

# What are the Functional and Phenotypic Qualities of Therapeutically Successful Anti-Tumor T Cells?

Nicholas P. Restifo, MD



iSBTc

October 31, 2009

# Major Points

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1. Cancer immunotherapies, especially those based on adoptive cell transfer (ACT) of tumor-specific T lymphocytes, can be curative in some patients with metastatic cancer.
2. ACT-based immunotherapies can be enhanced with immunoablation prior to therapy.
3. The type of tumor-specific T cell that is delivered and its level of differentiation can be manipulated to improve treatment outcomes.

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**C.K. (200cGy)**

**Pre**

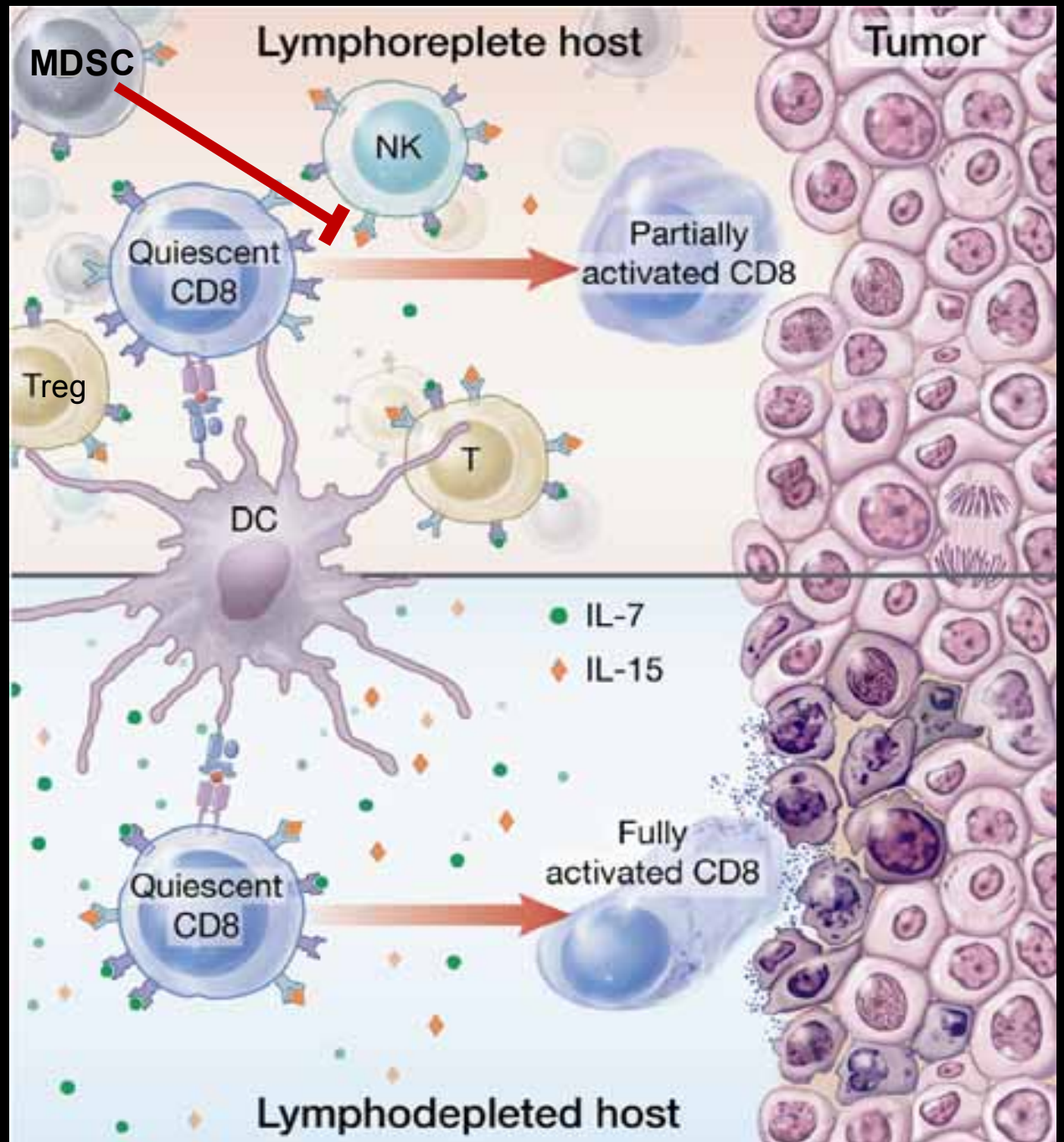
**12 days**



# “Sinks, Suppressors and Antigen Presenters”

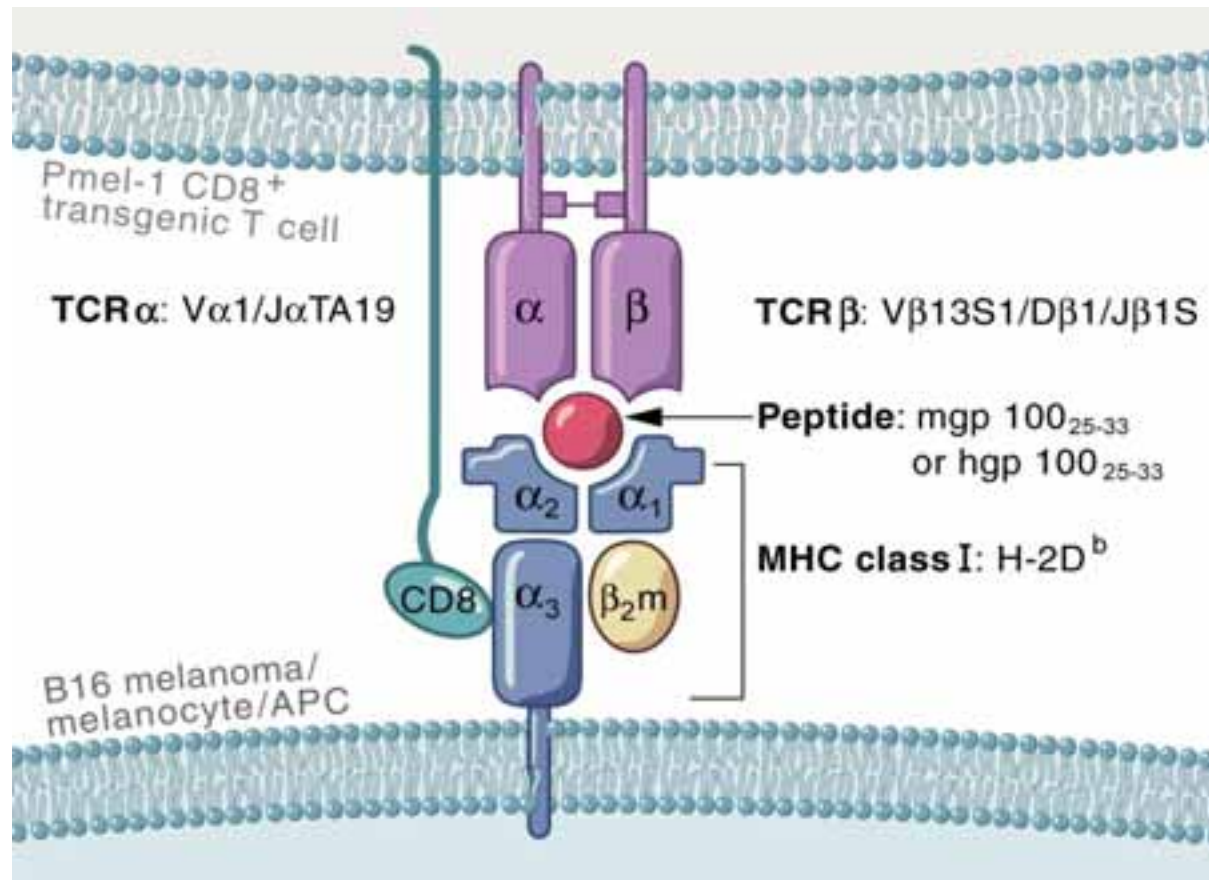
How lymphodepletion augments the function of T cell-mediated immunotherapy

