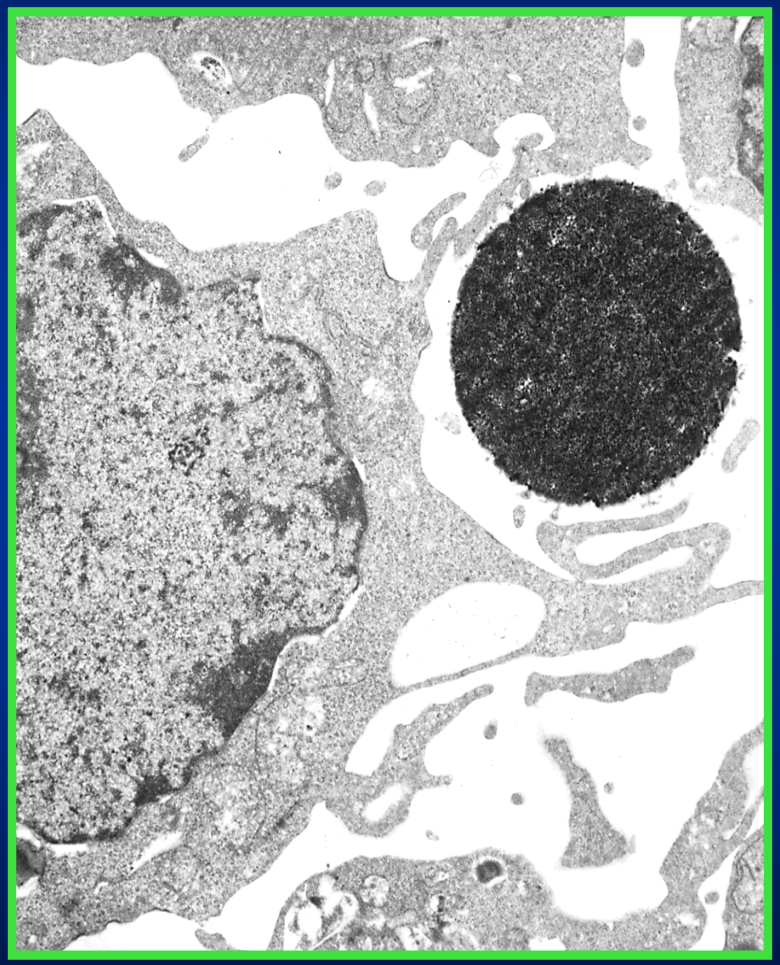


T Cell Culture Systems

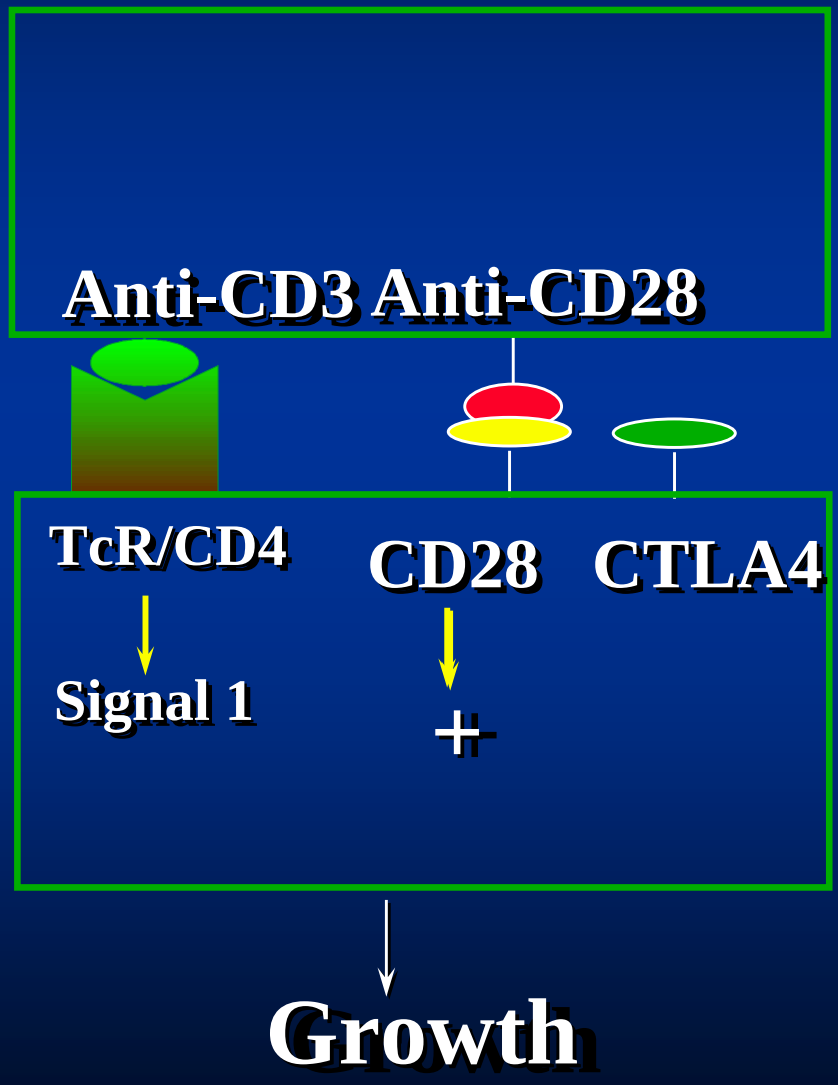
- physiologic
- pharmacologic



T Cell Artificial APC Culture System

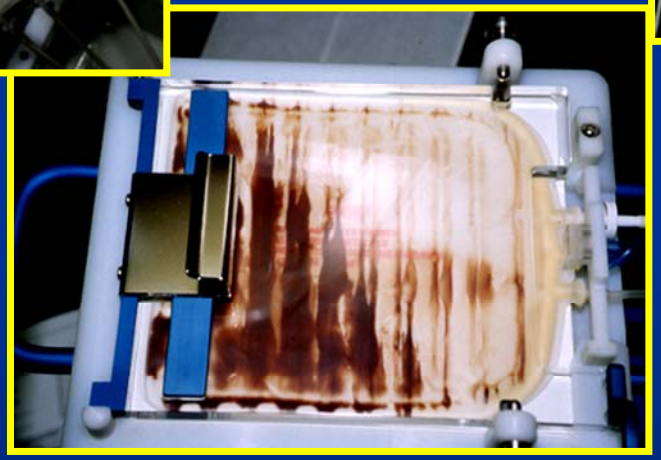


Artificial DC: Bead



Levine et al. *Science.* 1996; *272*:1939

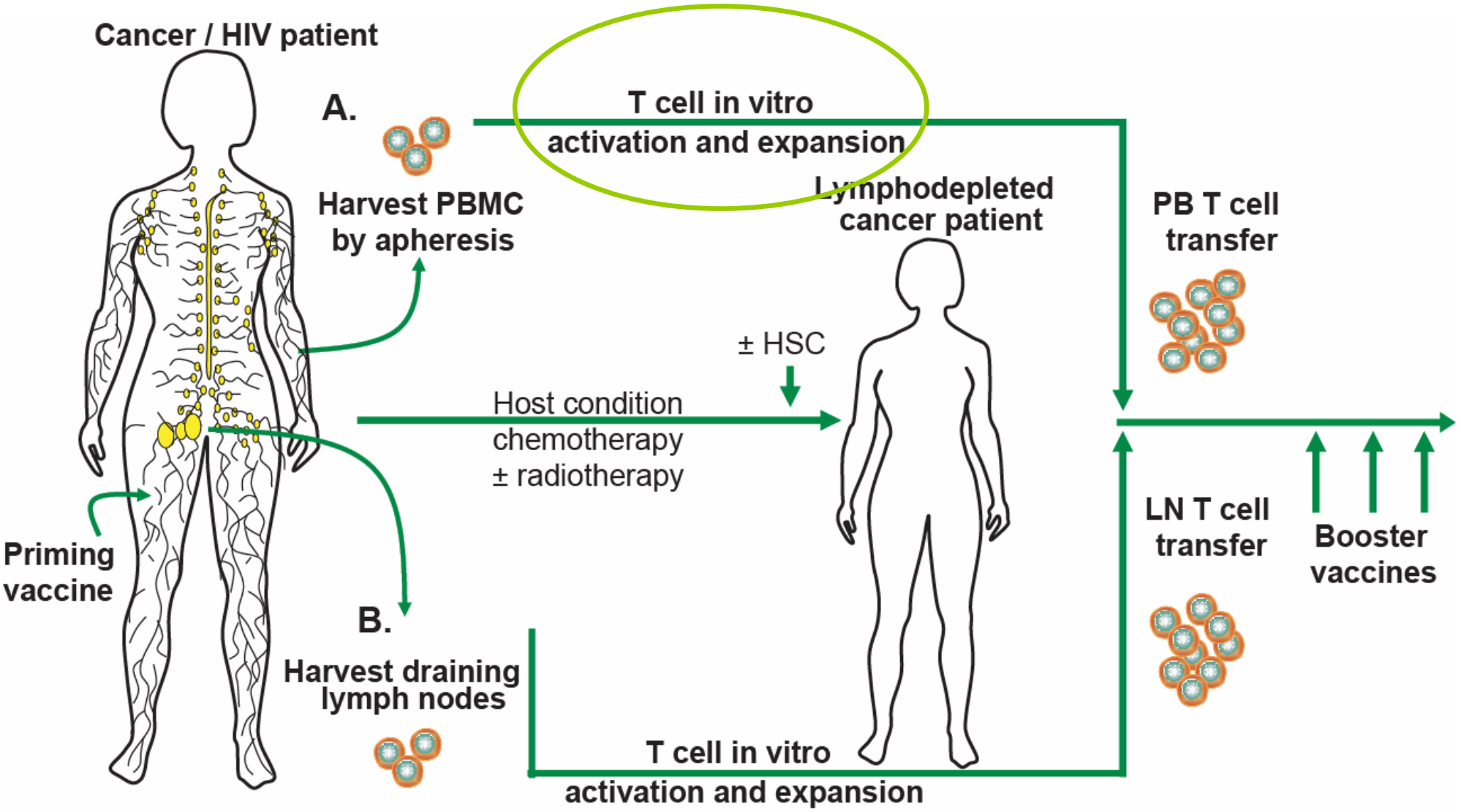
Clinical Scale T Cell Culture Process



Wave Bioreactors

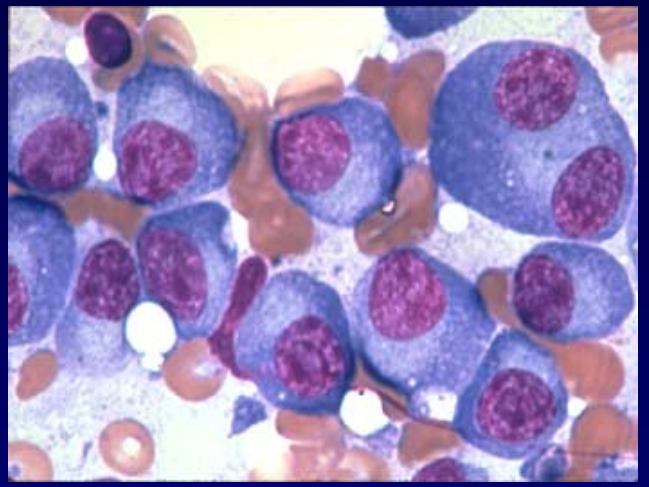
Levine et al. J Hematotherapy 1998: 7:437

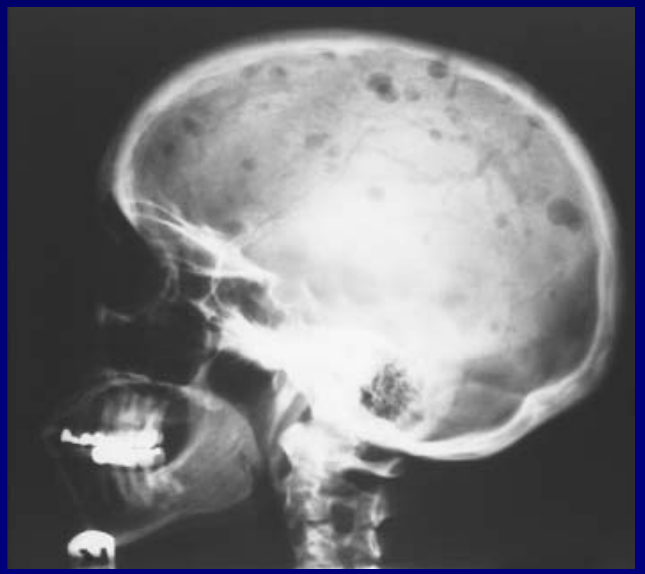
Combination immunotherapy: vaccination and adoptive transfer as a "prime-boost"



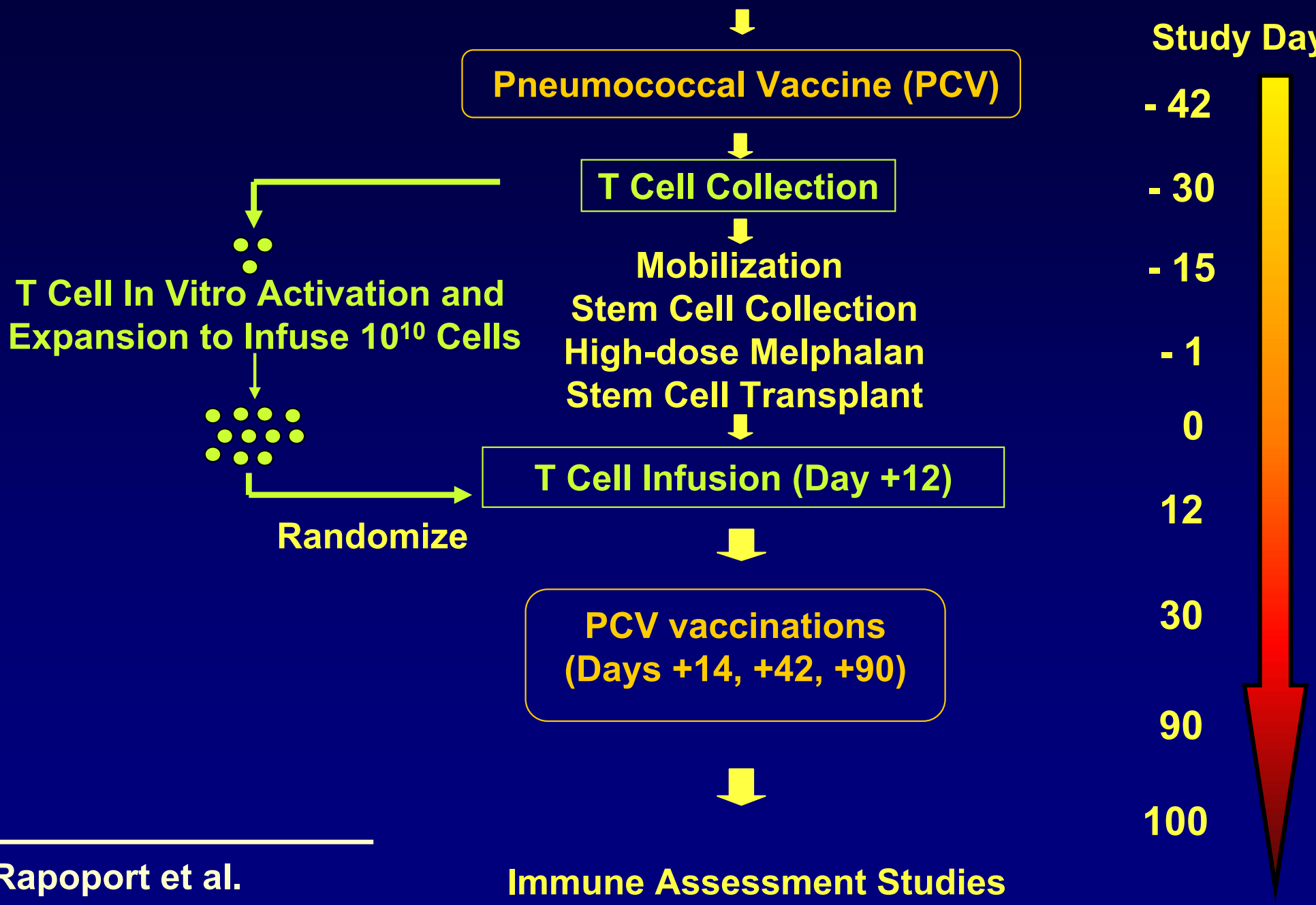
Multiple Myeloma

- Plasma cell neoplasm characterized by serum monoclonal Ab, osteolytic lesions, pathological fractures, anemia, hypercalcemia
- 15% of hematologic malignancies
- Autologous transplants are highly effective for tumor reduction (first line therapy), but *cures are infrequent*.
- GVM/GVT: Allogeneic transplants can induce cures, but *treatment-related risks are high*.





