

# Adoptive transfer strategies: impacting Tregs and vaccines

iSBTc Oncology Biologics Development Primer



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PENNSYLVANIA**

**Abramson Cancer Center**

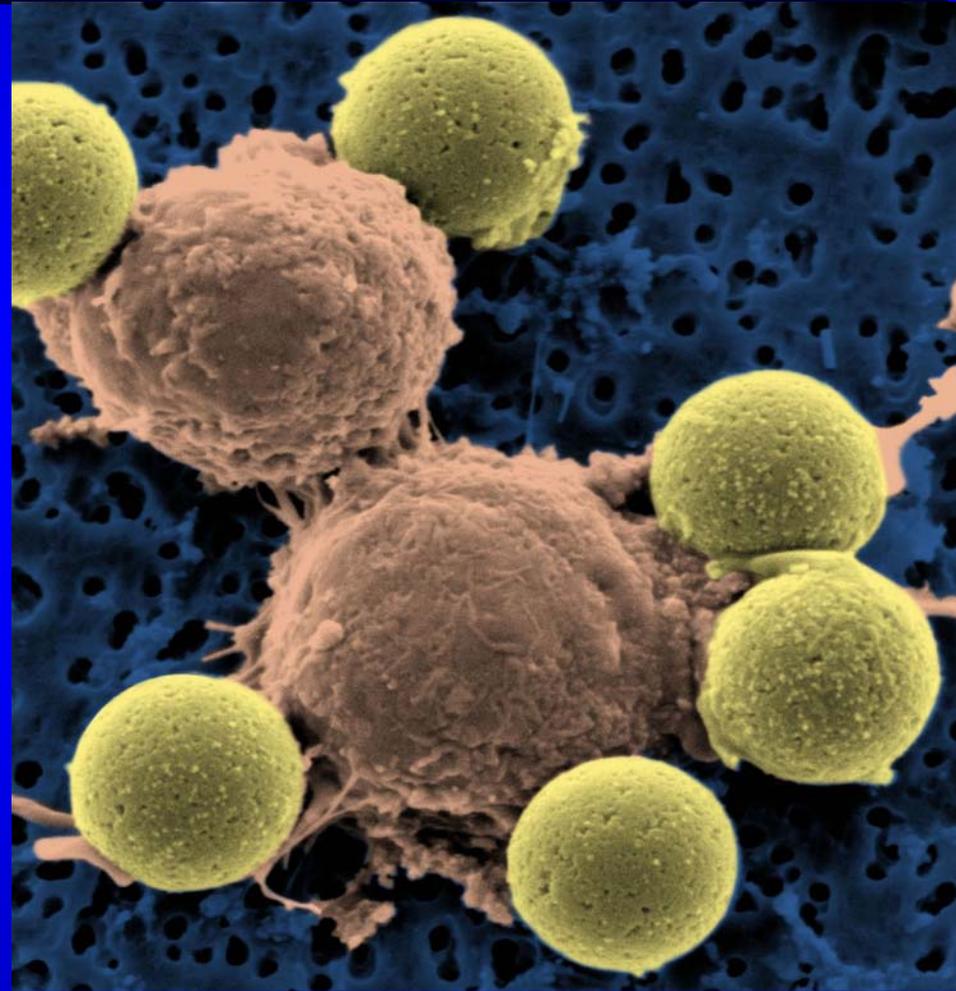
# Disclaimer

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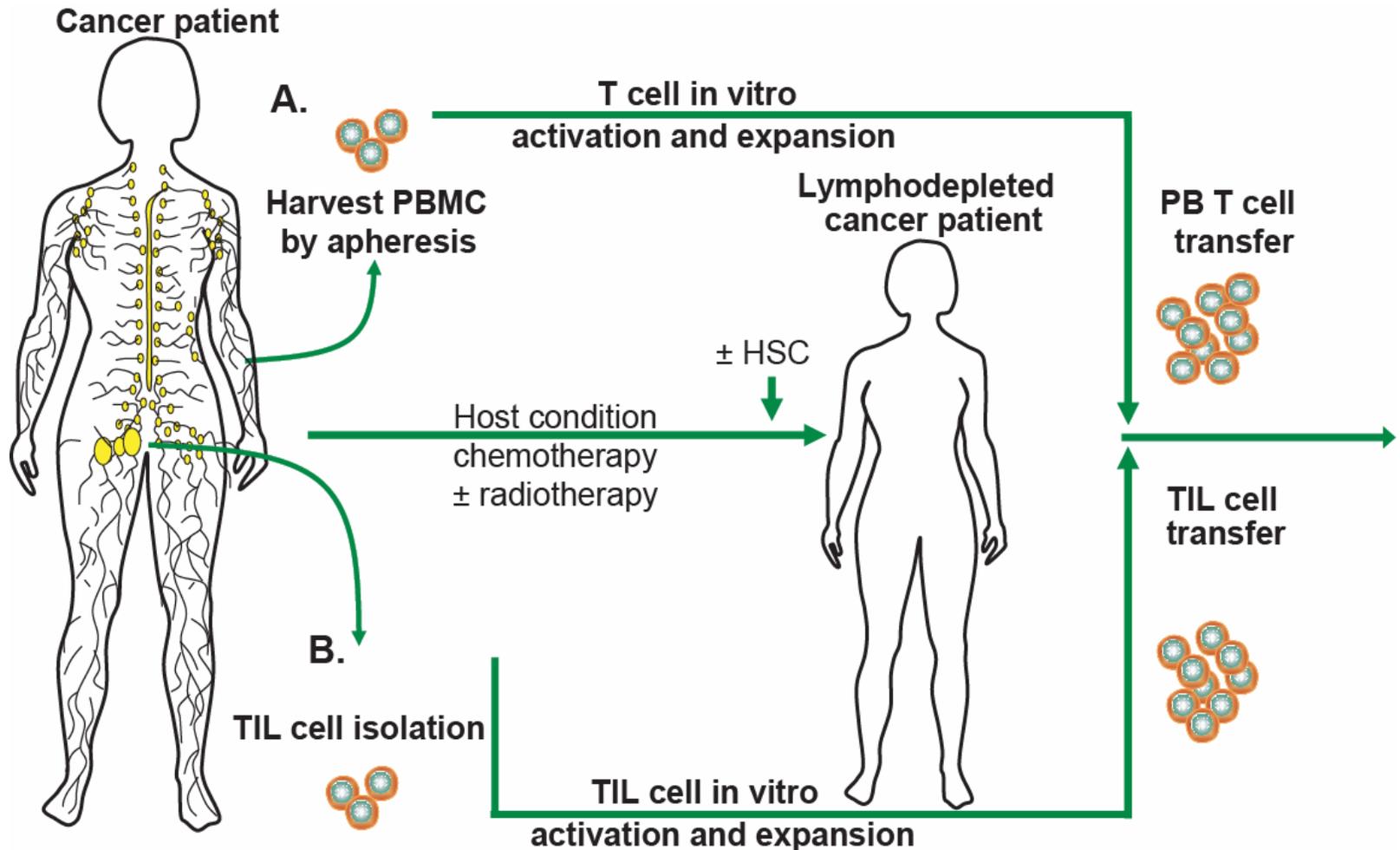
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# Overview: Adoptive T Cell Therapy