Klebanoff, Trends Immunol, 2005; Gattinoni, JEM, 2005



# The pmel-1 **CD8**<sup>+</sup> TCR transgenic model

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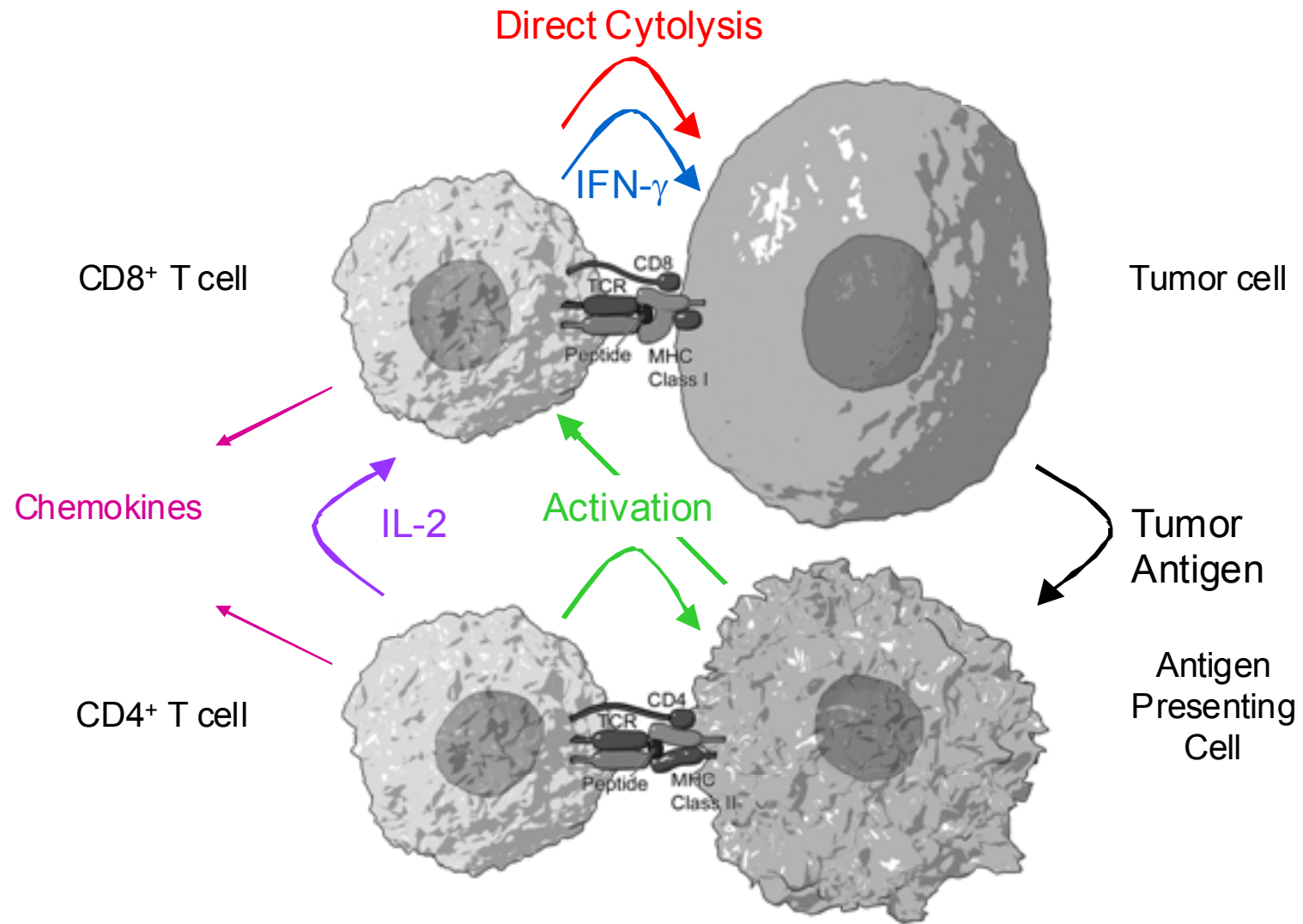