Myeloma Phase I/II Adoptive Transfer Trial



Rapoport et al.
Nat. Med. 2005; 11: 1230

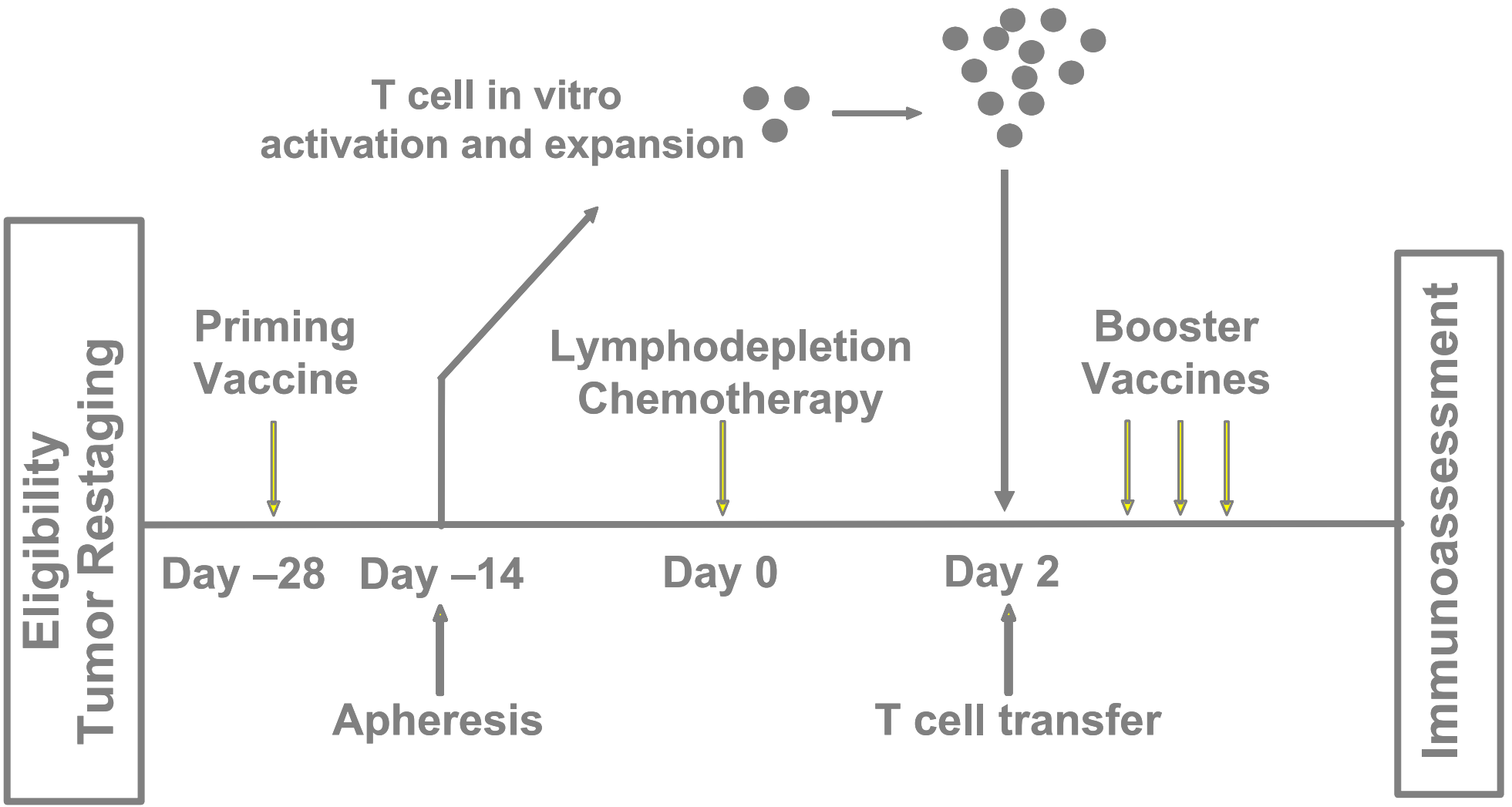
Adoptive transfer of vaccine primed T cells augments immunity in lymphodepleted hosts: Summary of first trial

- First successful randomized multicenter adoptive immunotherapy trial
- Accelerated recovery of CD4 and CD8 counts to normal levels by day 42 (P=0.004)
- Protective antibody levels established by day 30
- Improved proliferative capacity of CD4 T cells to vaccine carrier antigen (P<0.01) and to Staphylococcal enterotoxin B (P=0.004)

=> Adoptive transfer of vaccine primed T cells appears to facilitate establishment of CD4 T central memory cells

Rapoport et al. *Nat. Med.* 2005; 11: 1230

Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells



Phase I/II Combination Immunotherapy after ASCT for Advanced Myeloma of hTERT/Survivin Vaccination Followed by Adoptive Transfer of Vaccine-Primed Autologous T cells

PIs: Aaron Rapoport, U Maryland
Edward Stadtmauer, U Pennsylvania

INDs:
Vaccine (Vonderheide)
T cells (June)

Design: Randomized (biologic) comparison
1) Autologous T cells day 2 post ASCT
2) Vaccine + vaccine primed T cells

Status:
Protocol open to accrual
18 patients enrolled

SUMMARY OF MYELOMA TRIAL PATIENTS

As of October. 2007

	ID #	Age on Study	R	S	Study Arm	On Study Date	# hTERT Vaccines Received	Day 0	T cell Infusion	T cell Dose
1	MGCC610-MD001	55	AA	M	A	12/18/06	4	1/30/2007	02/01/07	2.63E+10
2	MGCC610-MD002	67	C	F	B	01/05/07	NA	02/21/07	02/23/07	3.75E+10
3	MGCC610-MD003	51	AA	F	A	12/26/06	4	2/12/2007	02/14/07	3.38E+10
4	MGCC610-MD004	57	C	M	B	03/27/07	NA	5/5/2007	05/10/07	4.18E+10
5	MGCC610-MD005	37	C	F	B	05/15/07	NA	6/26/2007	06/28/07	3.80E+10
6	MGCC610-MD006	51	A	F	A	05/25/07	3	7/11/2007	07/12/07	3.36E+10
7	MGCC610-MD007	60	C	F	B	12/26/06	NA	8/7/2007	08/09/07	4.81E+10
8	UPCC 13406-01	55	C	M	A	04/18/07	4	6/20/07	06/22/07	4.29E+10
9	UPCC 13406-02	45	C	M	A	05/30/07	4	7/24/07	07/26/07	4.41E+10
10	UPCC 13406-03	61	AA	M	B	05/02/07	NA	7/17/07	07/19/07	5.00E+10
11	UPCC 13406-04	47	C	F	B	06/13/07	NA	7/31/07	08/02/07	3.86E+10

hTERT vaccine + day 2 T cell trial: Interim Summary

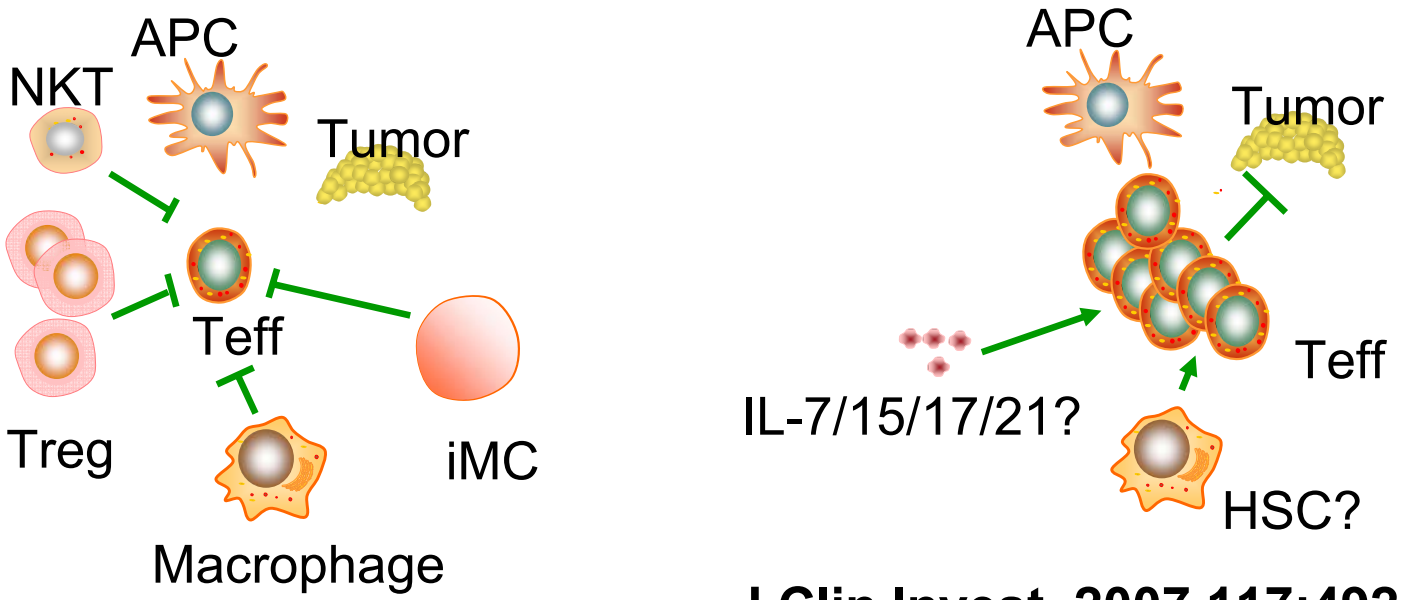
- 18 patients enrolled
- Safety to date: no HSC engraftment issues
- Unexpected adverse event:
  - T cell engraftment syndrome in 6 patients (skin rash, fever, diarrhea)
  - Lymphocytosis: sustained in many patients
- Above implies major *schedule dependent* (day 2 vs day 12) difference in T cell engraftment and effector functions

T Cell Expansion in Lymphopenic Hosts Enhanced CD8 Effector Function?

Potential mechanisms:

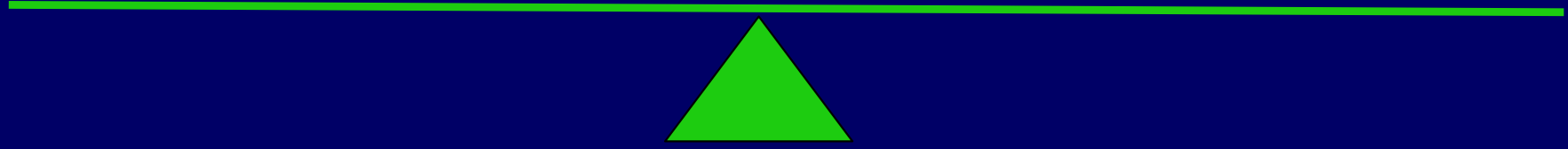
- Role of lymphopenia
- Depletion of Tregs, NKT, B cells?
- Removal of cytokine sinks?

  IL-2 vs IL-7/-15/-21 regulation
- Stem cell push?



J Clin Invest. 2007 117:492-501

Rationale for Adoptive T Cell Immunotherapy with Genetically Engineered T Cells



Natural T Cells	Gene-Modified T cells
Safety profile established	Safety profile scant
T cells have the potential to target cancer stem cells	Repertoire limitations can be overcome
Anecdotal responses observed to immunotherapy	Anecdotal responses observed in immunotherapy
Repertoire may be inadequate or lacking	Efficient *gene transfer* required
Immunosenescence a major issue in humans	Efficient *T cell culture* required

Oncoretroviral vs.Lentiviral vectors

O Maloney oncoretroviral vectors

Only transduces dividing cells

Insertional mutagenesis

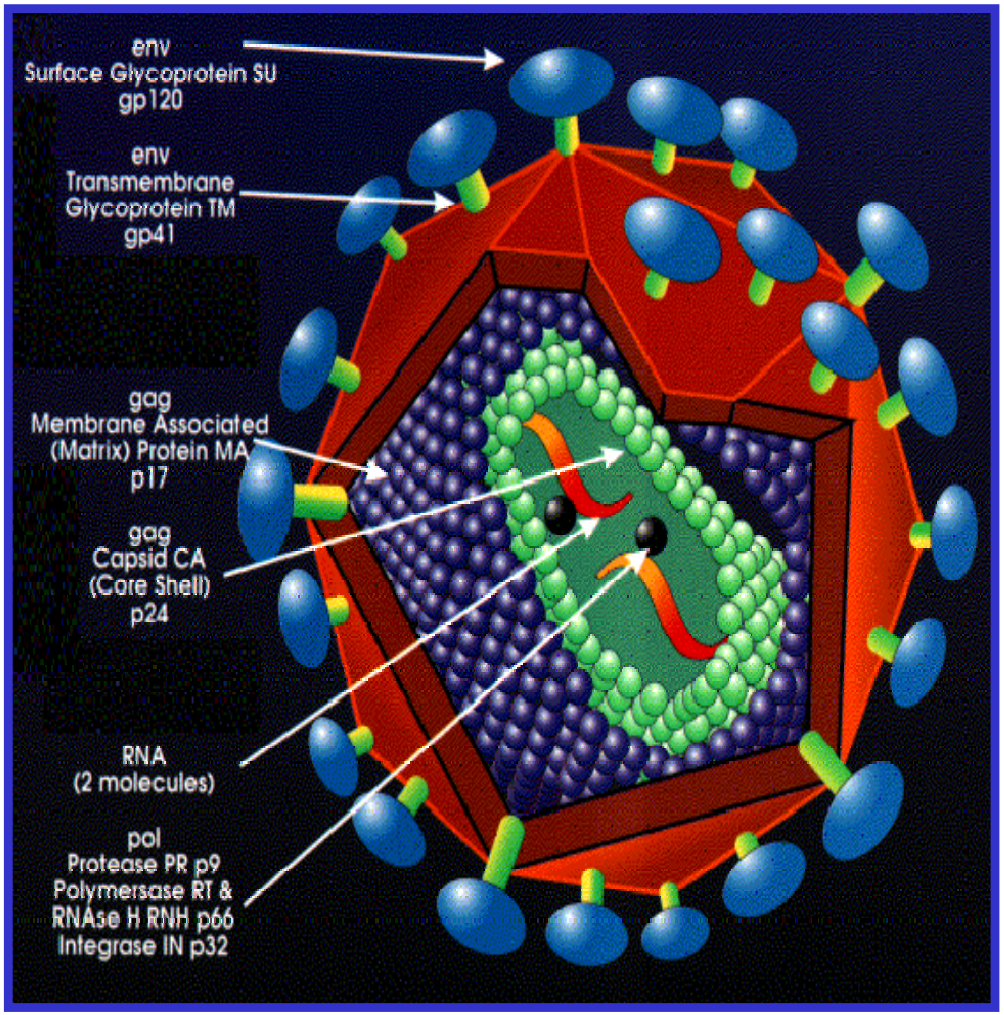
T cell leukemia in SCID (cγ chain)