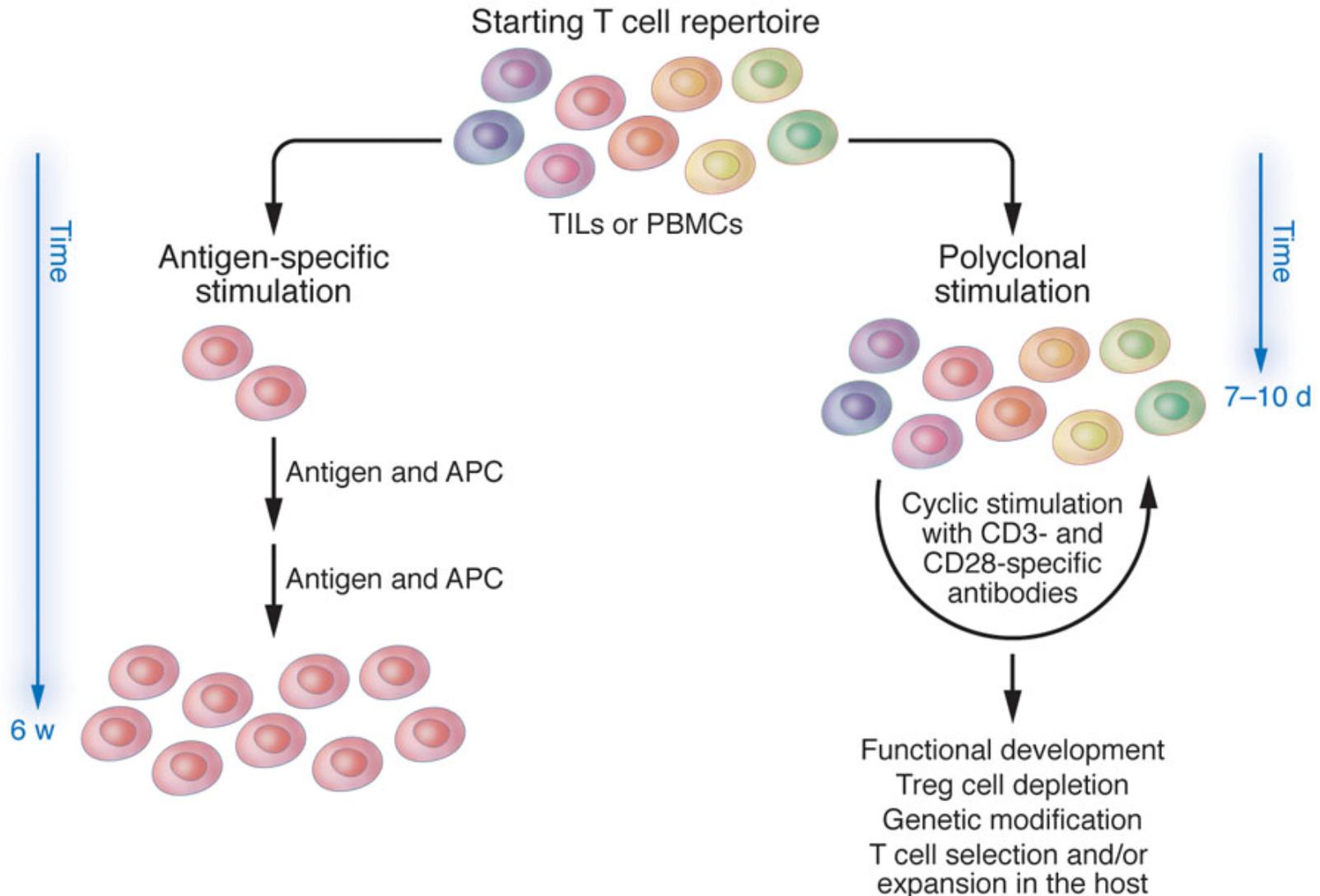
- Effector T cells: schedule and combination dependent effects
- Adoptive transfer of Tregs



# General Approaches for Adoptive T Cell Therapy



# Cell Culture Approaches for Adoptive T Cell Therapy



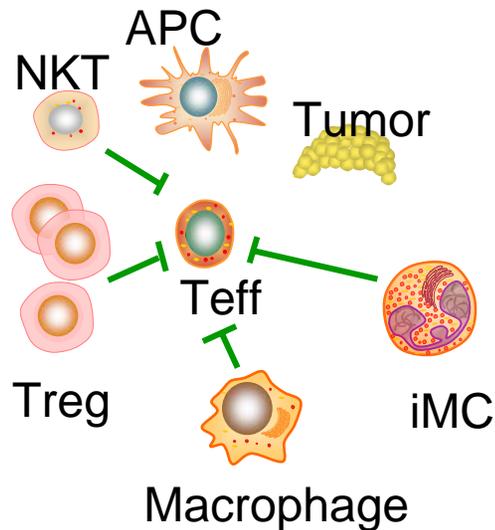
# T Cell Expansion in Lymphopenic Hosts Enhanced CD8 Effector Function

Potential mechanisms:

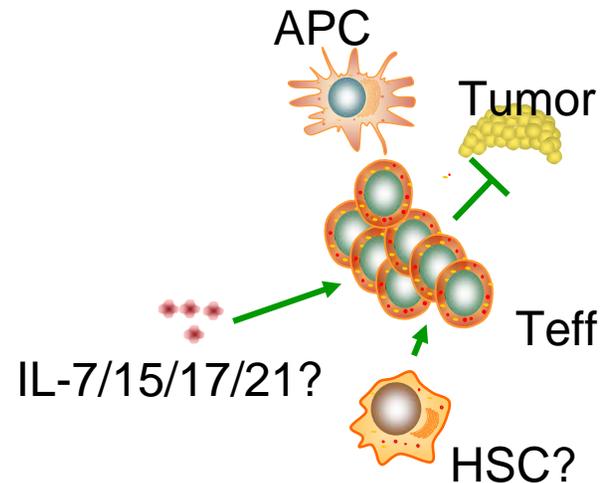
- Role of lymphopenia
- Depletion of Tregs, NKT, B cells?
- Removal of cytokine sinks?