Overwijk, WW, et al. *J Exp Med*, 1998

Overwijk, WW, et al, *J Exp Med*, 2003 [www.jax.org](http://www.jax.org)

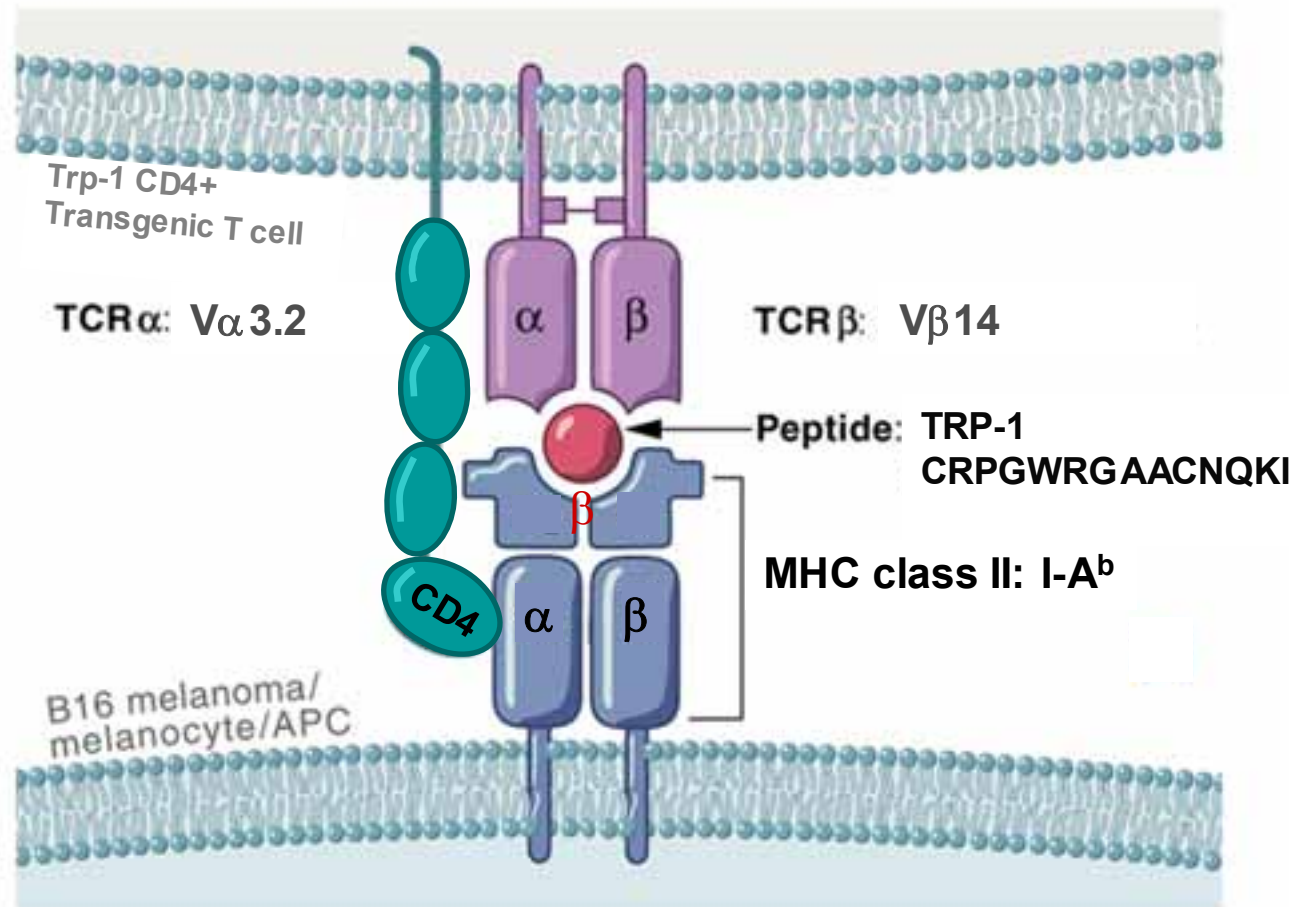
# Anti-tumor activities of $\alpha/\beta$ T cells