O Potential advantages of HIV based lentiviral vectors

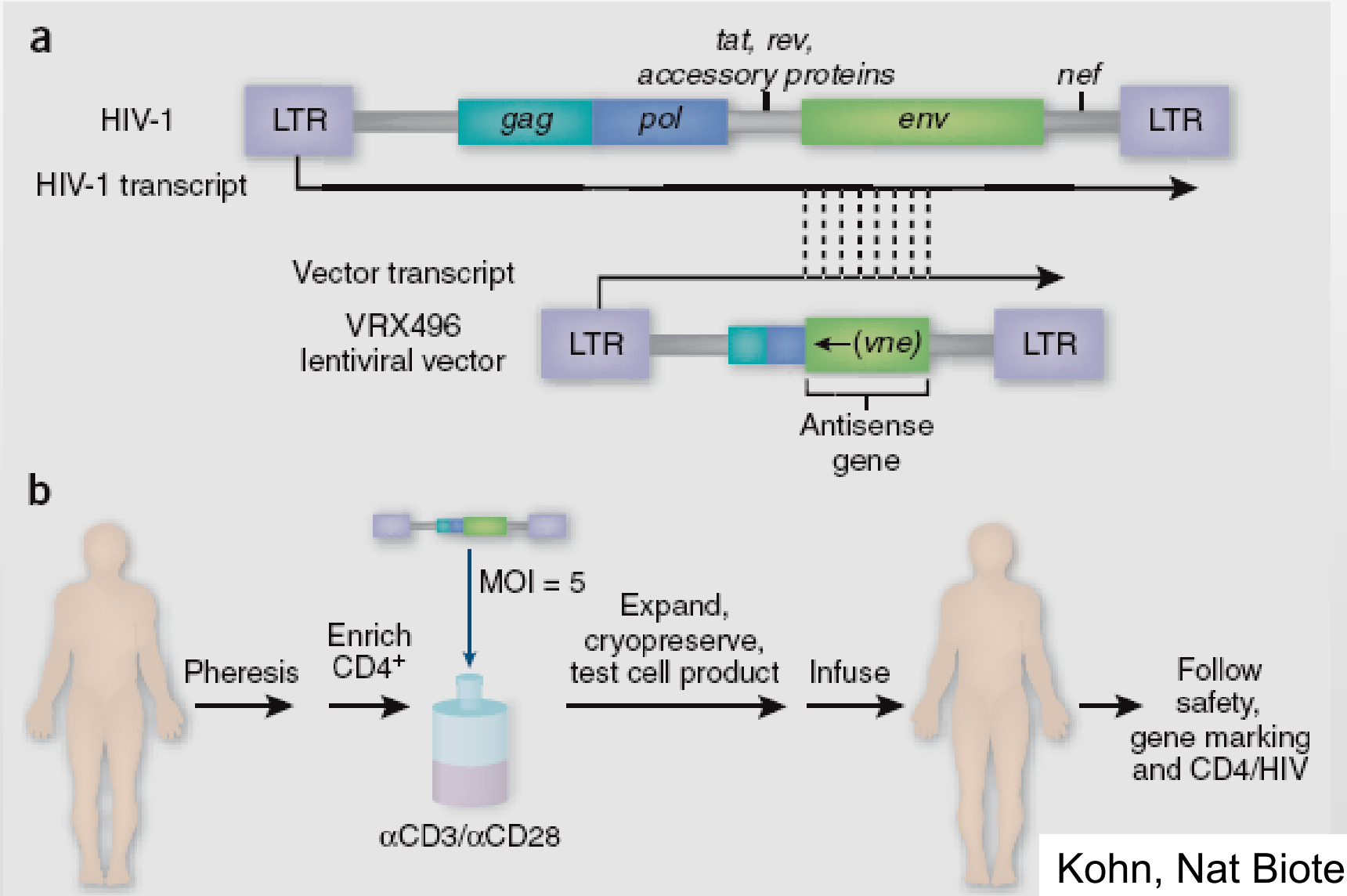
high efficiency transduction

long term expression ⇒ less susceptible to silencing

not yet tested in humans



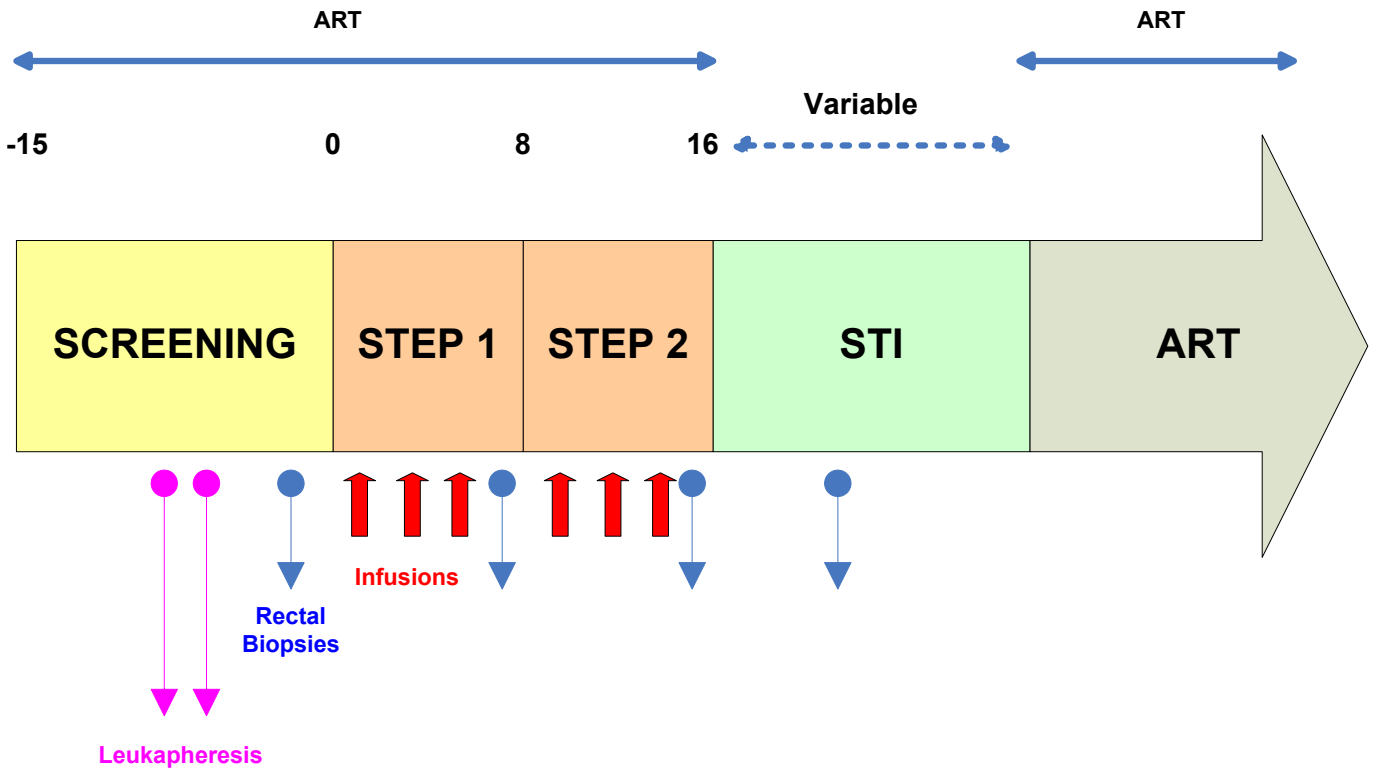
First in Humans Test of Lentiviral Vectors



Kohn, Nat Biotech, 2007

Levine et al. PNAS 2006

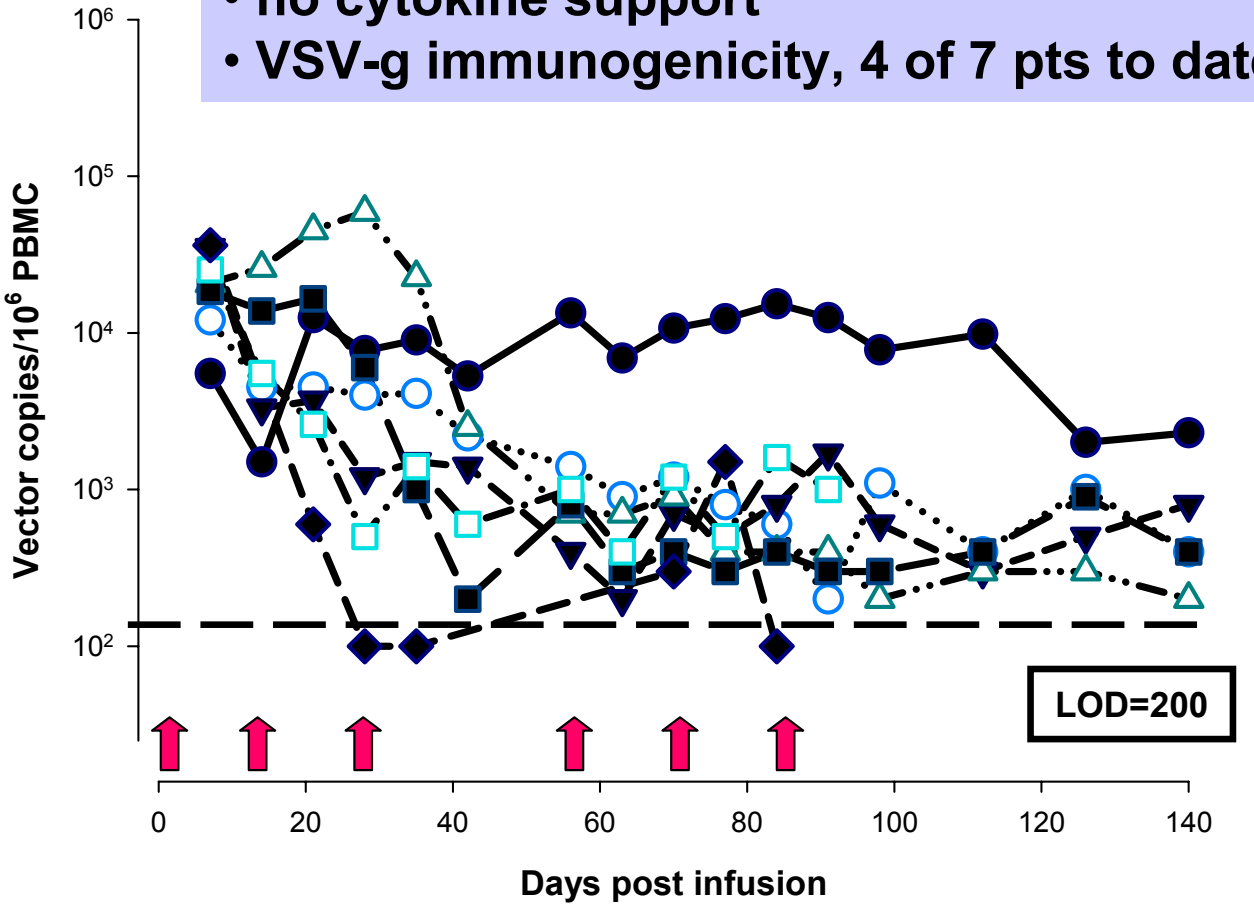
Penn/ViRxSys Multi-dose STI Study (Protocol #802456)

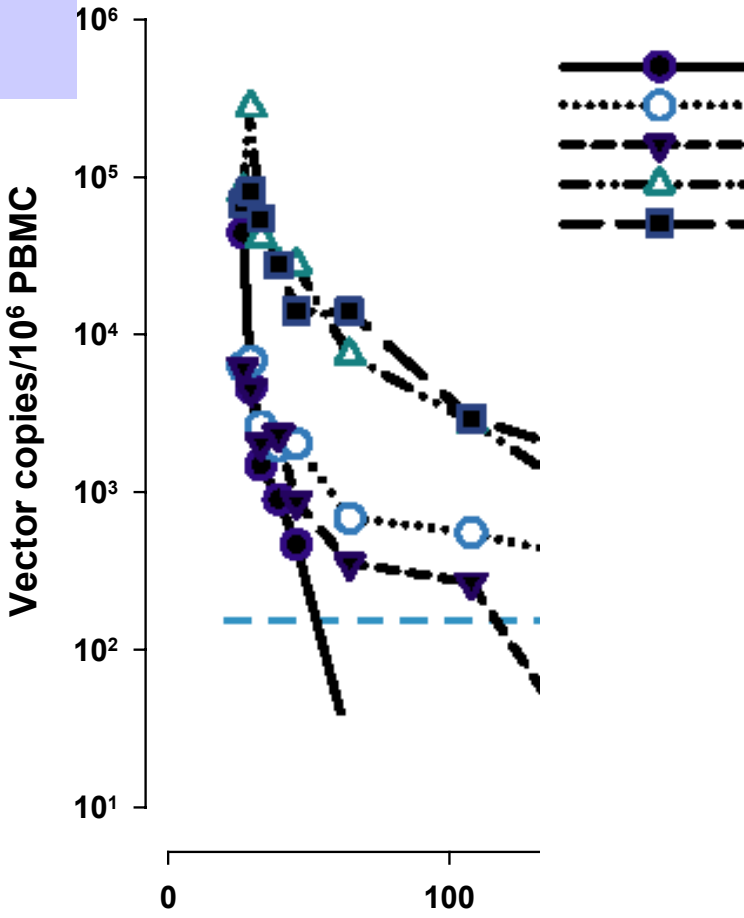


- Objectives: determine safety and trafficking of multiple infusions of CD4 T cells transduced with anti-sense HIV
- Status
  - study opened in August 2006
  - 11 patients enrolled and 9 patients infused

Lentivector Persistence in CD4 Cells

- long term persistence of non-selecting vector
- no cytokine support
- VSV-g immunogenicity, 4 of 7 pts to date