IL-2 vs IL-7/-15/-21 regulation

Day 12 p HSC

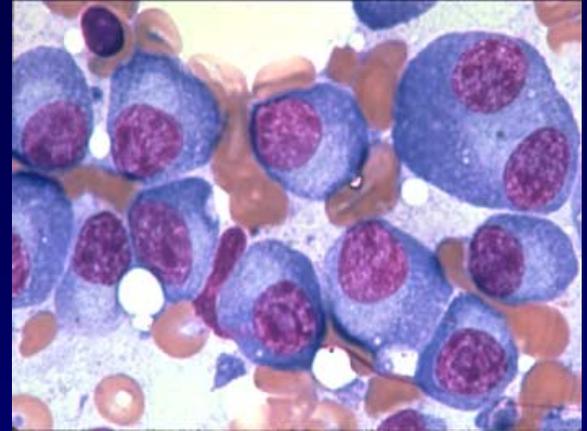


Day 2 p HSC



# 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*.



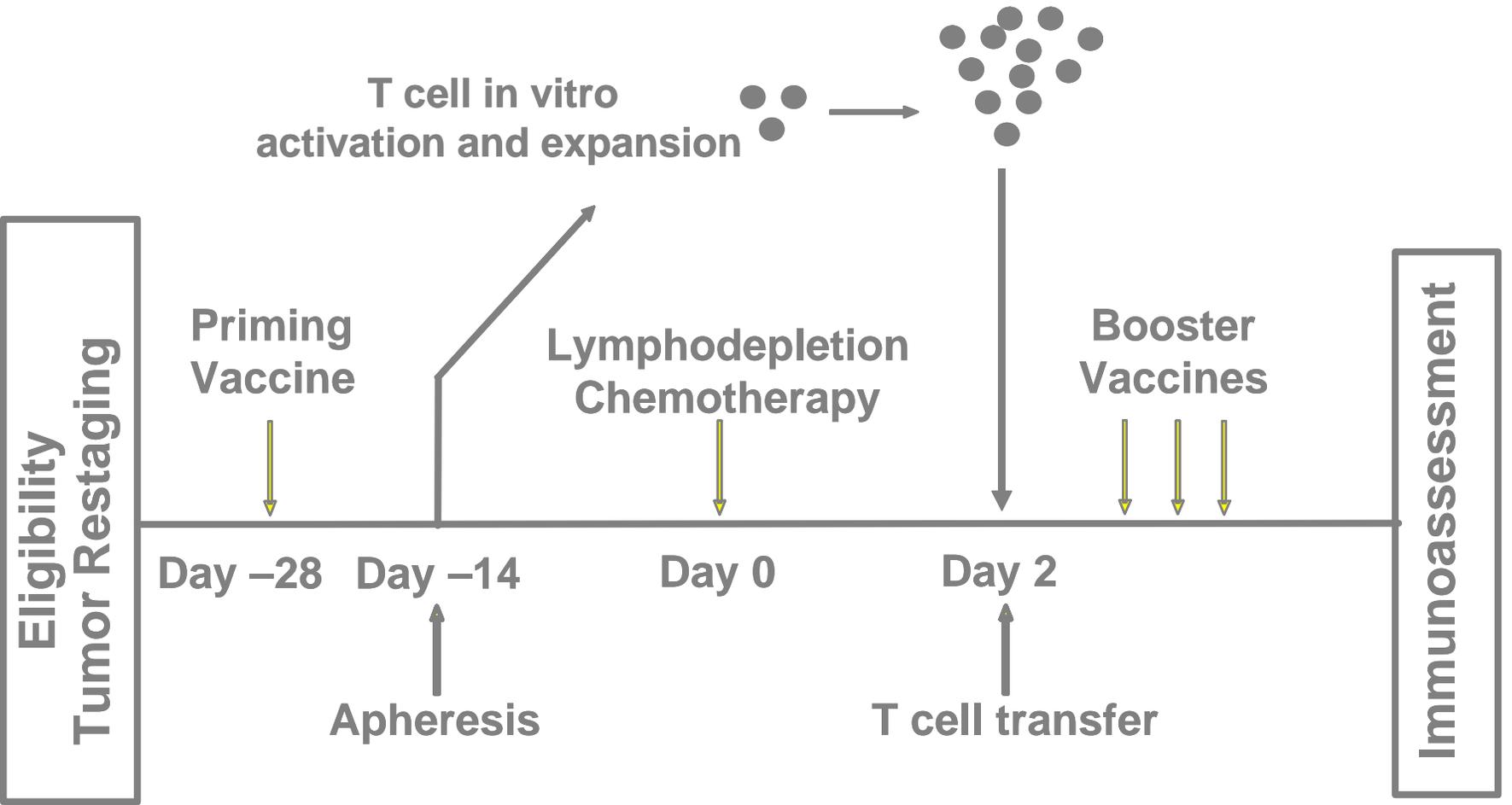
# Adoptive transfer of vaccine primed T cells augments immunity in lymphodepleted hosts: Summary of first trial

- First successful randomized multicenter adoptive immunotherapy trial
  - Accelerated recovery of CD4 T cells to normal levels by day 42 (P=0.0001)
  - Protective antibody levels established by day 30
  - Improved proliferative capacity of CD4 T cells to vaccine carrier antigen (P<0.0001) and to Staphylococcal enterotoxin A (P=0.004)
- => Adoptive transfer of vaccine primed T cells appears to facilitate reestablishment of CD4 T central memory cells

But what about tolerance?



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



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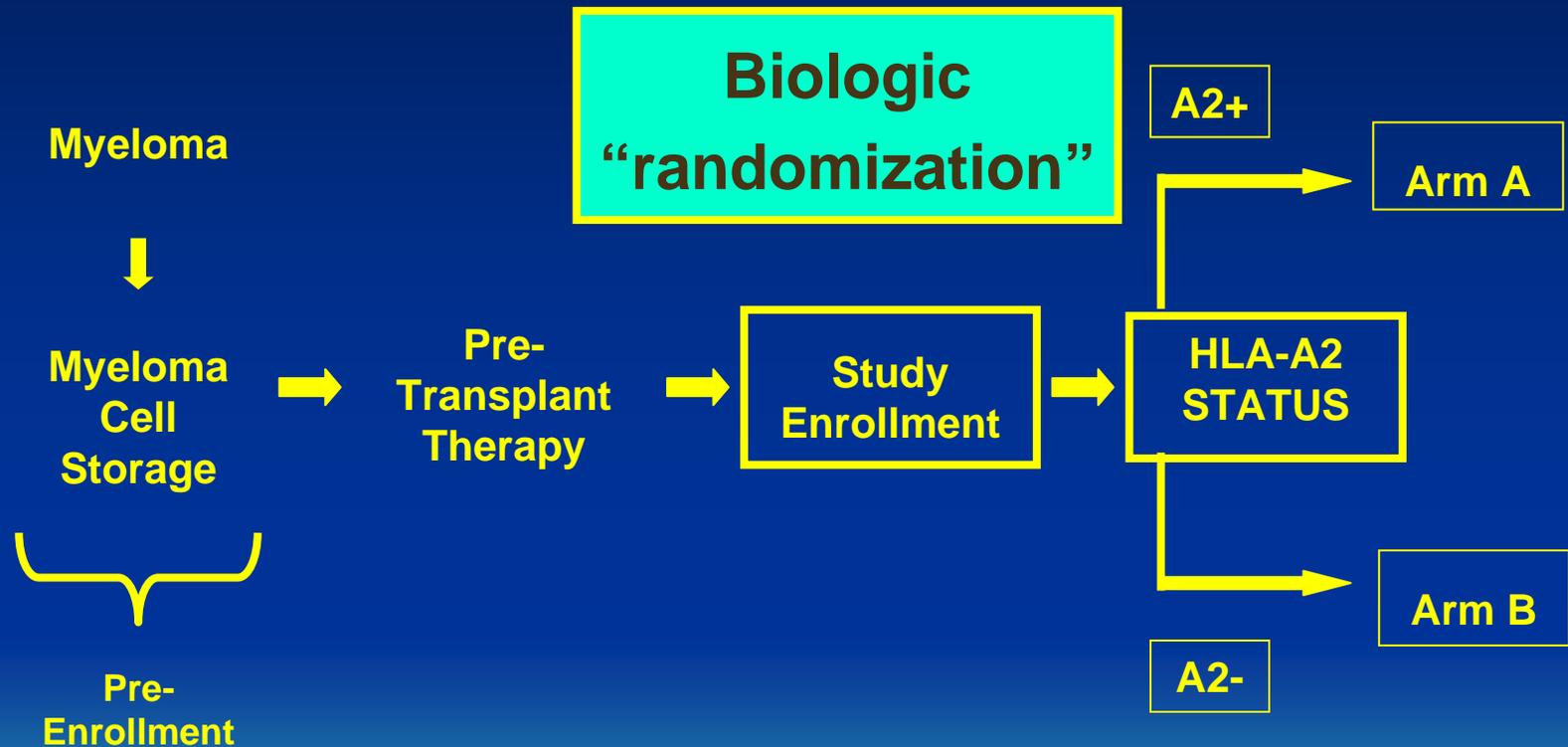
**PIs:** Aaron Rapoport, U Maryland  
Edward Stadtmauer, U Pennsylvania