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# The Trp-1 **CD4**<sup>+</sup> T cell transgenic model

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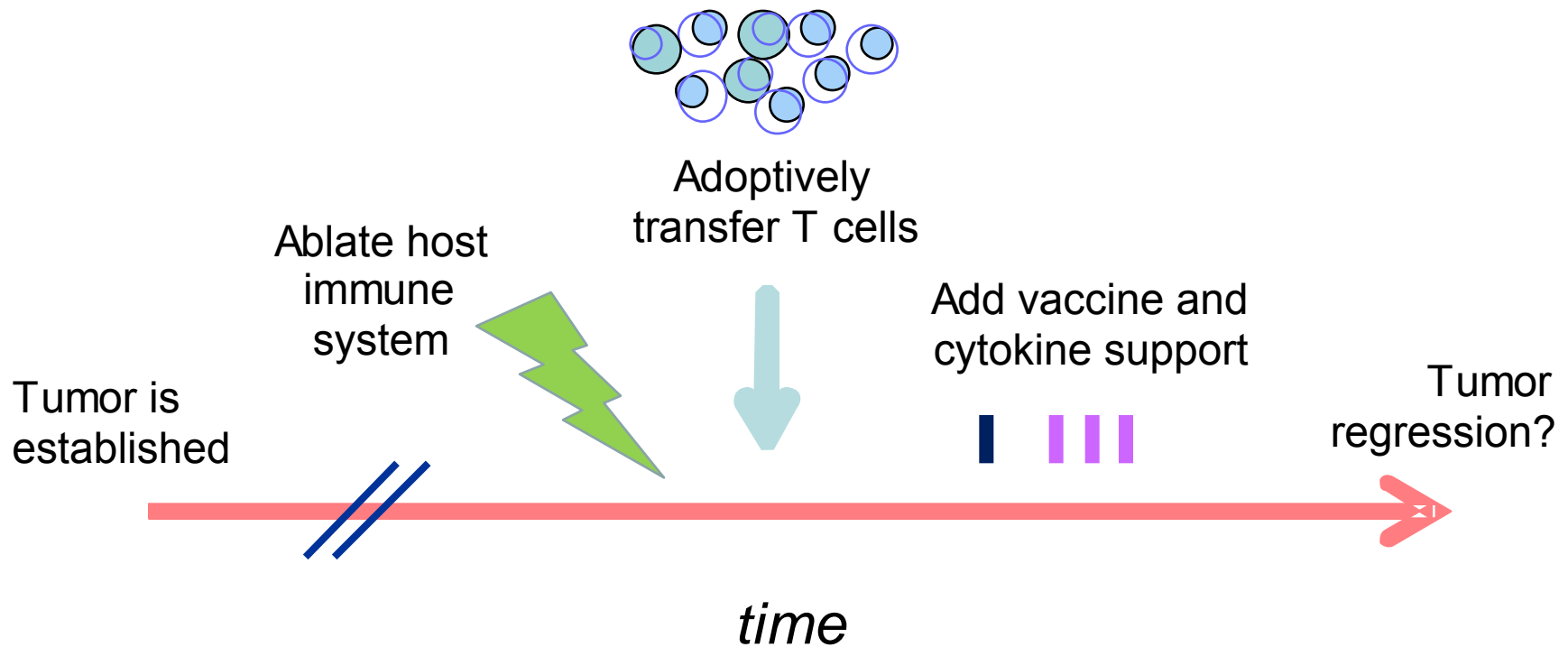


Muranski, P, et al, *Blood*, 2008

Estimated Available for Sale Date: 14-Dec-09 at [www.jax.org](http://www.jax.org)