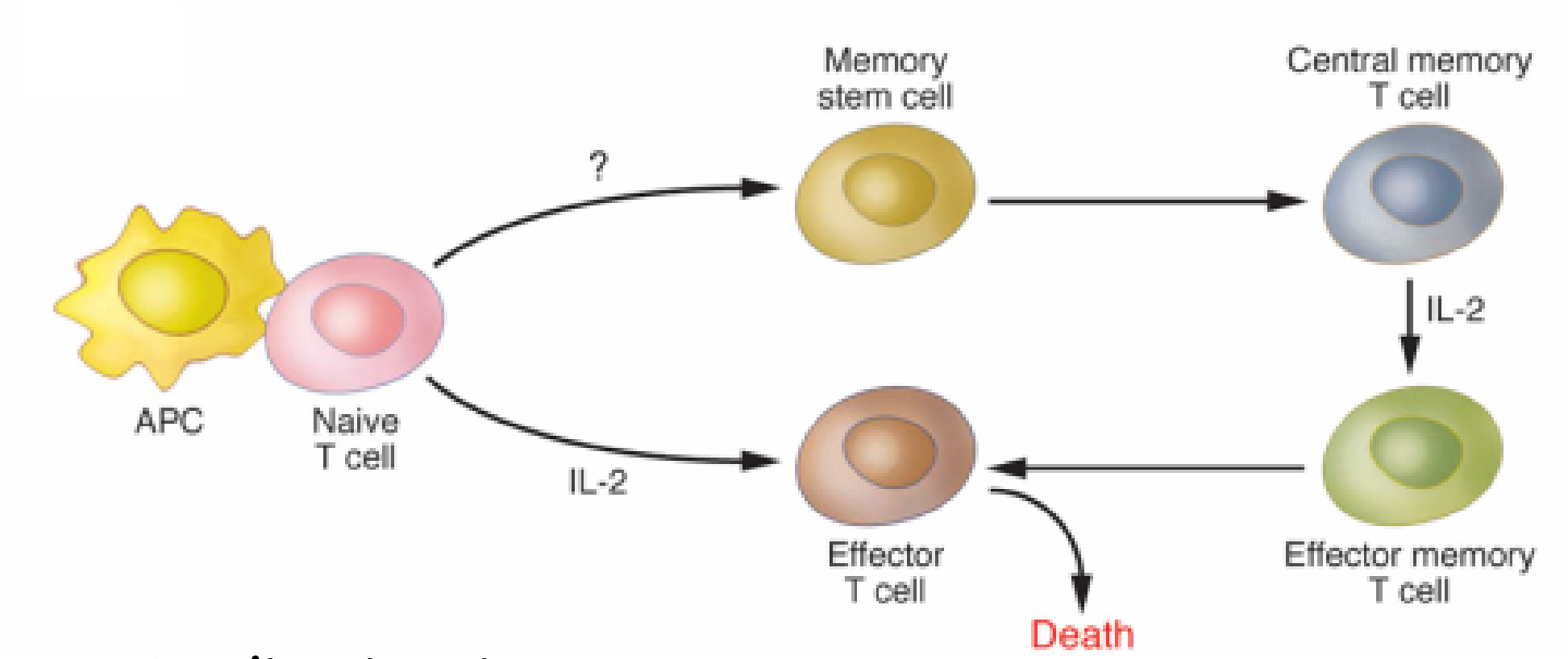




Multiple infusions

Single infusion:
T ½ =23.5 +/- 7.7 days

Memory Stem Cell Hypothesis



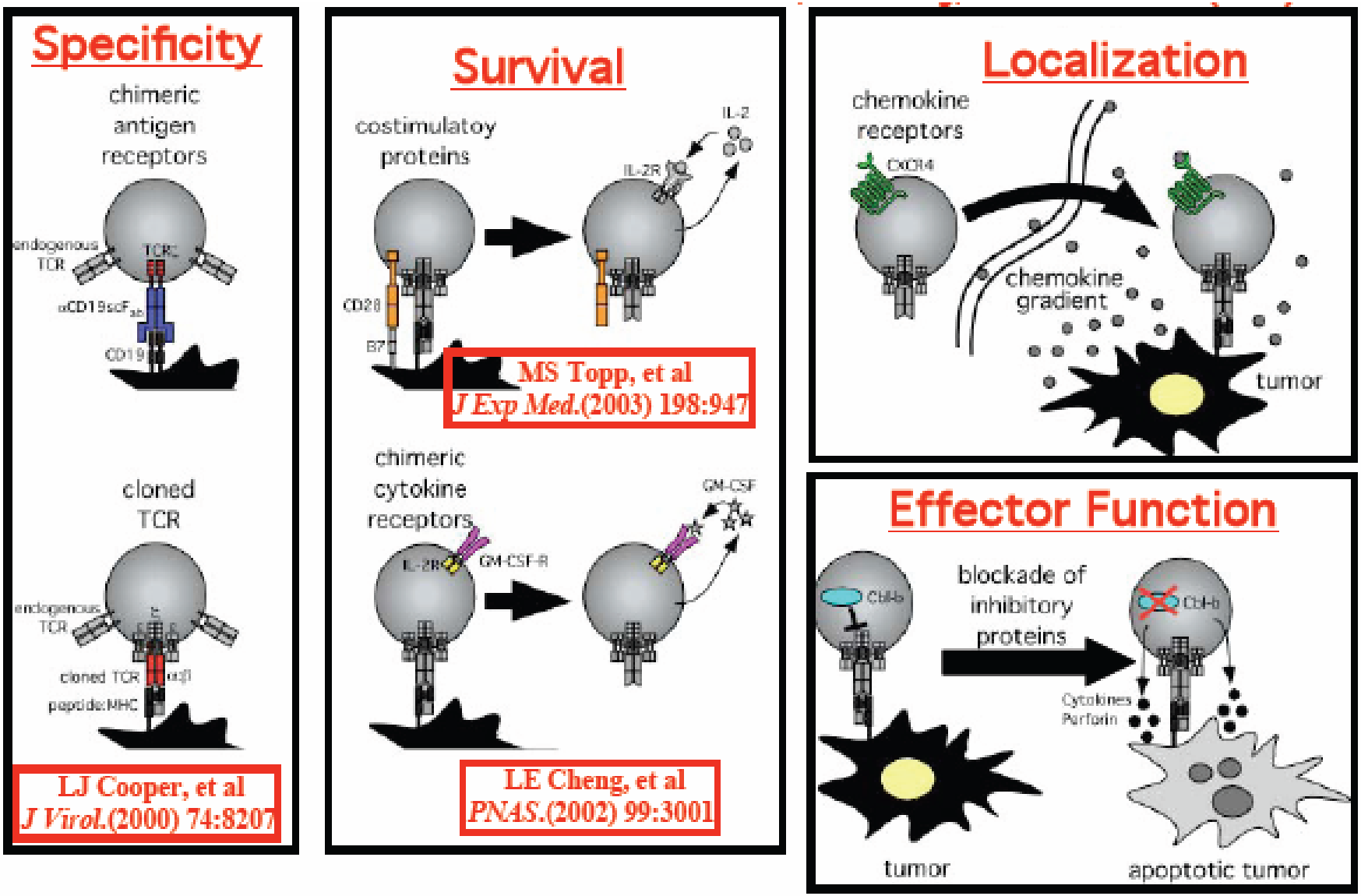
- **Studies in mice:**
  Fearon, *Science*. 293: 248
  Zhang, *Nat Med*. 11: 1299
- **Implications for human T cell gene therapy**

Lessons from the First Lentiviral Gene Transfer Trial - HIV

- HIV based vectors appear safe in 13 of 13 patients treated to date
- Promising engraftment with genetically engineered CD4 T cells
- No evidence for insertional oncogenesis
- HIV based vectors have promise for HIV and cancer therapy

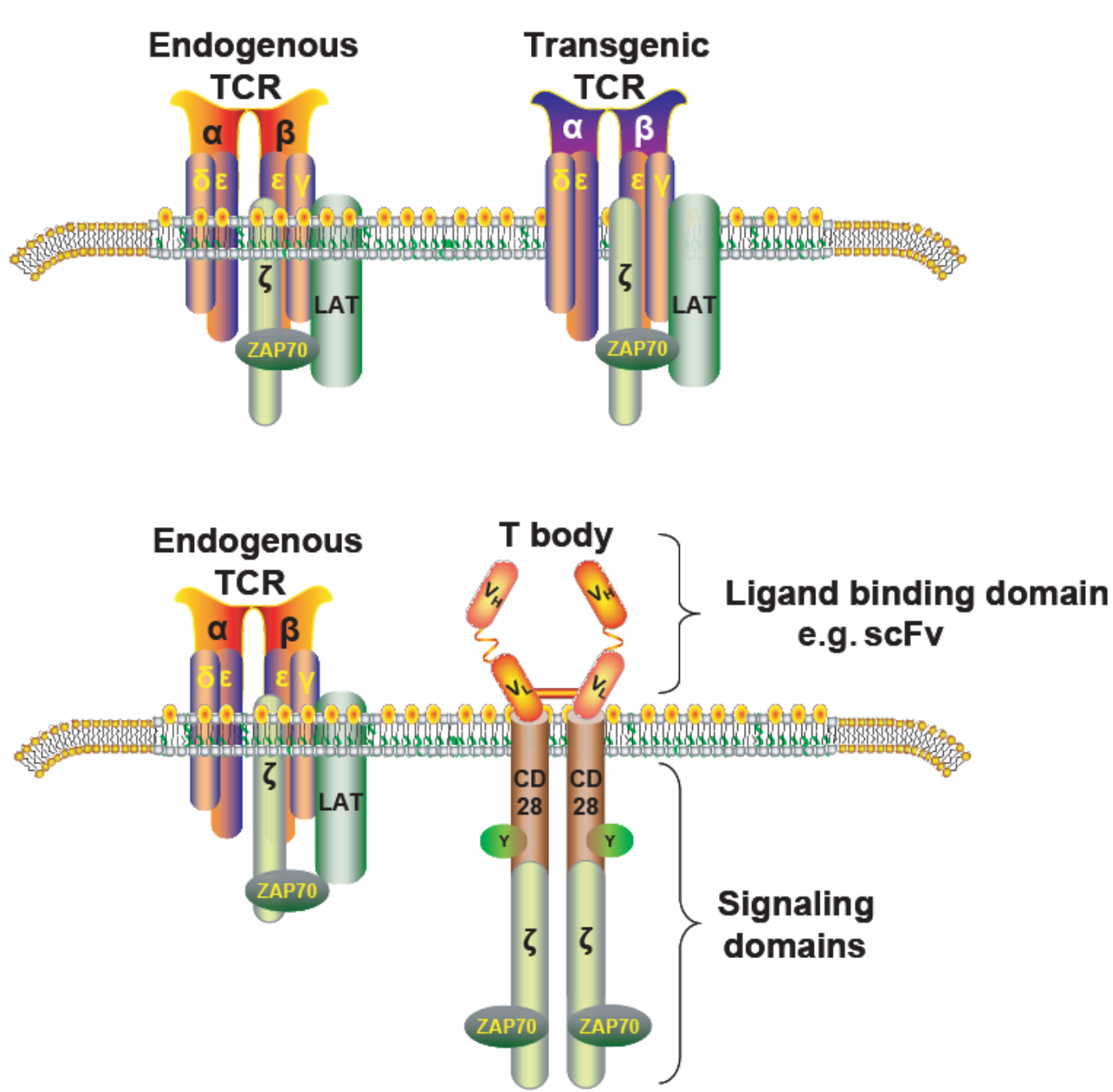
Levine et al. PNAS 2006

Strategies to Improve Adoptive Transfer of Tumor Specific T Cells Using Genetic Modification

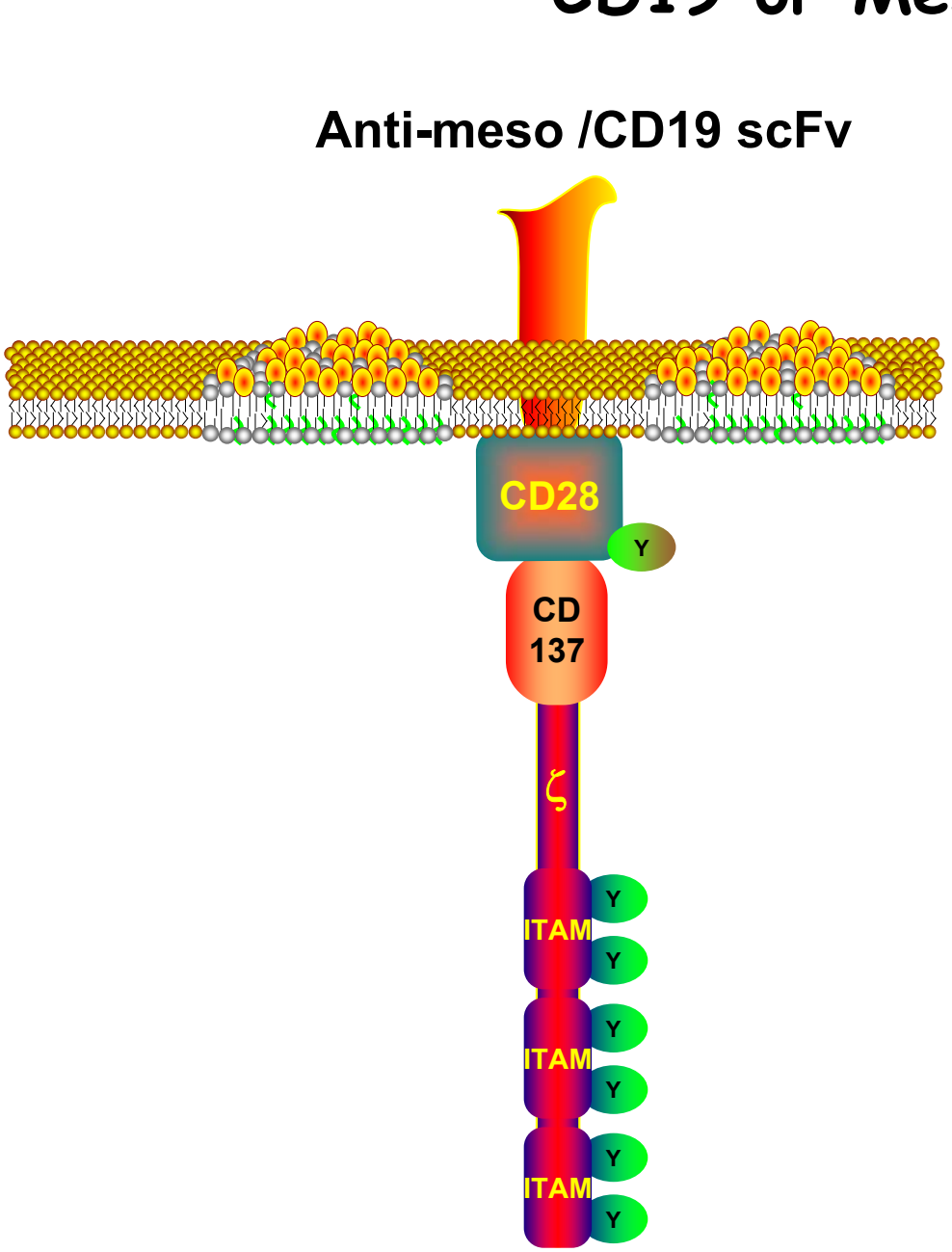


Ho, Greenberg et al, Cancer Cell (2003)

Redirected T Cell Approaches with engineered T cells



Lentiviral Redirected T Cells Targeting CD19 or Mesothelin



Carmine Carpenito
Michael Milone

Mesothelin as a tumor target for EOC

- **40 kDa gpi linked surface glycoprotein that is widely expressed in EOC, mesothelioma and pancreatic cancers (Hassan and Pastan, Clin Can Res 2004)**
  - Restricted expression in normal cells
- **Mesothelin cell biology**
  - Unique transcripts in serum of EOC patients (Scholler et al, PNAS 1999)
  - Mesothelin binds to Muc16/ CA125 (Rump et al, JBC 2004)
- **Mesothelin subject to immunosurveillance**
  - can be recognized by HLA class I restricted CD8 T cells (Thomas et al, JEM 2004)
  - 40% of patients with EOC have antibodies to mesothelin (Ho et al, Clin Cancer Res 2005; 129:515)

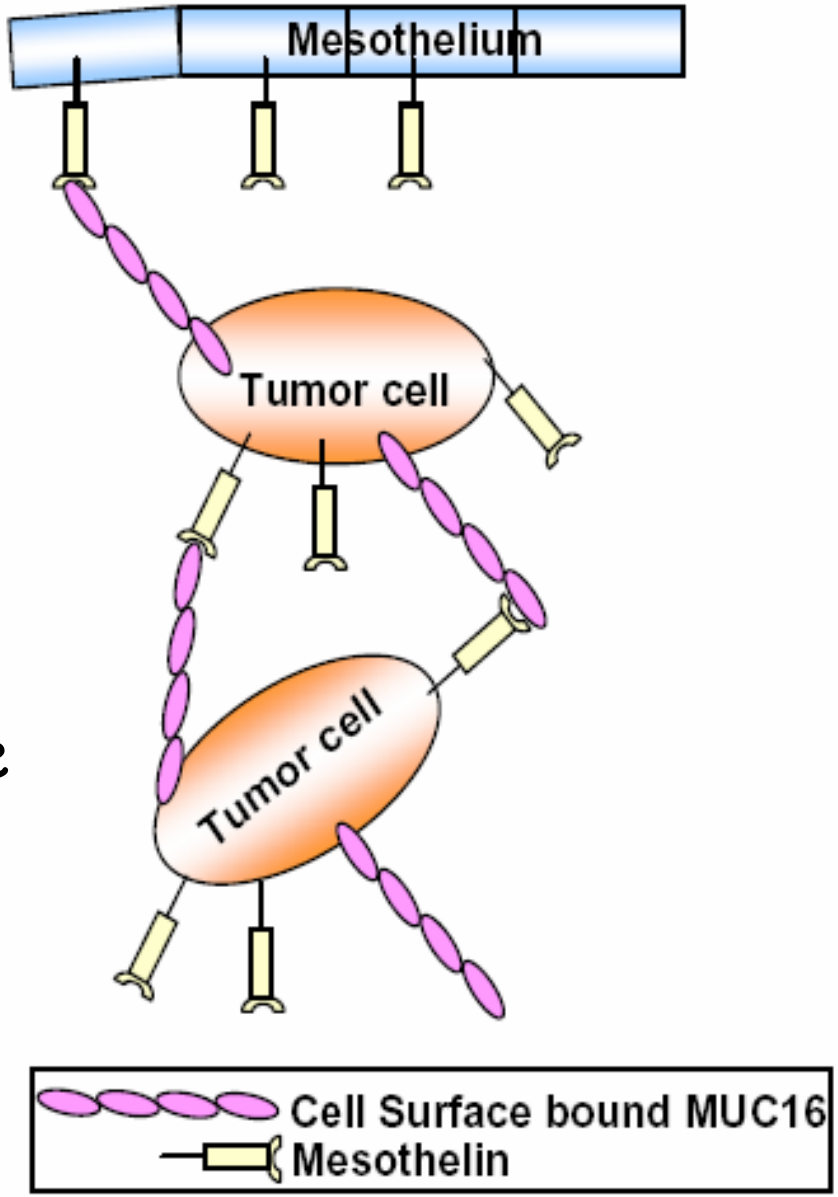
Mesothelin as a Target for Adoptive Transfer Therapy