**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

# Myeloma Trial #2 Protocol Flow



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

**HLA-A2- (Arm B)**

**Study Day**

- 42

- 30

- 15

- 1

0

12

30

90

100

**TERT, Survivin, CMV, PCV**

**PCV**

**T Cell Collection**

**T Cell In Vitro Activation and  
Expansion to Infuse  $10^{10}$  Cells**

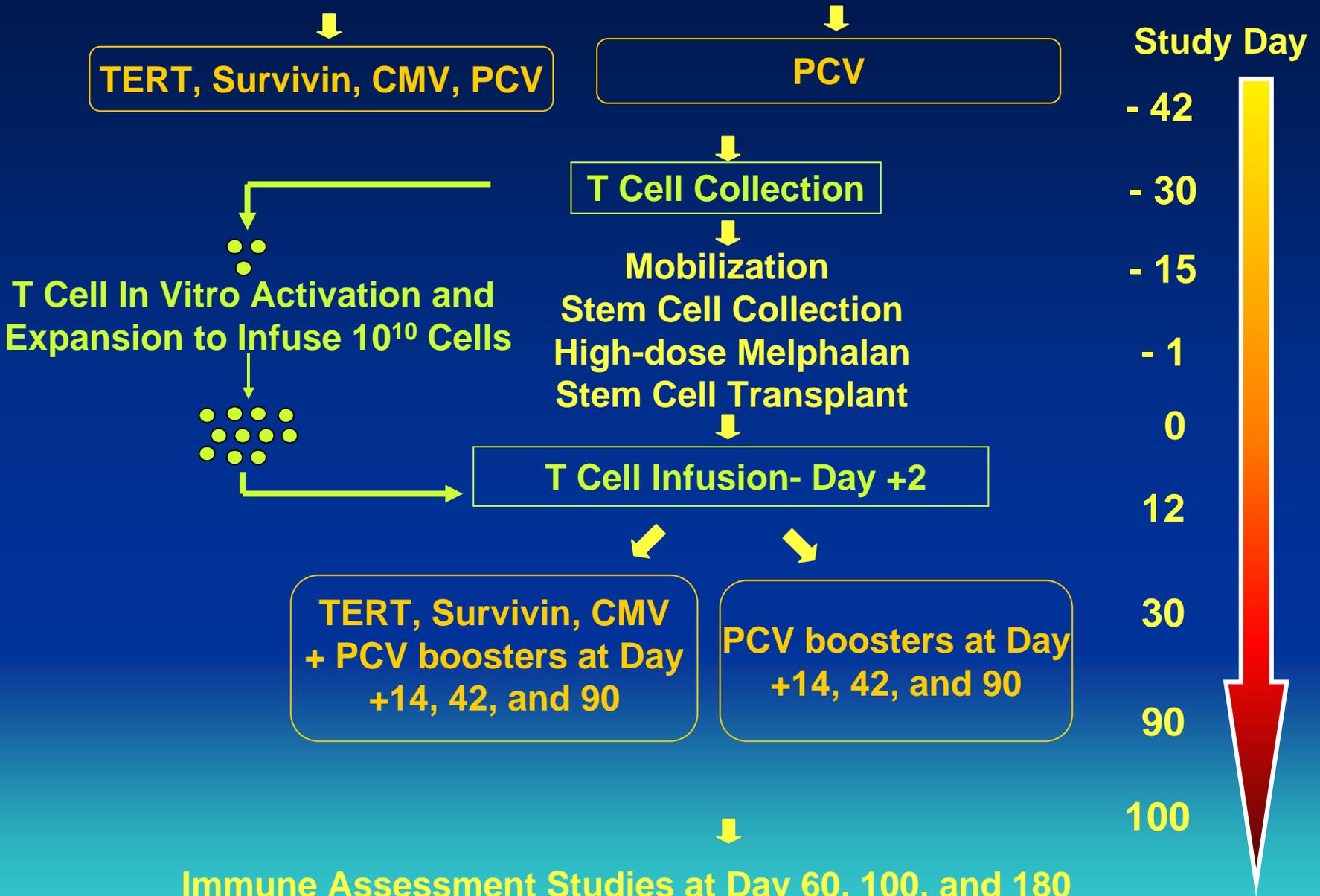
**Mobilization  
Stem Cell Collection  
High-dose Melphalan  
Stem Cell Transplant**

**T Cell Infusion- Day +2**

**TERT, Survivin, CMV  
+ PCV boosters at Day  
+14, 42, and 90**

**PCV boosters at Day  
+14, 42, and 90**

**Immune Assessment Studies at Day 60, 100, and 180**



# T-cell Recovery - Myeloma Trial #2

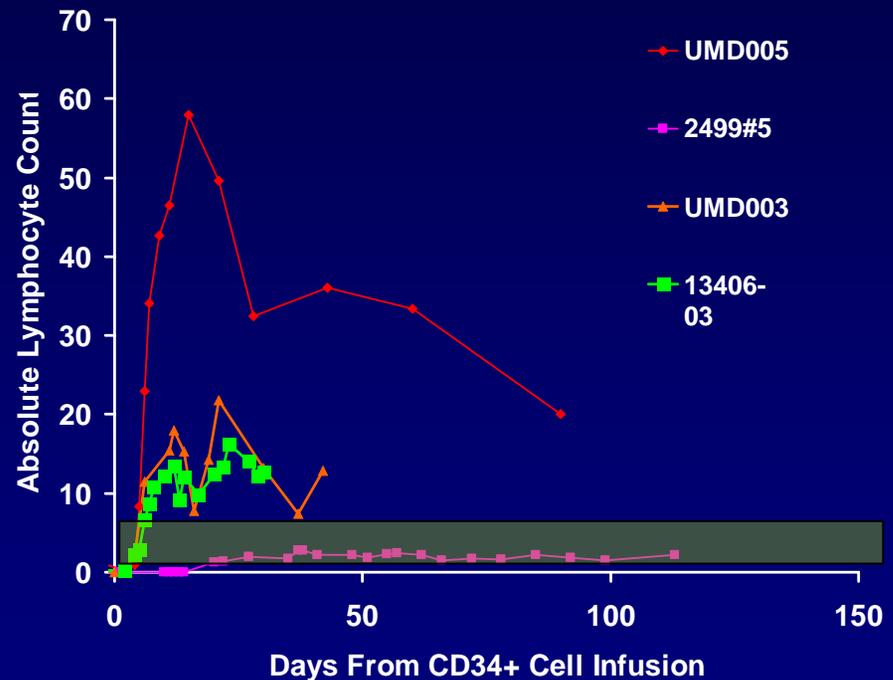
CD 4	Day	Mean	Median	MIN	MAX	N
	14	1846	1349	516	7668	17
	60	2085	1993	851	4517	10
	100	1313	1264	382	2309	10

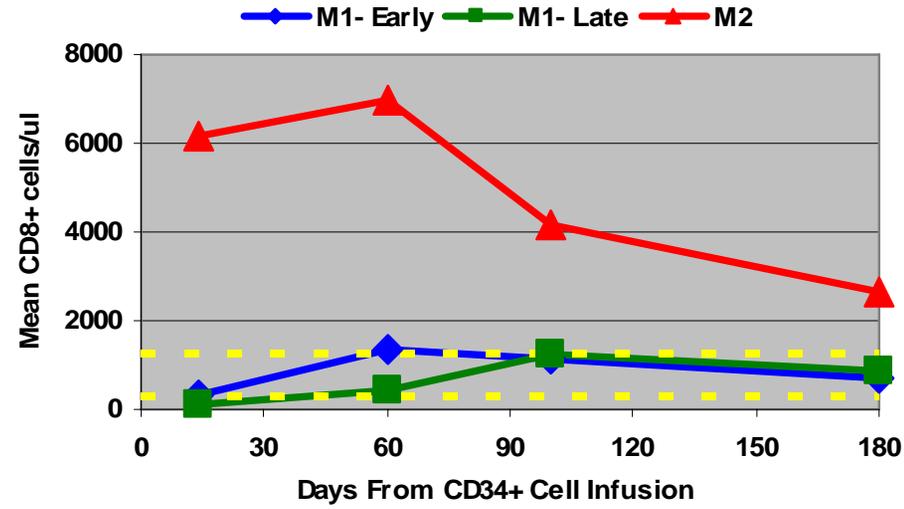
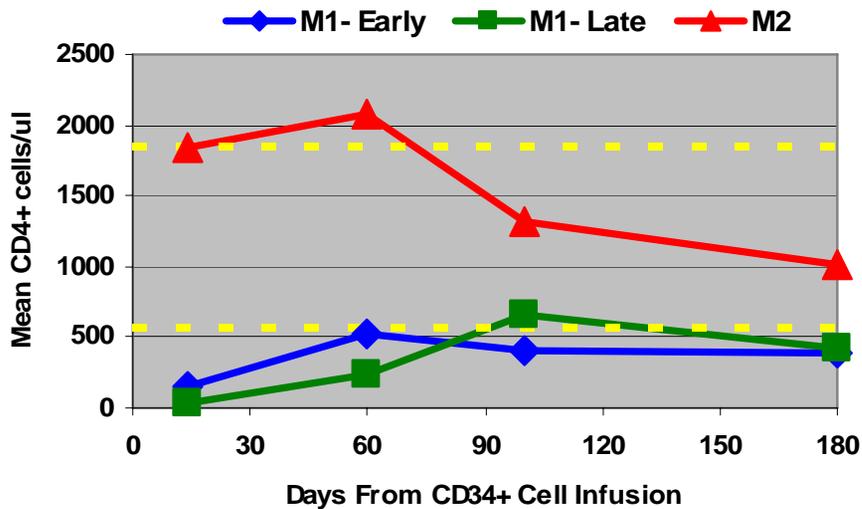
CD 8	Day	Mean	Median	MIN	MAX	N
	14	6153	3233	1271	39354	17
	60	6952	5308	2065	23863	10
	100	4169	2998	1111	12599	10