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

Mesothelin cell biology

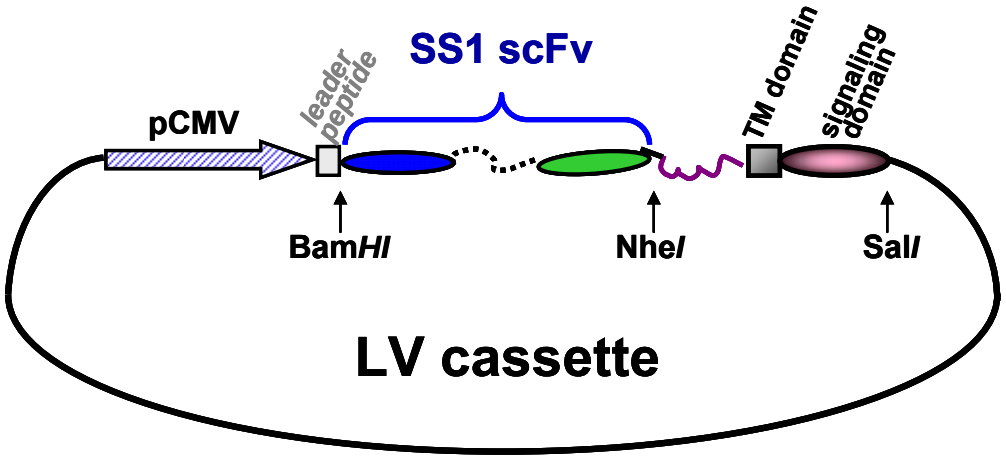
meso$^{-/-}$ mice are healthy and fertile

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

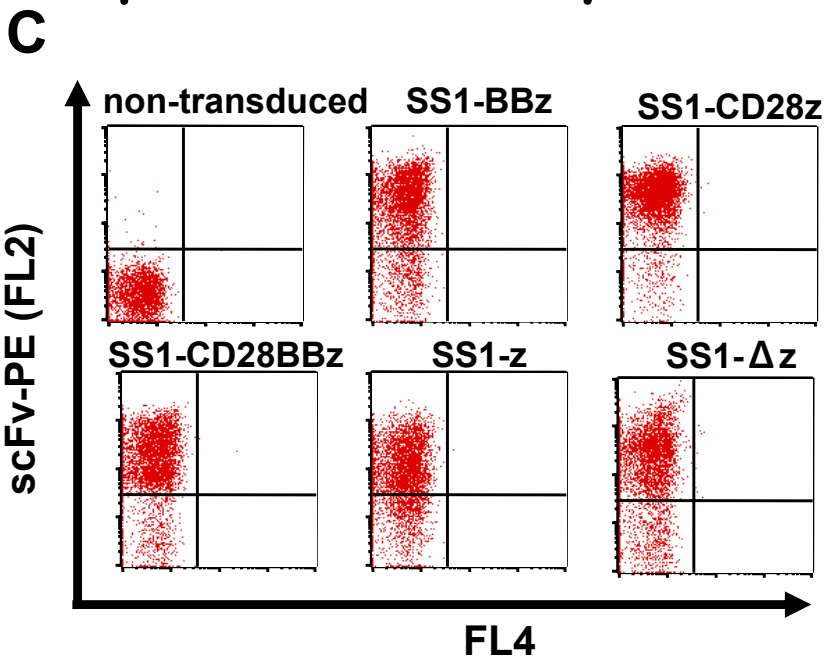


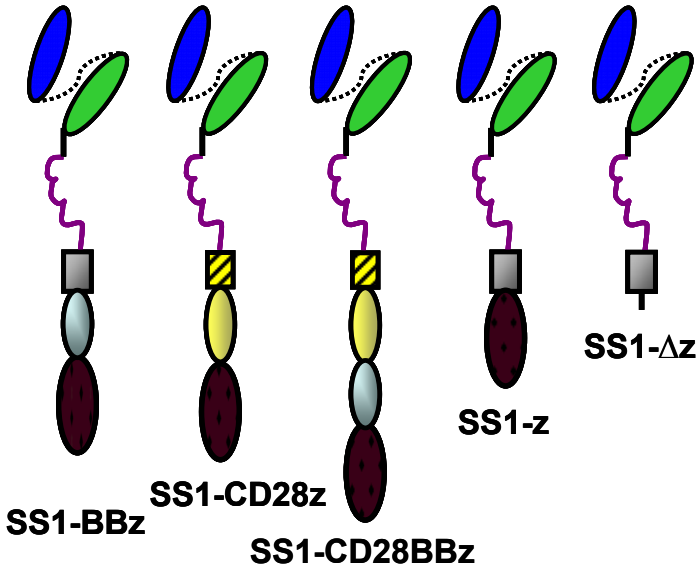
Gubbels *et al*
Mol Cancer 5(1):50

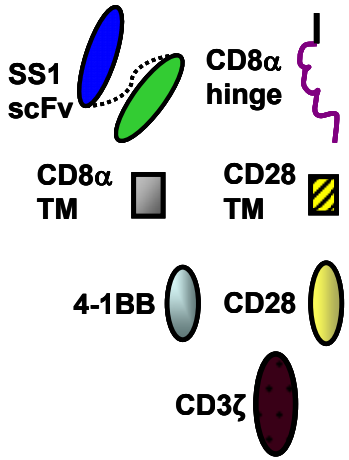
Lentiviral Redirected T Cells Targeting Mesothelin



Expression: Primary T Cells







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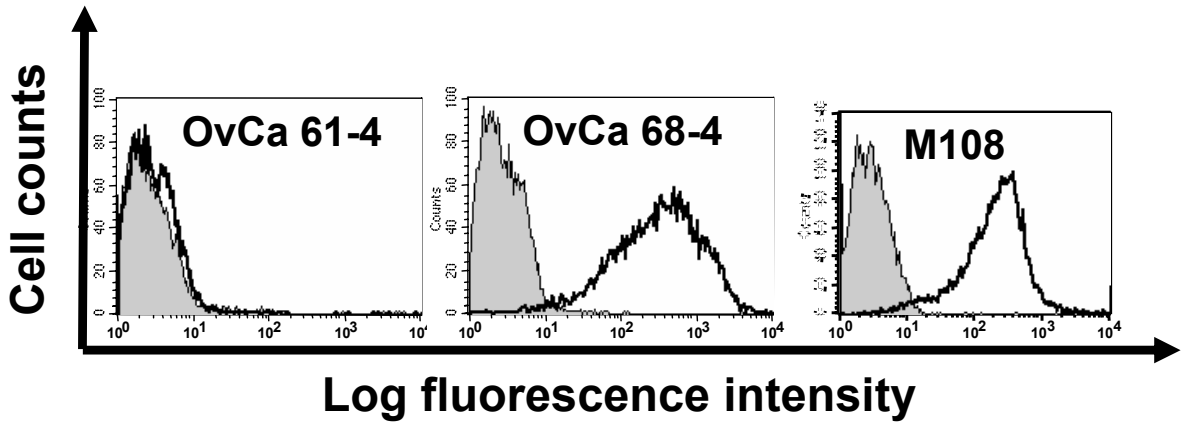
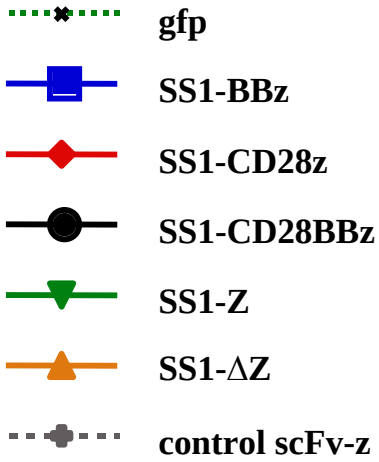
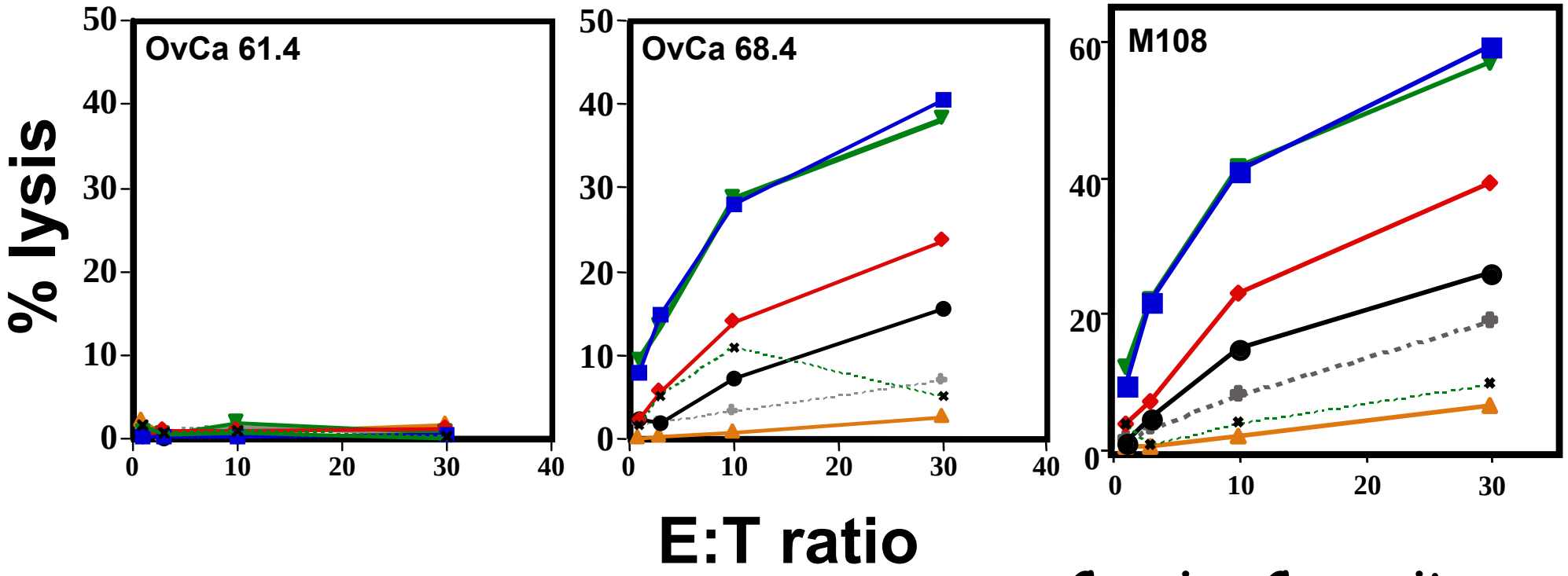
Mesothelin Redirected T Cells Kill Primary Ovarian and Mesothelioma Cells In Vitro







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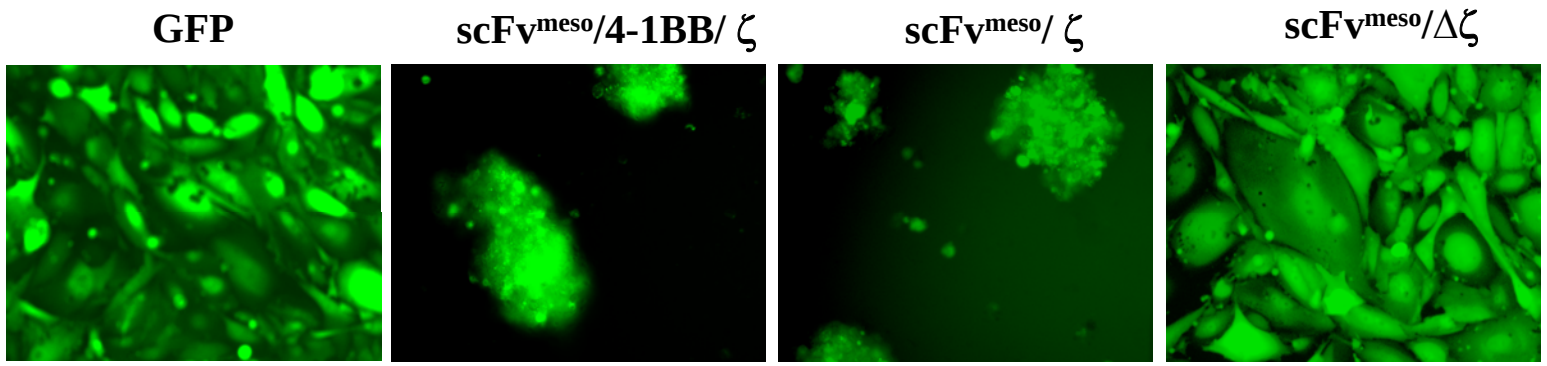
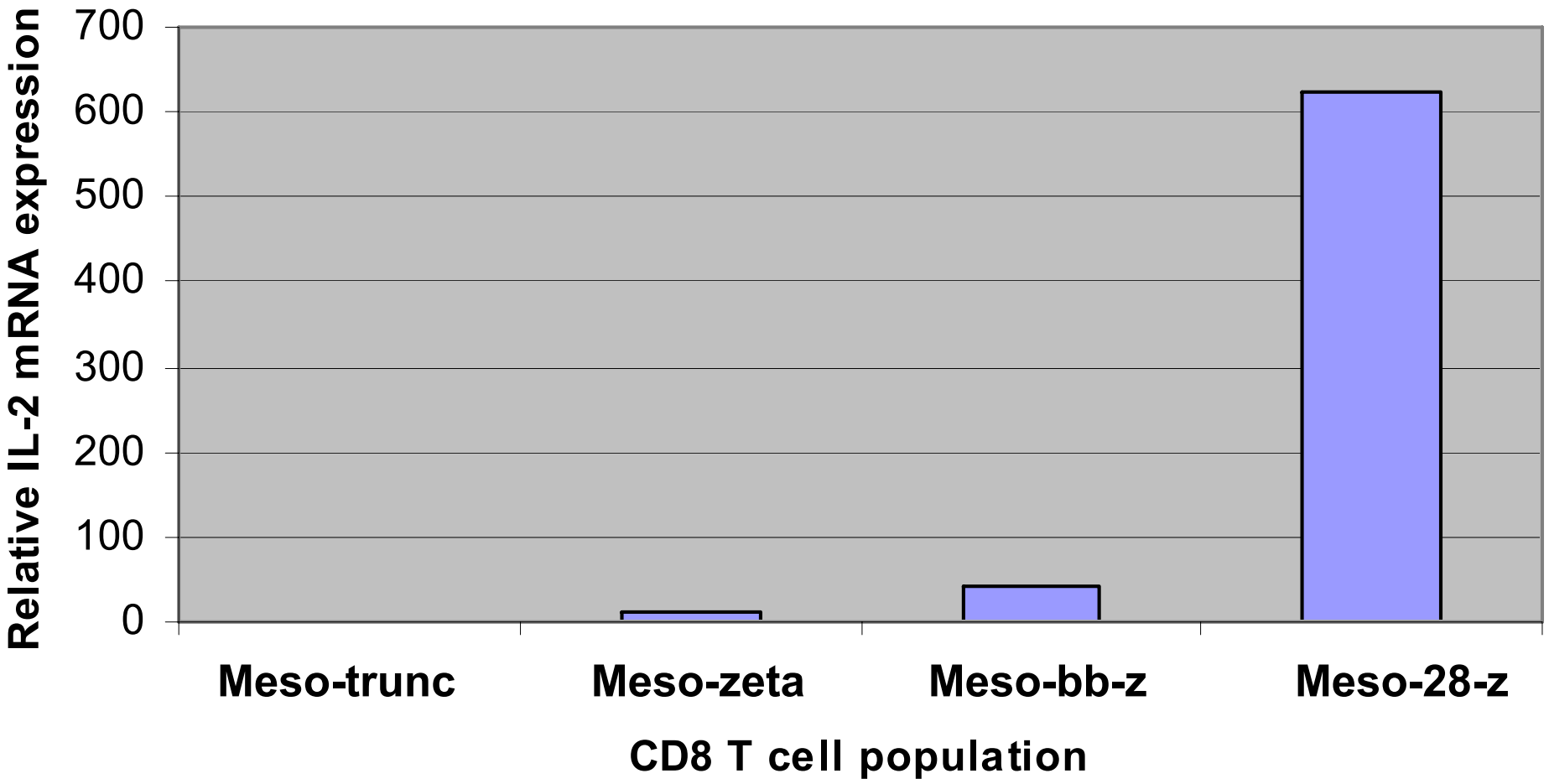
CD8 T cells expressing scFv^{meso} kill pat108.gfp cells at low E:T ratios

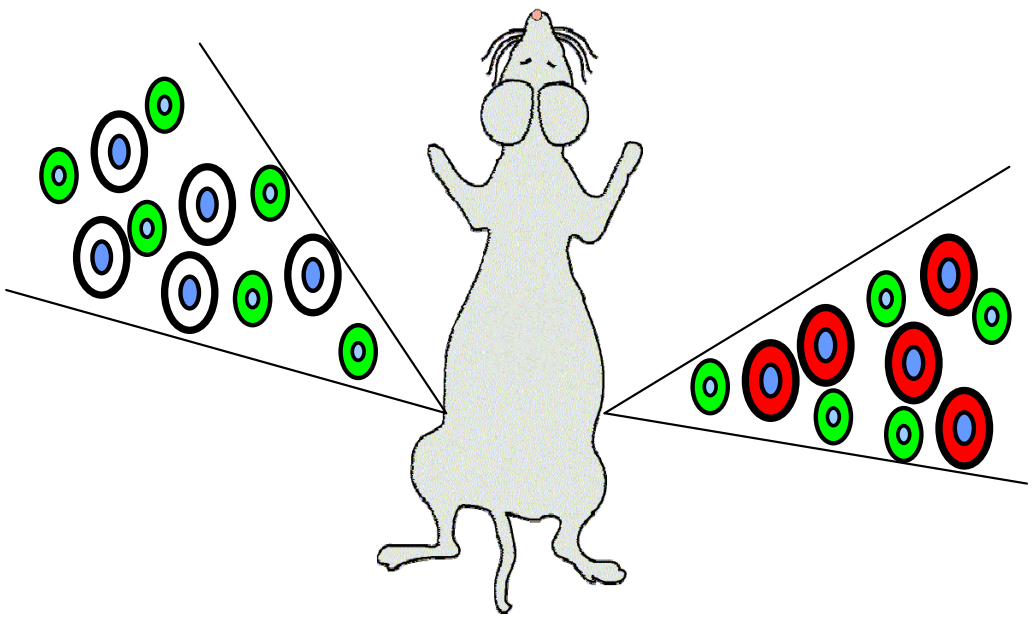


Day 0: 1 T cell added per 20 tumor cells
Day 2: Photograph

Co-stimulatory Domains Induce Cytokine Secretion in CD8 T Cells in Response to Mesothelin



SCID-Winn Assays: Summary



T cell

A431

A431.meso

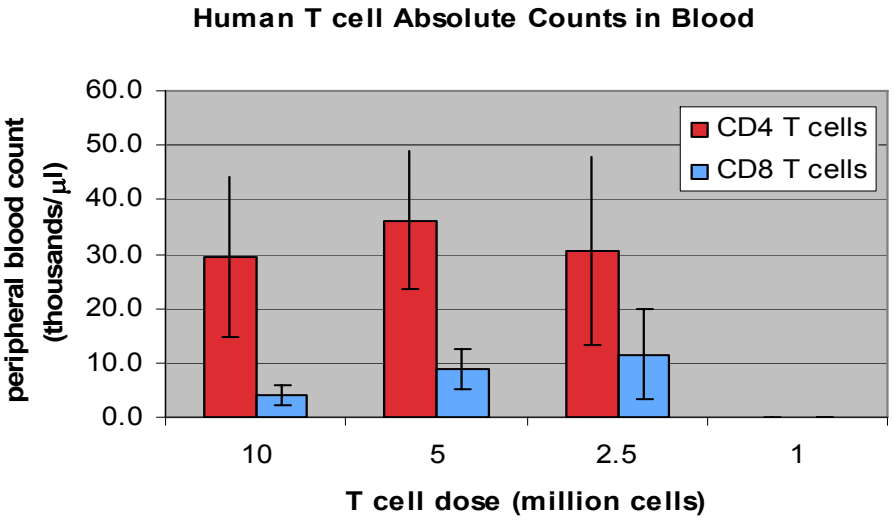
combine 1 million A431 or A431.meso cells with varying numbers of T:scFv^{meso}Zeta and inject into opposite flanks of Rag2 γ -/- mice

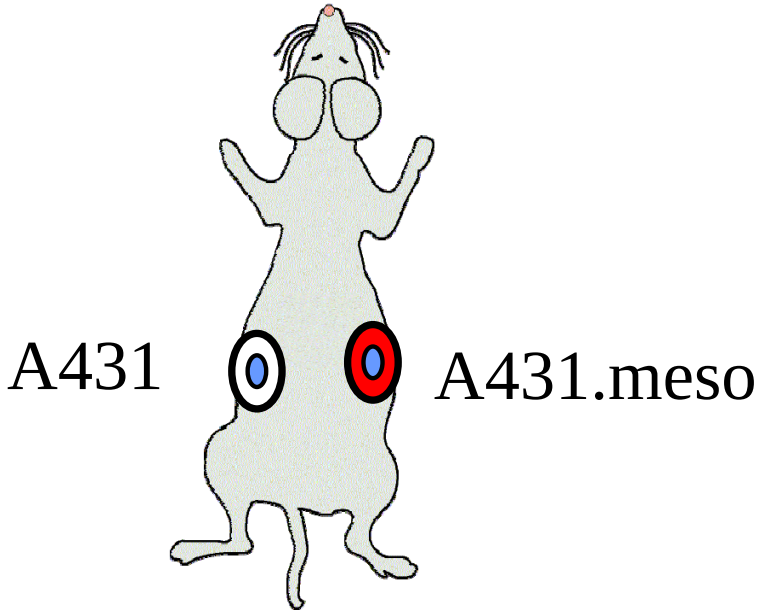
T cells expressing scFv^{meso} are able to kill mesothelin^{+} tumor cells

T cells expressing scFv^{CD19} do not kill mesothelin^{+} tumor cells

at E:T ratio (1:2), redirected T cells inhibit A431.meso tumor cells (serial killing)

NOD-SCID-β2$^{-/-}$ Mice
Human T Cells and Tumor Xenografts

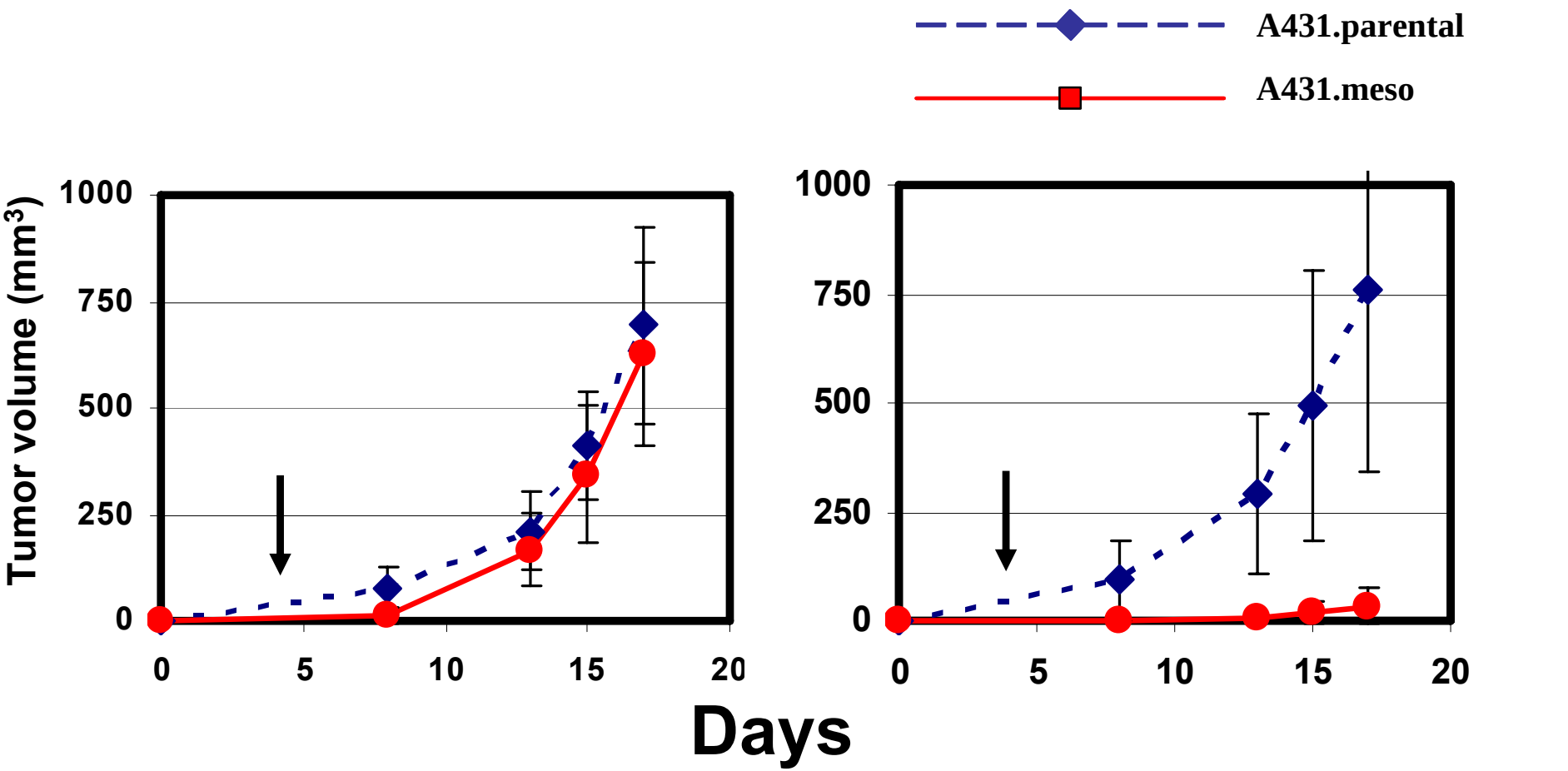




day 0 : inject A431 tumor cells s.c.

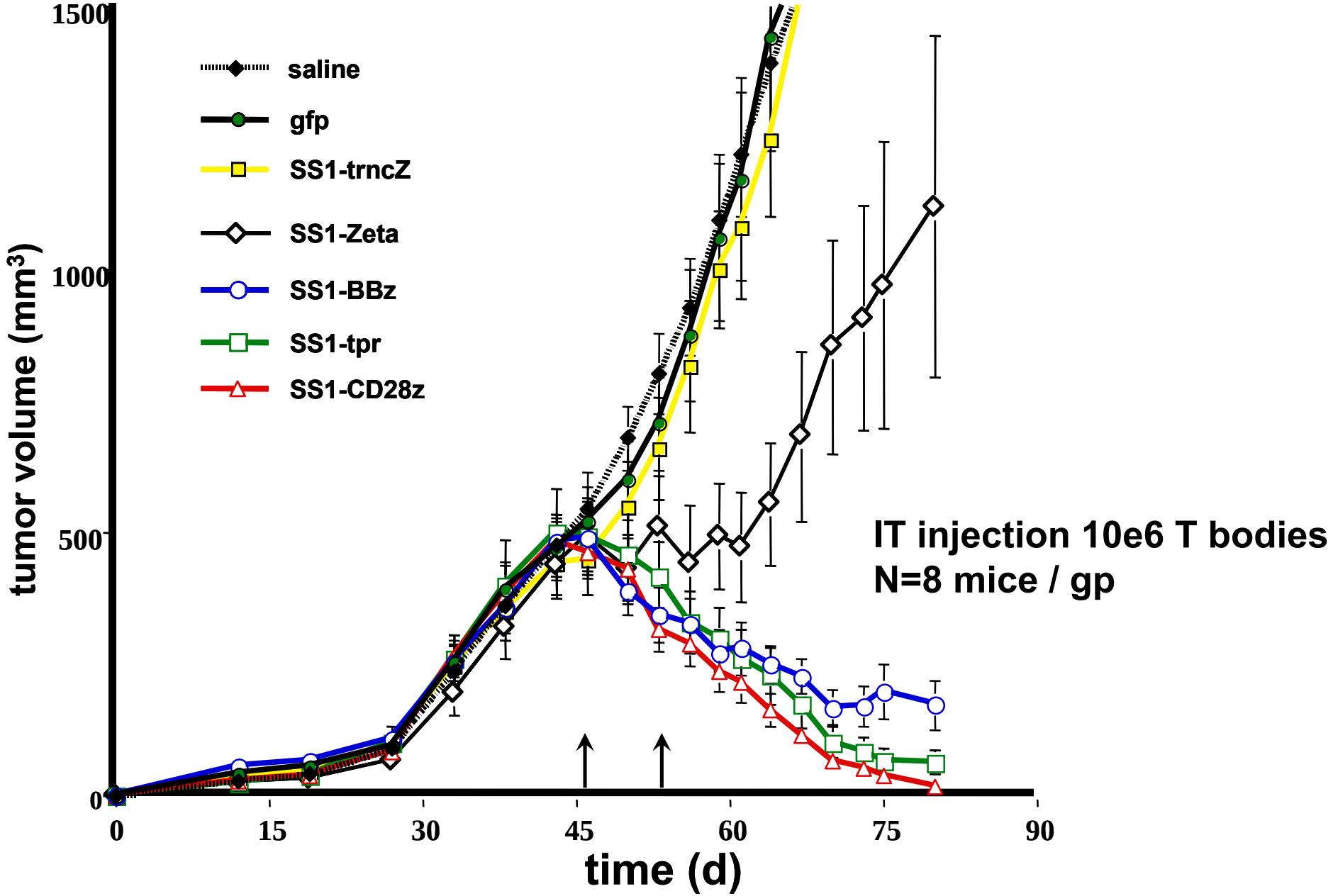
day 4 : inject scFv^{meso} T cells i.v.