# T Cell Leukocytosis Post Day 2 Adoptive Transfer

- **Schedule dependent effects of costimulated T cell infusion**
  - **Prolonged T cell leukocytosis in patients after day 2 T cell infusion**
  - **Rapid normalization of T cell counts with homeostasis after day 12 T cell infusion**



# CD4/CD8 T-cell Recovery – Comparison to Previous Adult Myeloma Trial



## CD4 Recovery

Day +2 (RED) – Current Study

Day +12 (BLUE) – Prior Study

Day +100 (GREEN) – Prior Study

## CD8 Recovery

Day +2 (RED) – Current Study

Day +12 (BLUE) – Prior Study

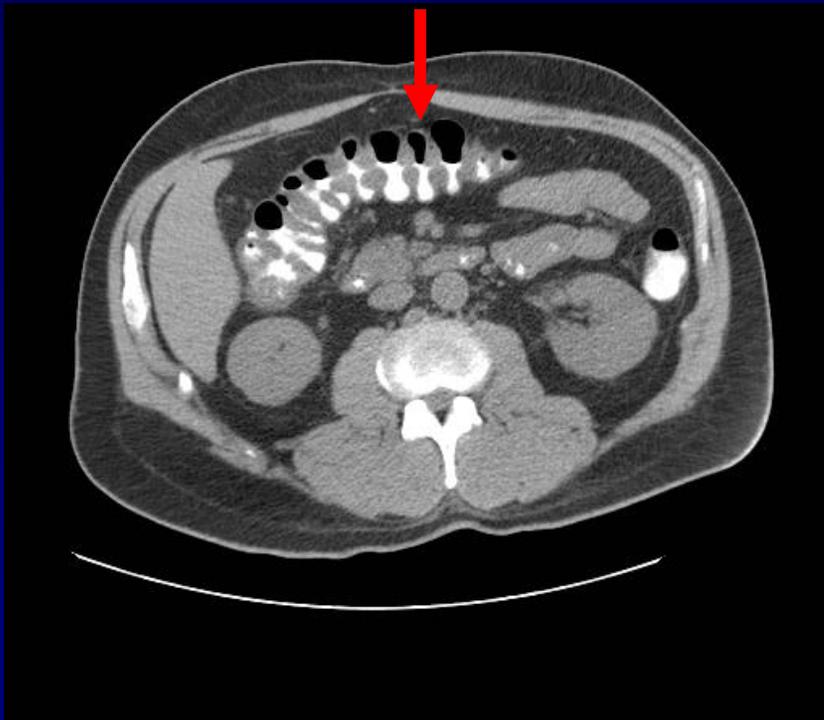
Day +100 (GREEN) – Prior Study

# Vaccine + day 2 T cell boost trial: Myeloma Interim Summary

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

# T Cell Engraftment Syndrome and auto-GVHD with day 2 autologous T cells

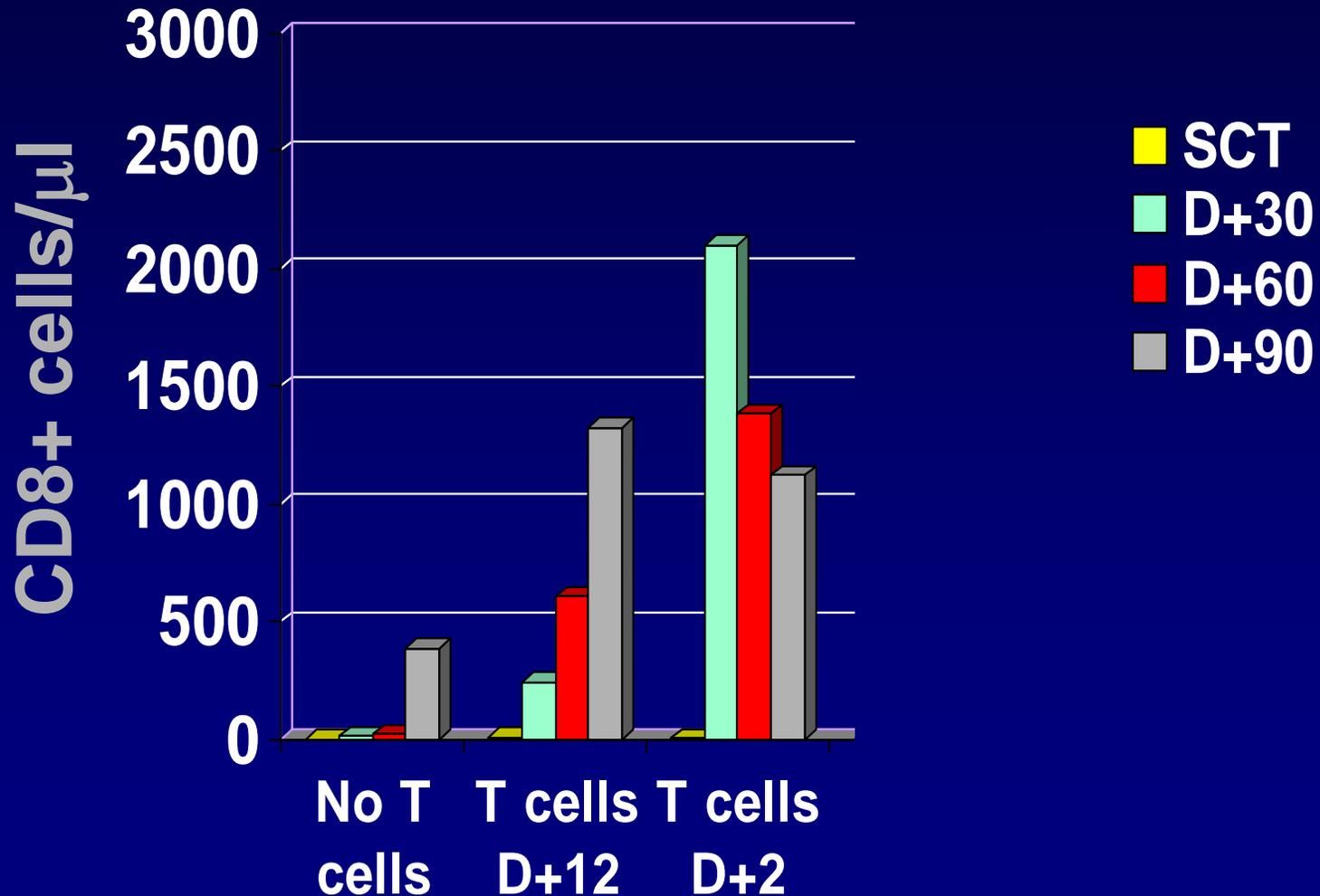
- T cell engraftment syndrome: onset by day 14 w rash, diarrhea, fever (n=6).
- Steroid responsive (n=3).



- Day 13 → N/V/Diarrhea, T=38
- Day 14 → 800cc stool, T=37.6
- Day 15 → 1300cc stool, T=38
- Day 16 → 900cc stool, T=38.1
- Day 17 → 500cc stool, T=38.1
- Day 18 → 300cc stool, T=37.7
- Day 20 → no diarrhea/fever

UMD-011 – Day +14

# Schedule Dependent Effects of T cell transfer on CD8 count



# “Engraftment Syndrome”

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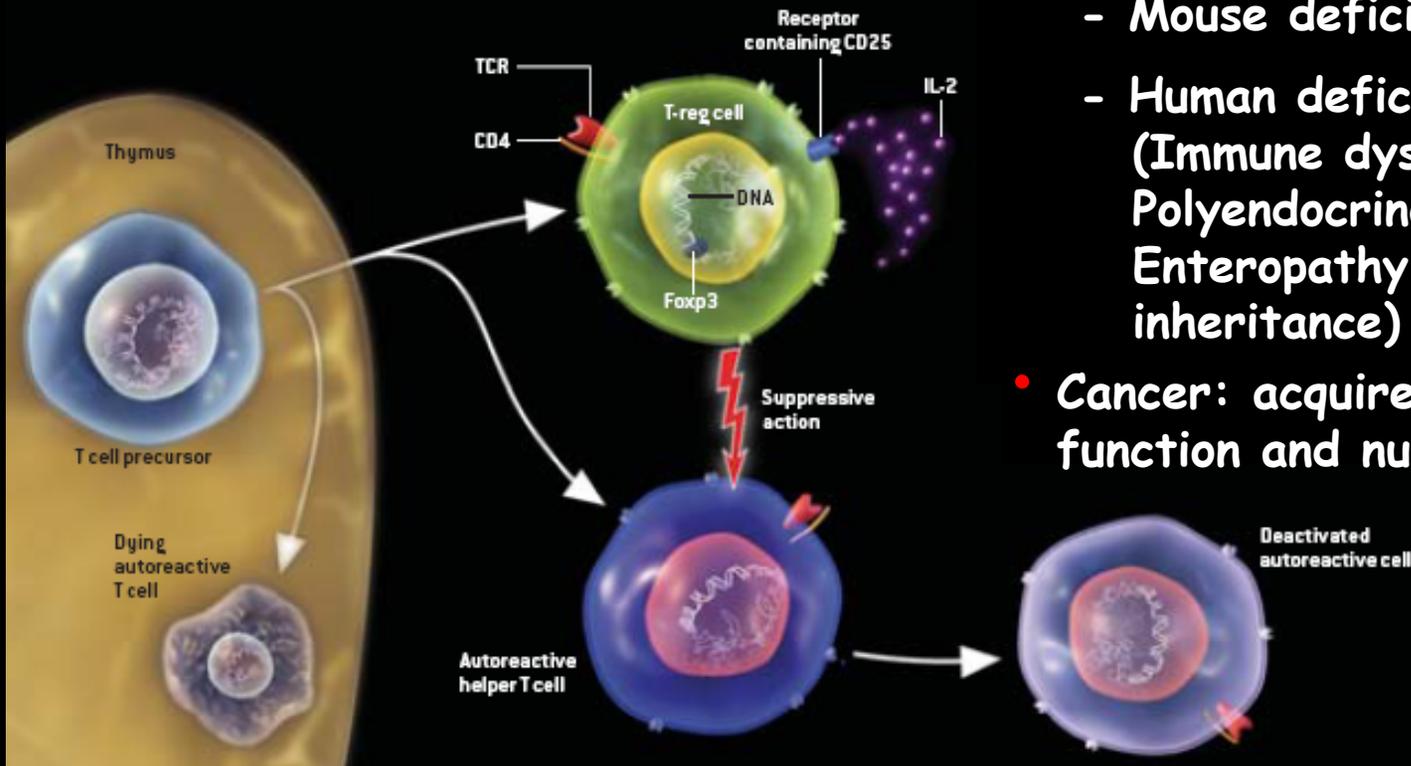
- **GVHD-like features with or without fever**
- **Not seen in pts receiving d+12 or d+90 T cells**
- **No delay in hematopoietic recovery after Day +2 transfers of costimulated T-cells**
- **T cell recovery is accelerated compared to randomized controls and is schedule dependent (day +2 vs day +12)**
- **T cell recovery shows sustained levels above normal, suggesting that early recovery may not be subject to normal homeostatic mechanisms**

# Issues To Be Addressed: T Cell Leukocytosis And Engraftment Syndrome

- **Schedule dependent immune reconstitution, toxicity and/or anti-self/tumor effects. Is this a good thing?**
- **Why does it occur with day 2 and not post day 12 infusions?**
- **Potential mechanisms**
  - **r/o trivial (microchimerism with allo)**
  - **Homeostatic cytokine milieu day 2 vs day 12**
  - **Treg depletion or Th17 generation on day 2?**

# Treg Tolerance Mechanisms

- Subsets: nTregs and iTregs
- Act to limit effector response to self-antigens by blocking cytokines and proliferation
- FoxP3 required for Treg function
  - Mouse deficiency: Scurfy
  - Human deficiency: IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked inheritance)
- Cancer: acquired gain of function and number of Tregs



# Use of Adoptive T Cell Immunotherapy To Tip the Balance of Teff and Tregs in vivo

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## Treg depletion

Adoptive transfer of T cells  
depleted of Tregs might  
increase effector T cell  
function in vivo

Potential use for vaccine  
adjuvant and cancer  
patients

Safety profile: unknown risk of  
autoimmunity

## Treg augmentation

Adoptive transfer of Tregs might  
induce immunosuppression  
or tolerance

Potential uses for GVHD,  
autoimmunity and organ  
transplantation

Safety profile: unknown risk of  
immunosuppression

# Potential Forms of Adoptive Cellular Immunotherapy with Tregs

## Wudqviuhg#F hœv

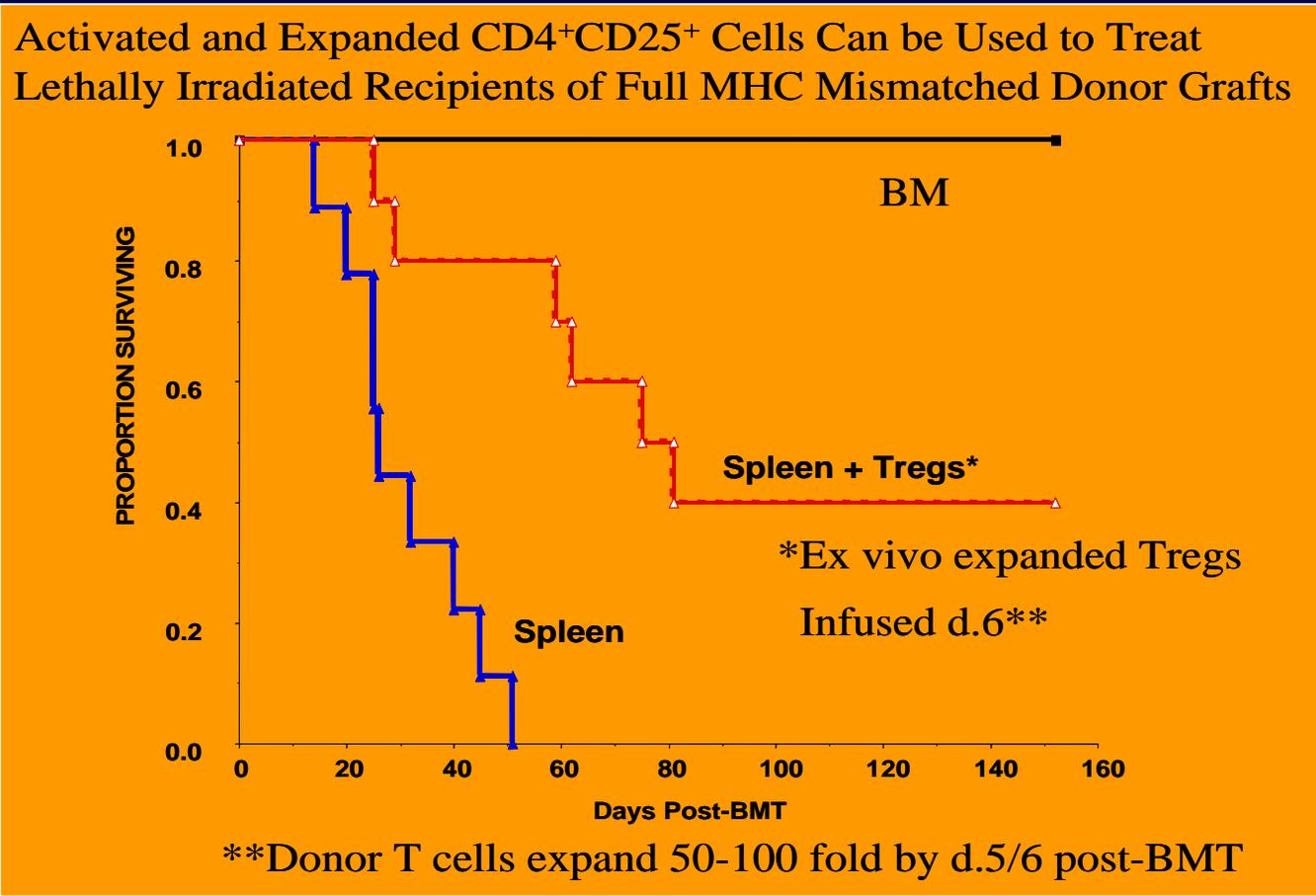
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W u h j v
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W u h j v
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W u h j v