Specific Killing of Mesothelin Tumor in NOD-SCID-β2$^{-/-}$ Mice: Day 4 Challenge



5 mice / gp
1x10^6 A431 cells
0.5x10^6 T cells day 4

Meso Redirected T Cells: In Vivo Killing of Large Established Primary Mesothelioma Xenografts: Day 45 Challenge

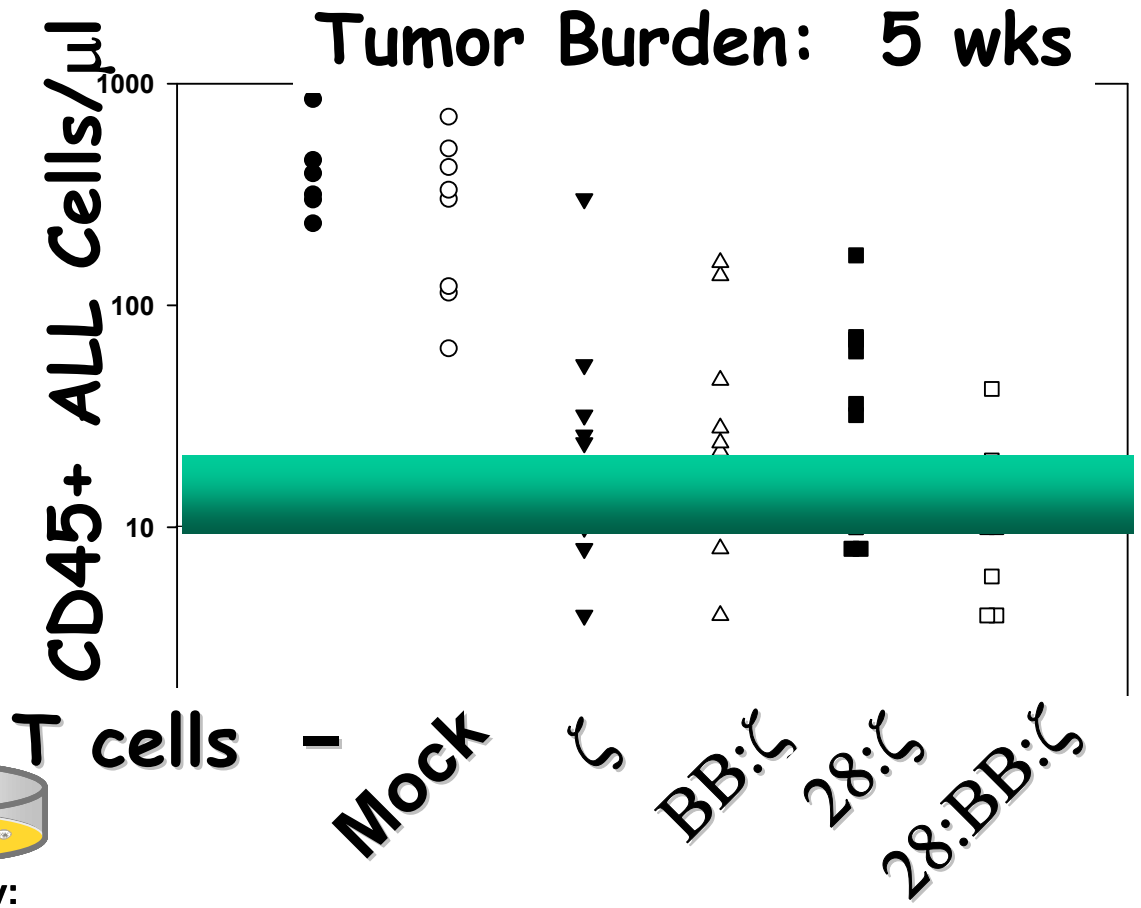


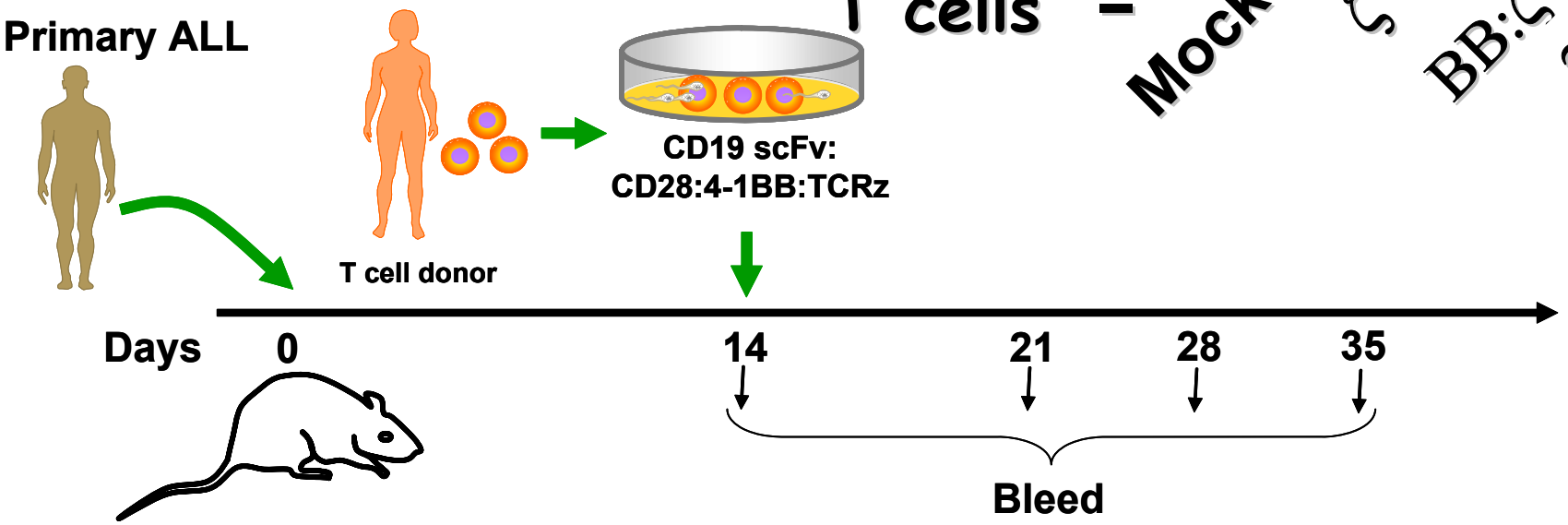
Summary - II

- Lentiviral vectors provide an efficient means to engineer human T cells with artificial antigen receptors
- CD8+ T cells armed with mesothelin-specific T-bodies efficiently lyse mesothelin + tumor cells including primary tumor cells
- T-bodies can trigger T cell proliferation
- Addition of co-stimulatory signal transduction domains to TCR-ζ containing T bodies enhance cytokine production
- Human T cells engineered with a minimal anti-meso T-body can suppress the development of tumors in a NOD-SCID-$\beta 2^{-/-}$ model of ovarian cancer
- anti-Meso T cells eradicate vascularized (45 days) xenografts in humanized NOG mice

Efficacy of anti-CD19 Lentiviral Redirected T Cells Day 14 Challenge Model w Primary Leukemia Xenografts

- $3x10^6$ gene-modified T cells/mouse
- ALL and T cell enumeration in blood performed by BD TruCount





UPenn Protocol #805313: Competitive Repopulation Strategy to Test Signaling Domains in Redirected Autologous T Cells

Leukapheresis: patient with CD19+ leukemia or lymphoma

CD3 enrichment, parallel T body transduction and expansion

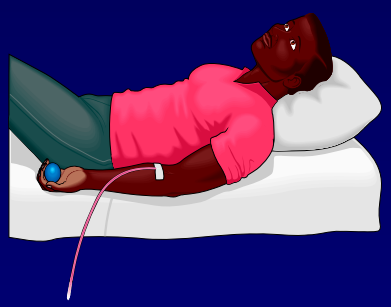
CD19-CD3ζ-4-1BB

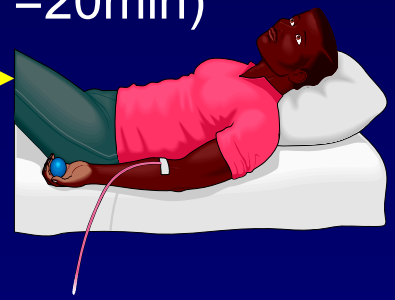
CD19-CD3ζ

Normalize RAT-19 cell numbers

Combine RAT-19 cells during infusion

Baseline (T=20min)

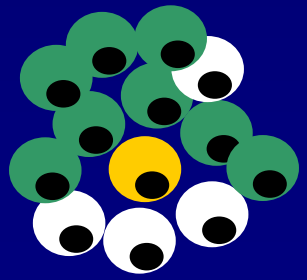




T cell genotype

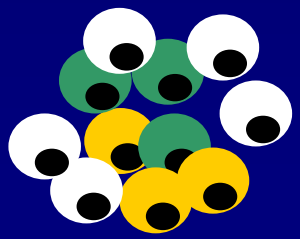
- Wild type
- RAT-19 CD3ζ-4-1BB
- RAT-19 CD3ζ

Outcomes (T=28 days)



CD19-CD3ζ-4-1BB better

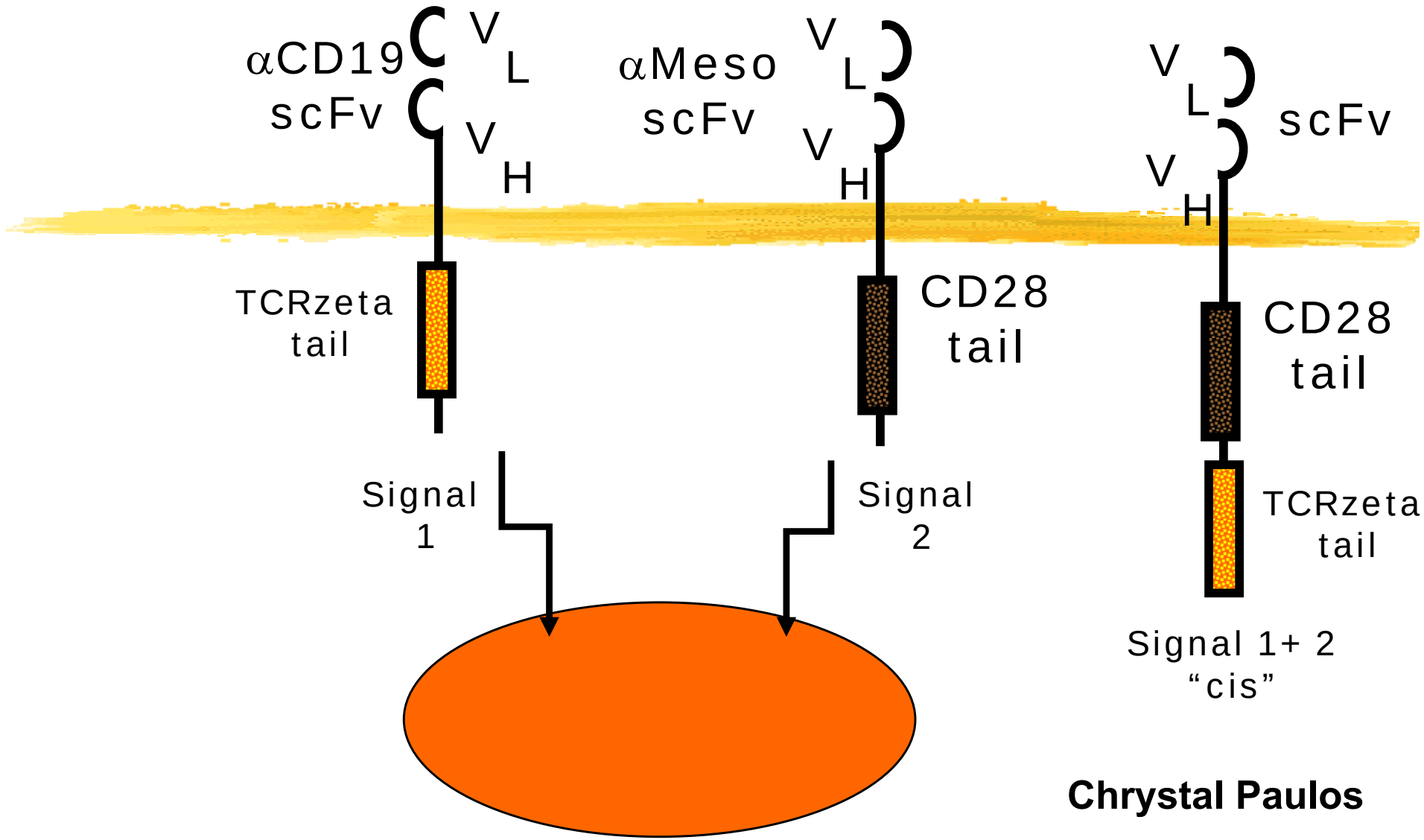
OR



No difference

Monitor expansion and persistence (blood) and trafficking (bone marrow)

Conditionally Retargeting T bodies using "Cis" and "Trans" Costimulatory Domains



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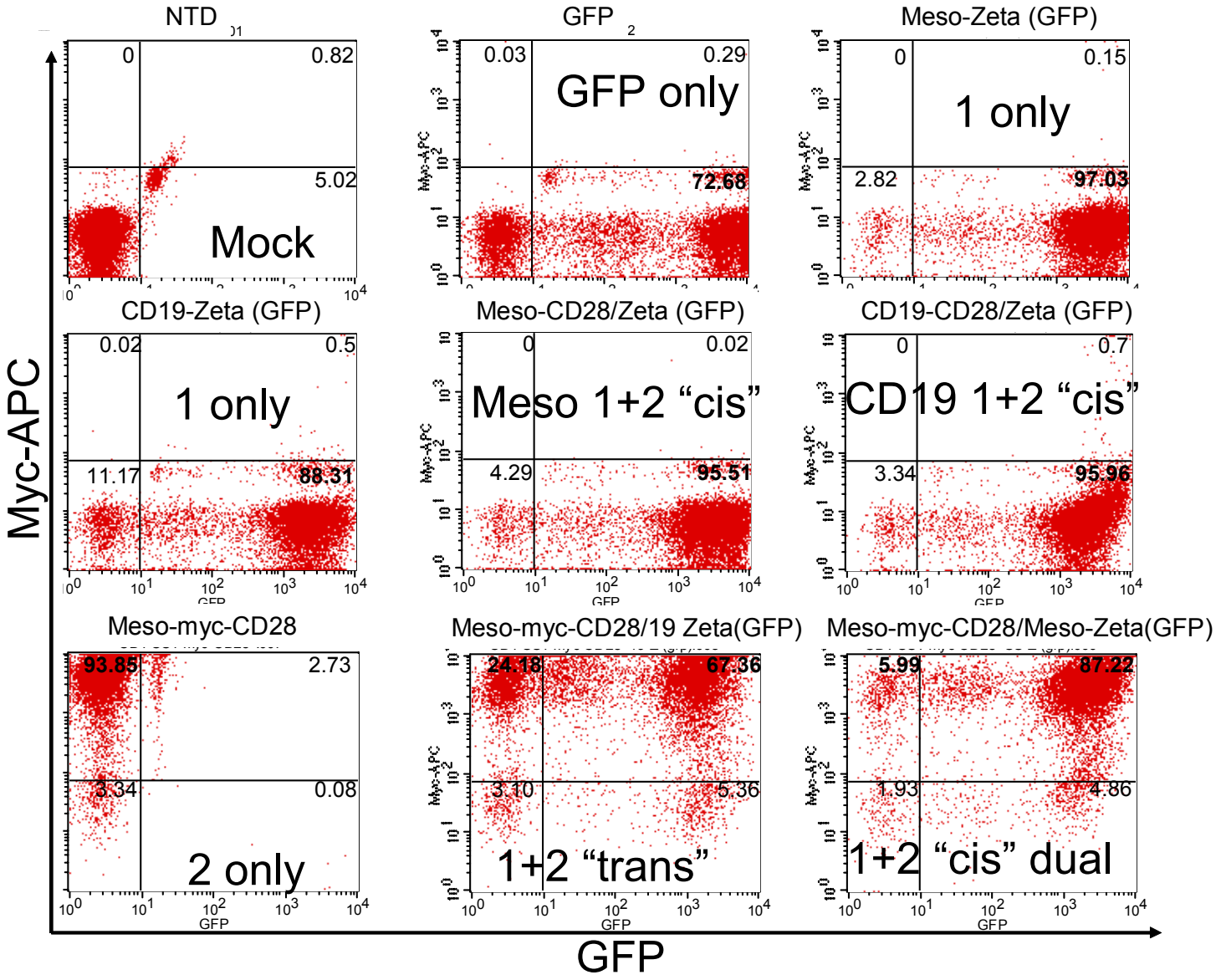
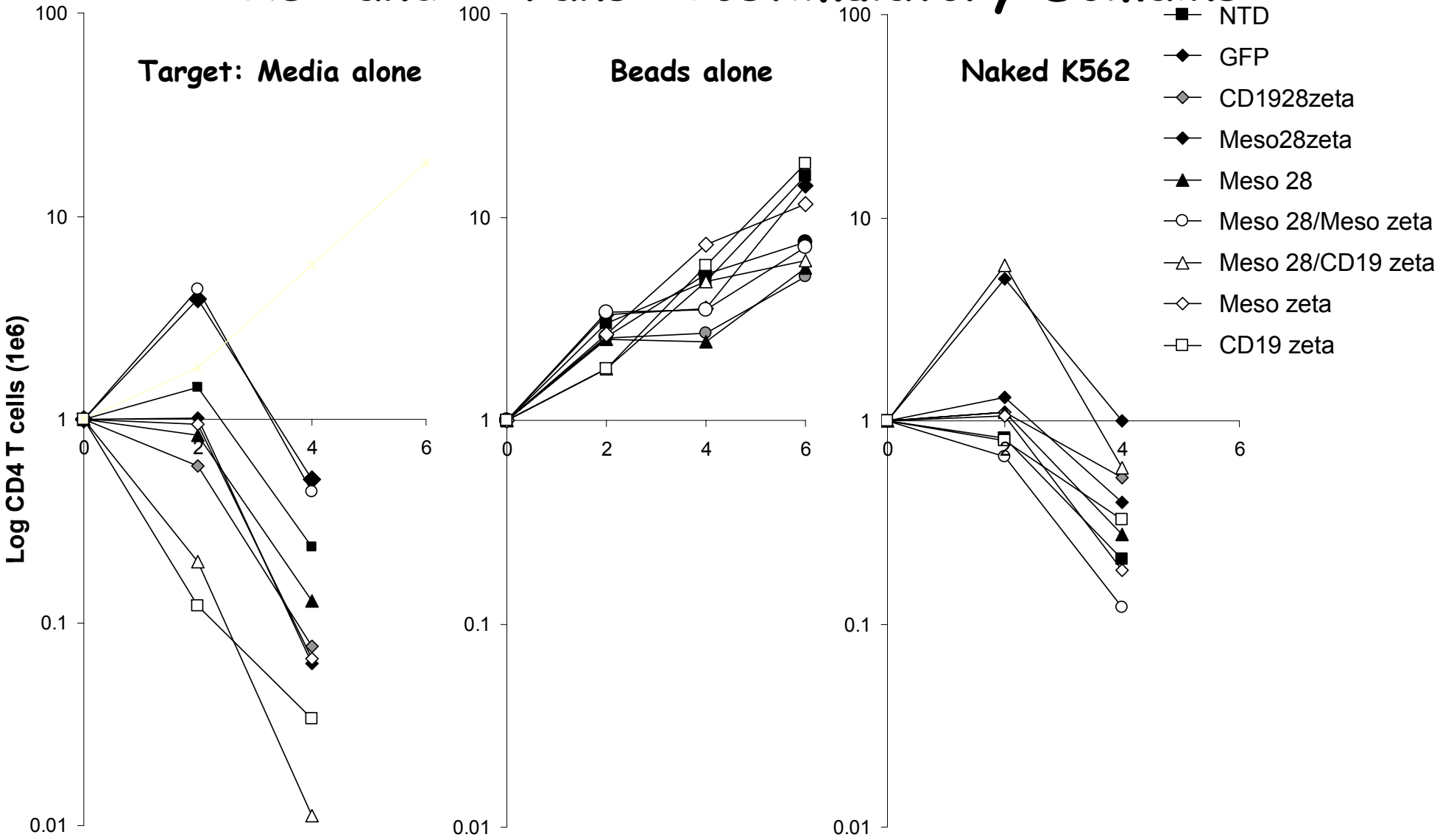
High transduction efficiency can be achieved in human CD4 T cells using lentiviral vector CD28 and TCR Zeta constructs