## I q g l f d w l r q

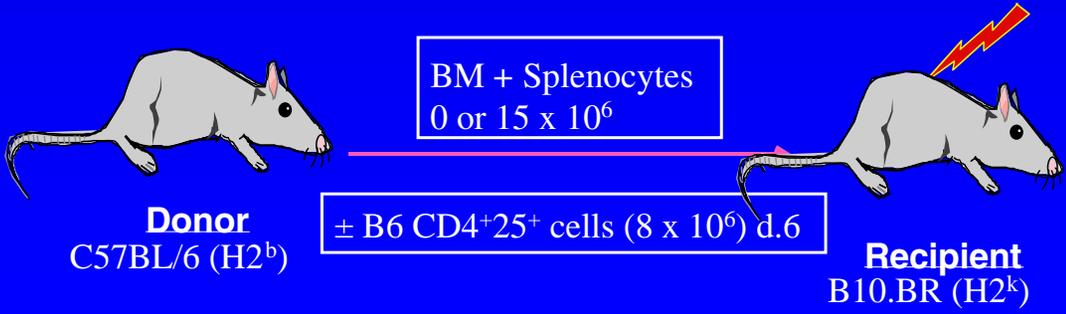
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w u d q v s œ l q w d w l r q
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I q i h u w l o l w |
- ~ Y d u l r x v

# Ex Vivo Expanded Mouse Tregs for GVHD Treatment



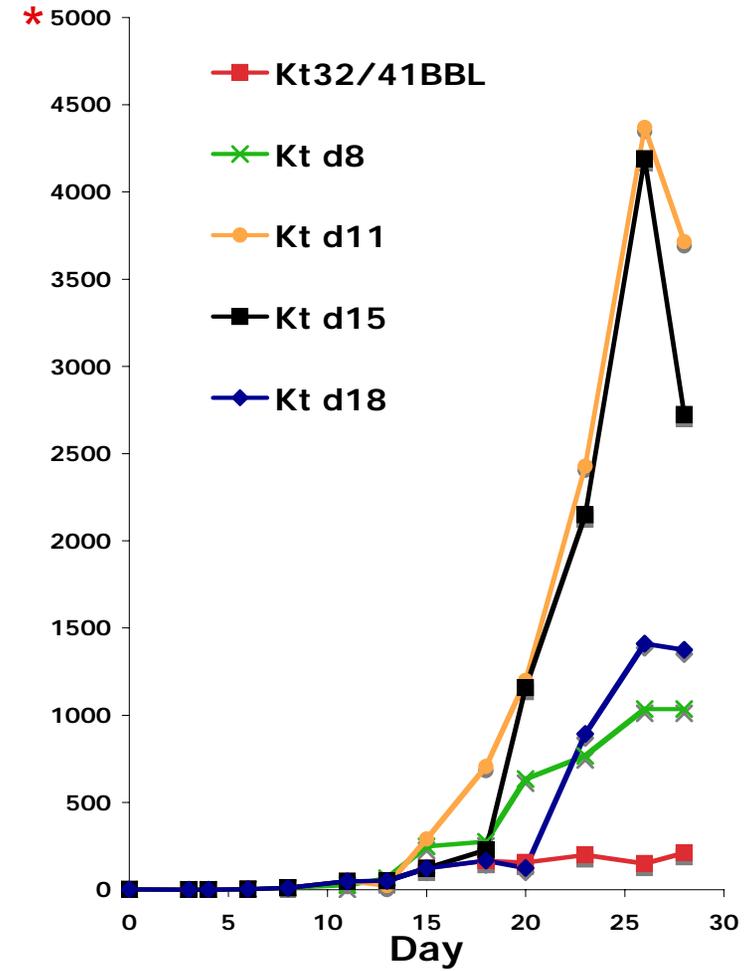
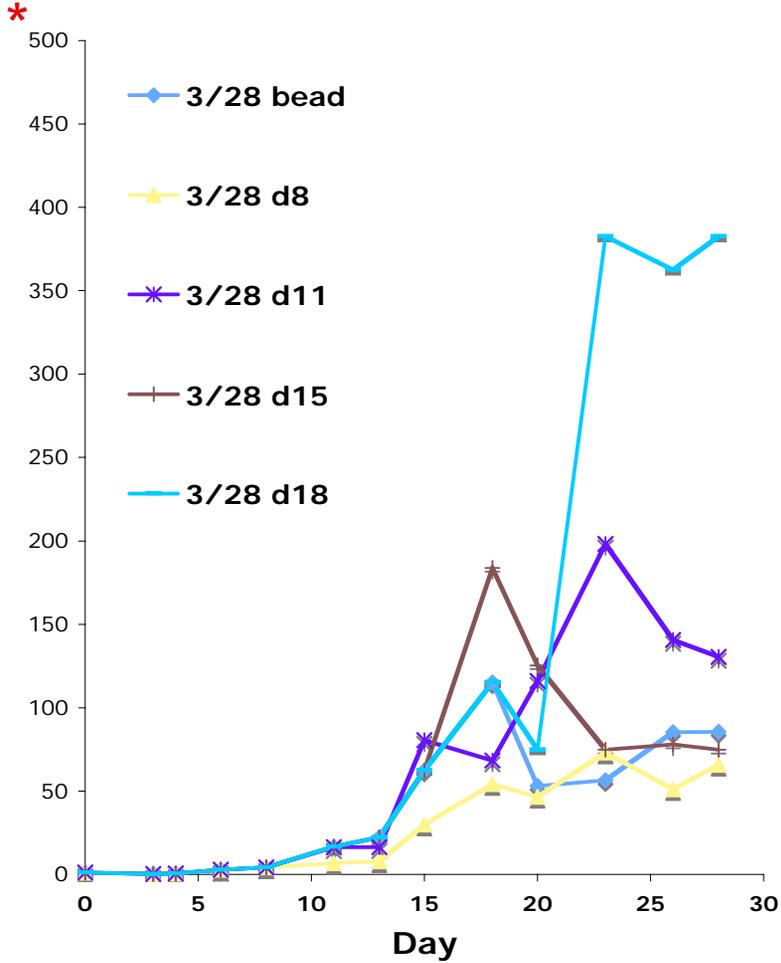
Taylor, et al.  
Blood. 2002;99:3493