Single and double transduction constructs: surface expression 67-97% efficacy

Retention of TCR induced proliferation, and absence of autonomous proliferation Directed by "Cis" and "Trans" Costimulatory Domains



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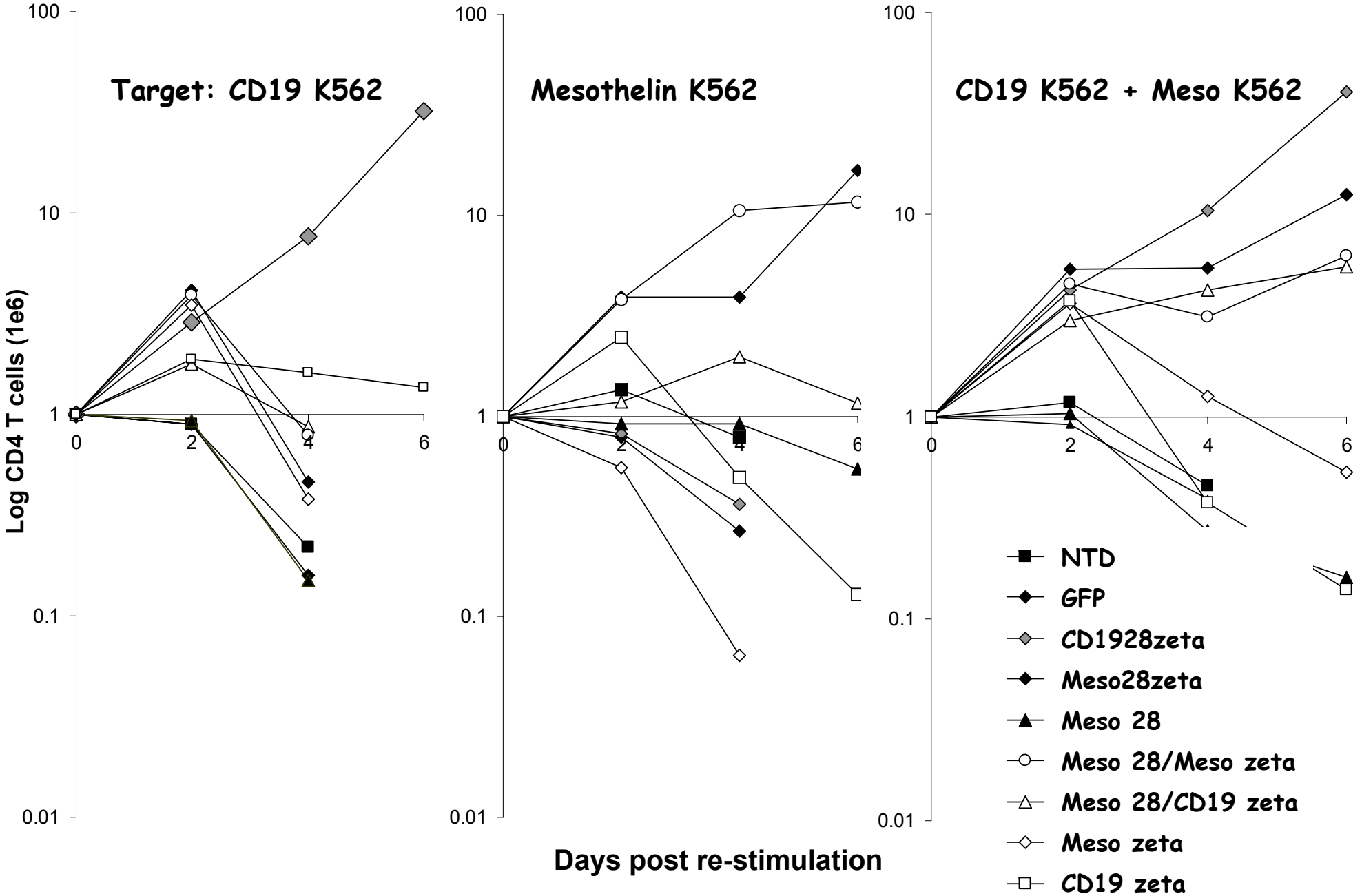
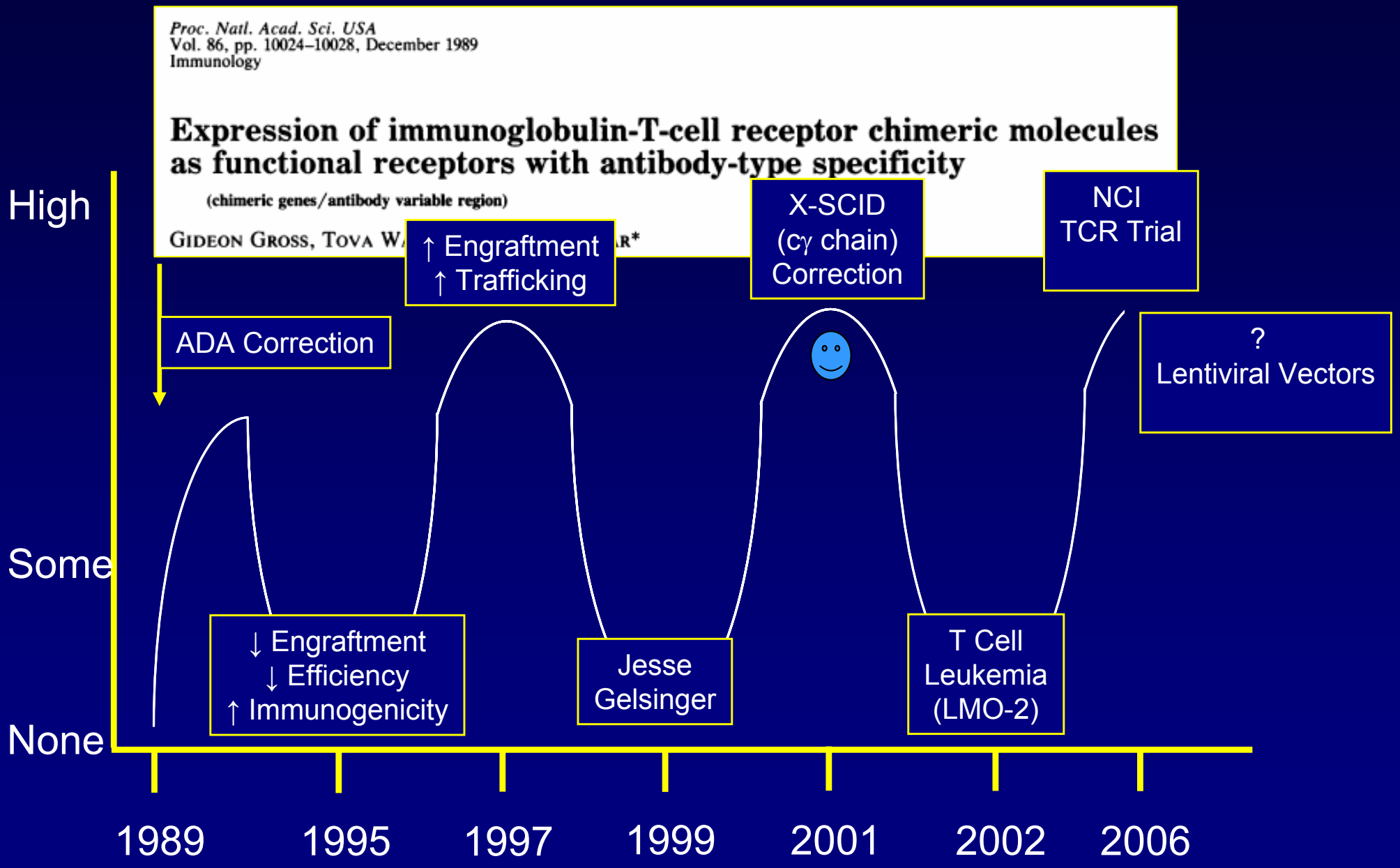
Selective Proliferation Directed by "Cis" and "Trans" Costimulatory Domains



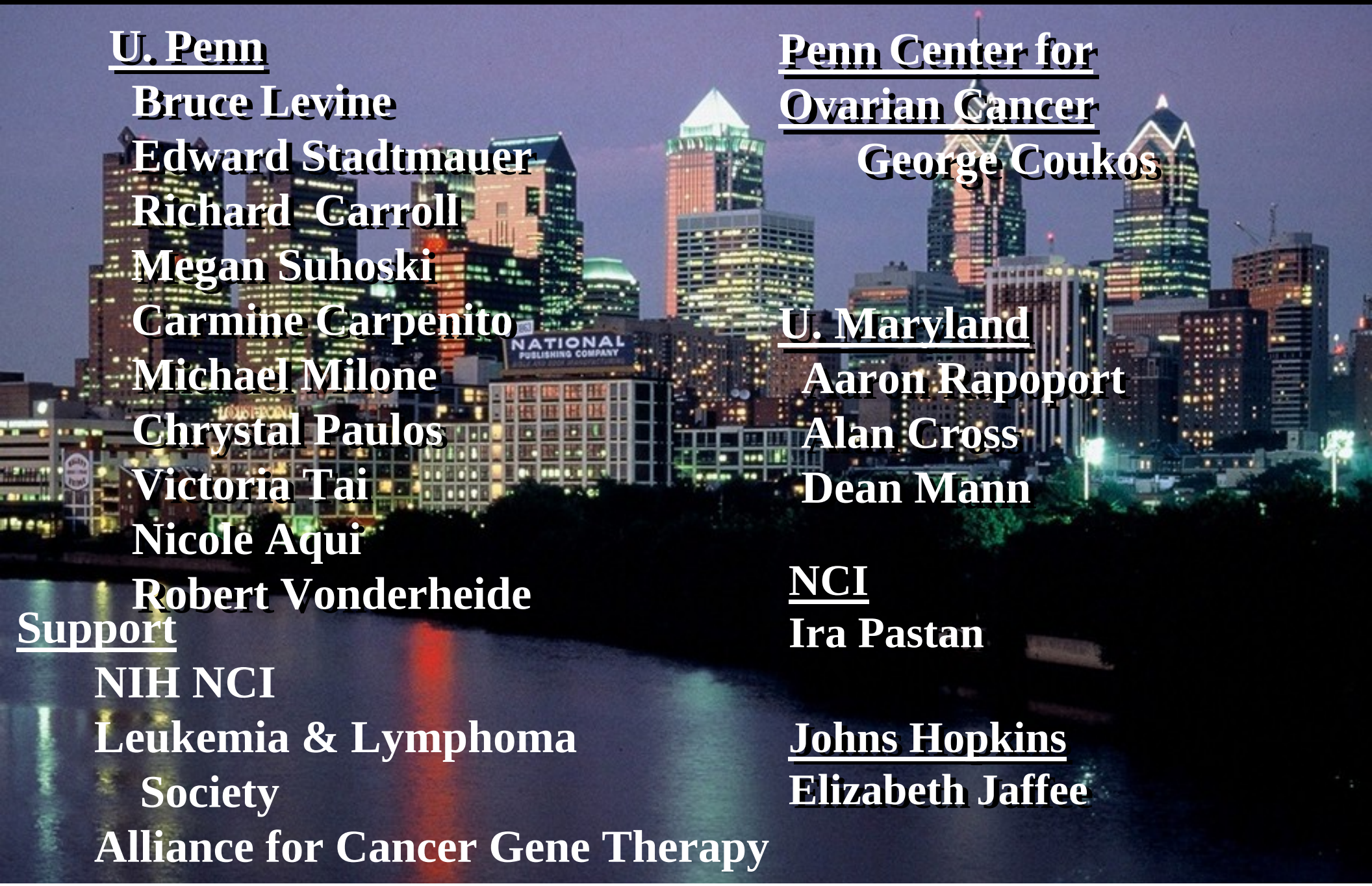
Towards Personalized Medicine: T Cell Adoptive Transfer Immunotherapies

- T cells have a number of properties to be the elusive "weapon of mass destruction" for cancer and chronic infections
  - Targeting/trafficking to tumor and sites of infection demonstrated
  - Long term persistence and stem cell like qualities of central memory T cells
  - Strategies to enhance function of T cells by genetic engineering
- Barriers to widespread utilization
  - Efficient T cell culture systems
  - Efficient T cell engineering

Enthusiasm for T Cell Gene Therapy



Collaborators and Acknowledgements



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