Hoffman, et al.  
J Exp Med. 2002;196:389



# Development of Human Treg GMP Compliant Culture Systems

## CD3/28 Bead aAPC or KT32/4.1BBL aAPC



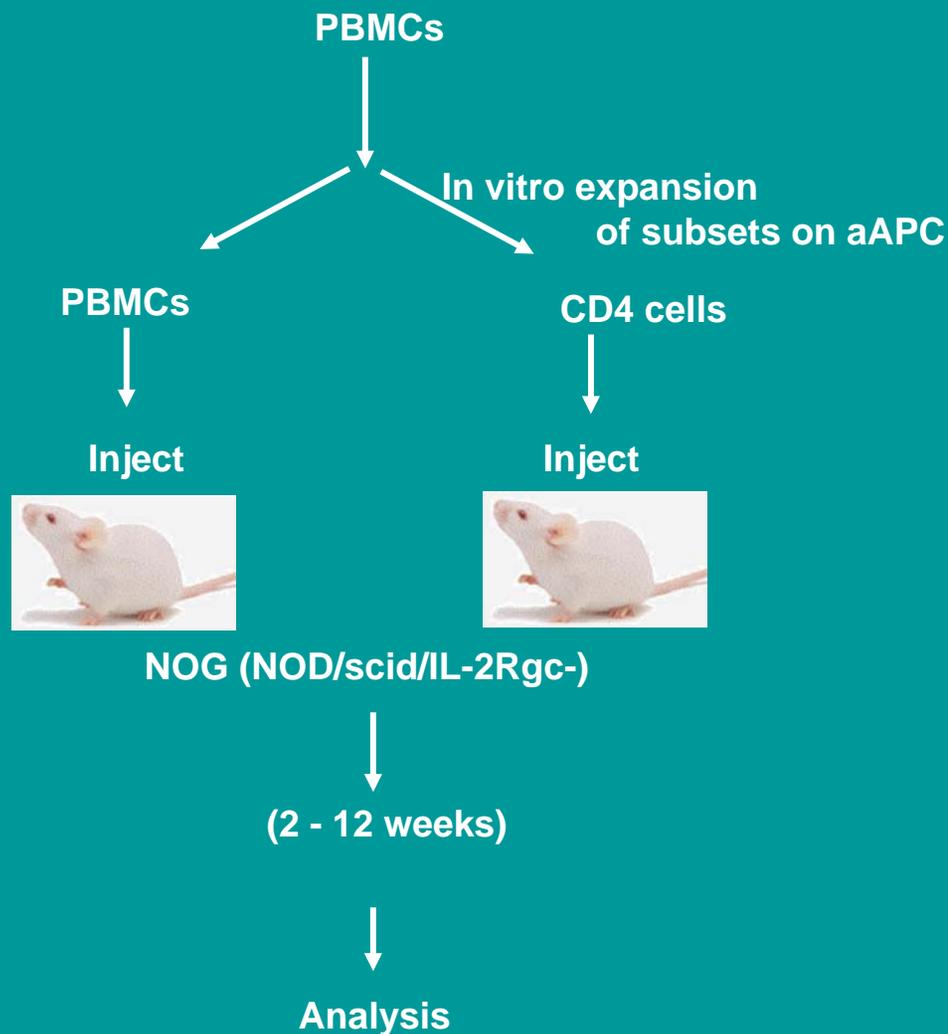
\* Note difference in scale

# Development of an in vivo model to test expanded human Treg cell function

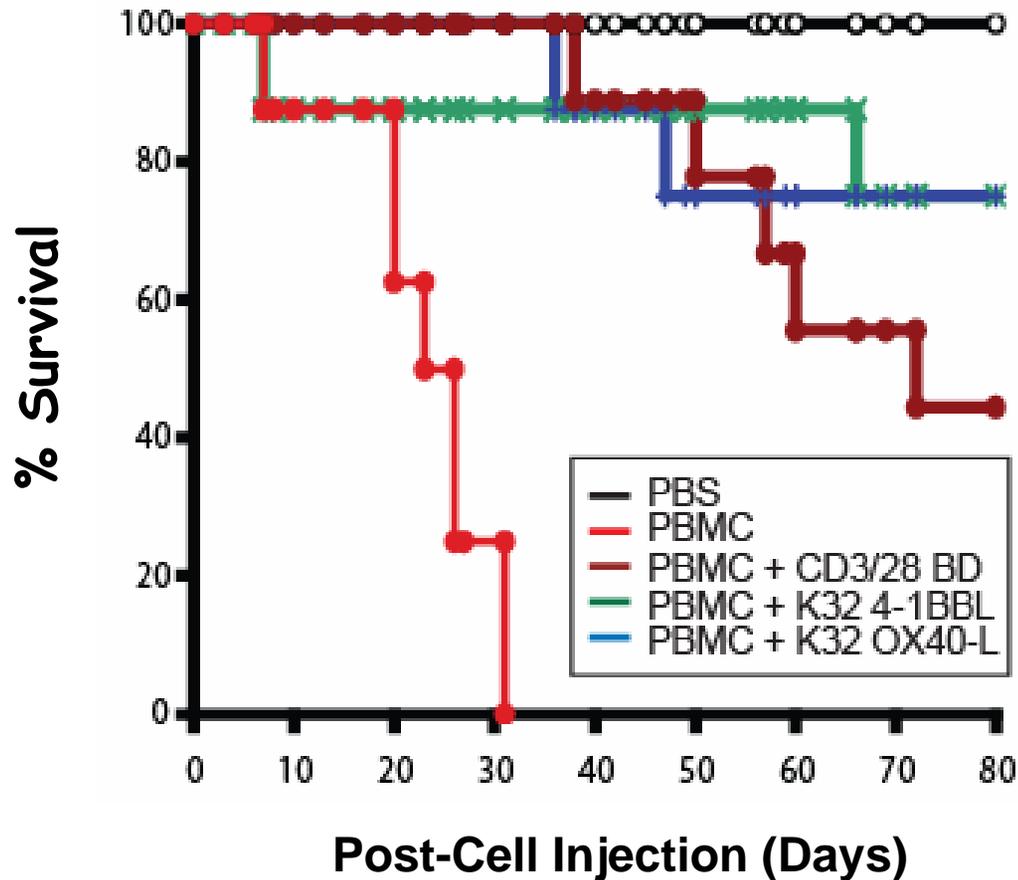
CD4<sup>+</sup>CD25<sup>+</sup> were transduced w GFP lentiviral vector and expanded by KT86 aAPCs and rapamycin for 21 days

## Analysis:

1. In vitro suppression assay and phenotype
2. Weight and visual inspection for GVDH.
3. Ratio of GFP to non-GFP positive CD4 T cells
4. T cell infiltration into lung and liver



# Ex Vivo Expanded Human Treg Prevent Lethal Xeno-GVHD in NOG mice



NOG mice (8 wks) were injected IP with 10 million PBMCs and 2 million expanded nTregs (6 mice per group).

# Treg Summary

- **Human Tregs prevent xeno-GVHD immunopathology in NOD/ $\gamma$ C<sup>-/-</sup> mice**
- **GMP compliant cell culture systems permit efficient ex vivo expansion of polyclonal CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> nTregs.**
- **These cells are currently in phase I clinical trials at the University of Minnesota.**

# Lessons Learned: Effector T Cell Transfers

- Schedule dependent effects uncovered “engraftment syndrome” with autologous T cells
  - Subset of patients develop a T cell “engraftment syndrome” with features of GVHD
  - Relationship to chemotherapy
  - Host lymphopenia
- Combination dependent effects (neuroblastoma trial)
  - Cluster of Transplant Associated Microangiopathy (TAM)
  - Associated with irradiation, isotretinoin, and T cell infusions
- Pre-clinical models in mice are poorly predictive for the above

# Case Studies: Lessons and Issues

- **Key Strategic Decisions**
  - Gene therapy or not?
- **Impact of Regulatory Interactions**
  - FDA and NIH/RAC very helpful
  - Redundancy and poor harmonization of reporting requirements
- **Financial Considerations: Projected Costs vs. Reality**
  - Academic development:
    - Advantages, can take on longer term projects and are less risk adverse than small biotech
    - Disadvantages: resource constrained. No grant budget can support a cell based therapy trial
- **Lessons Learned**
  - Teamwork required
  - Environment is critical

# Collaborators and Acknowledgements

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