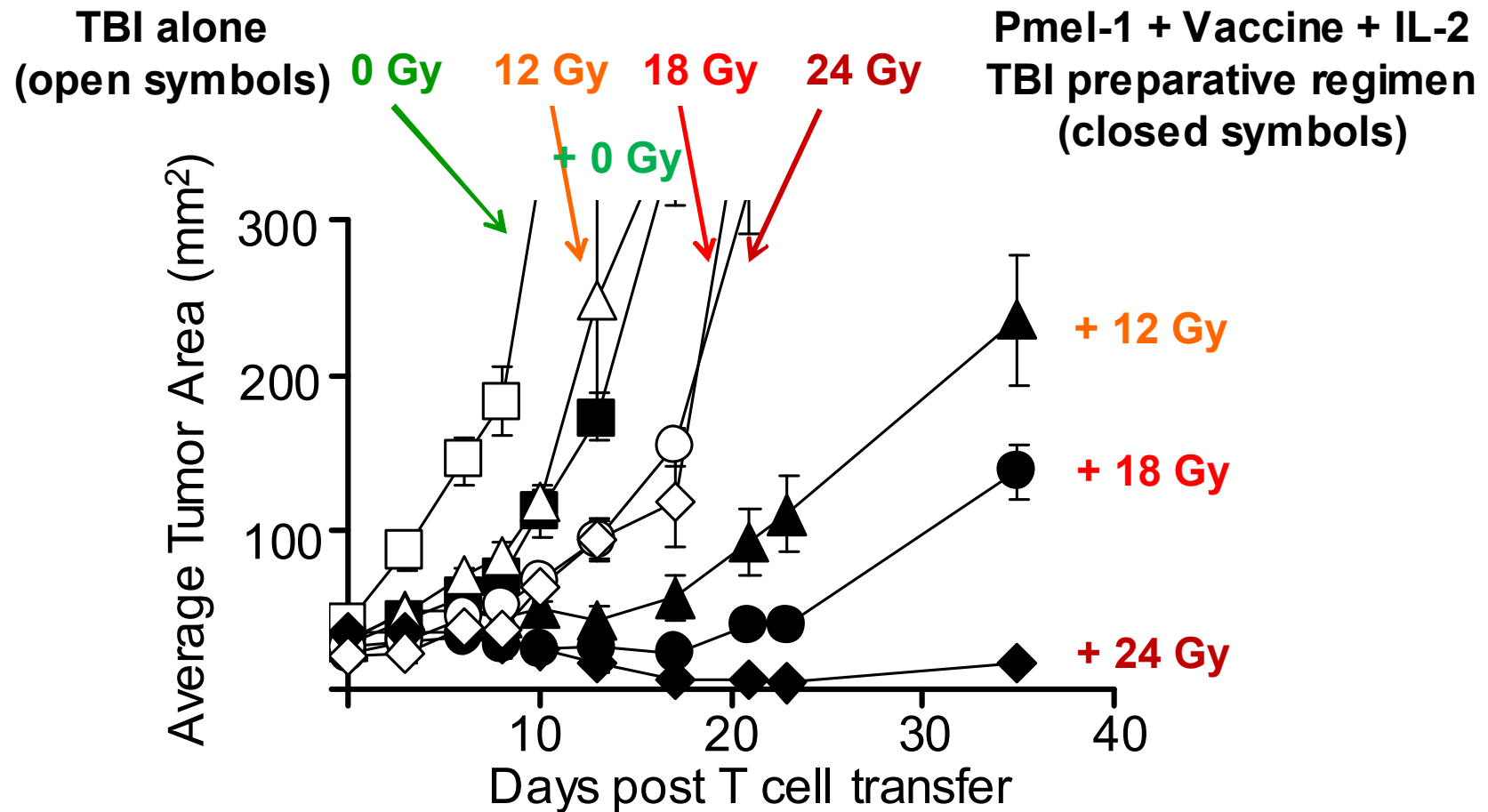
# Adoptive cell transfer (ACT) in mouse and man

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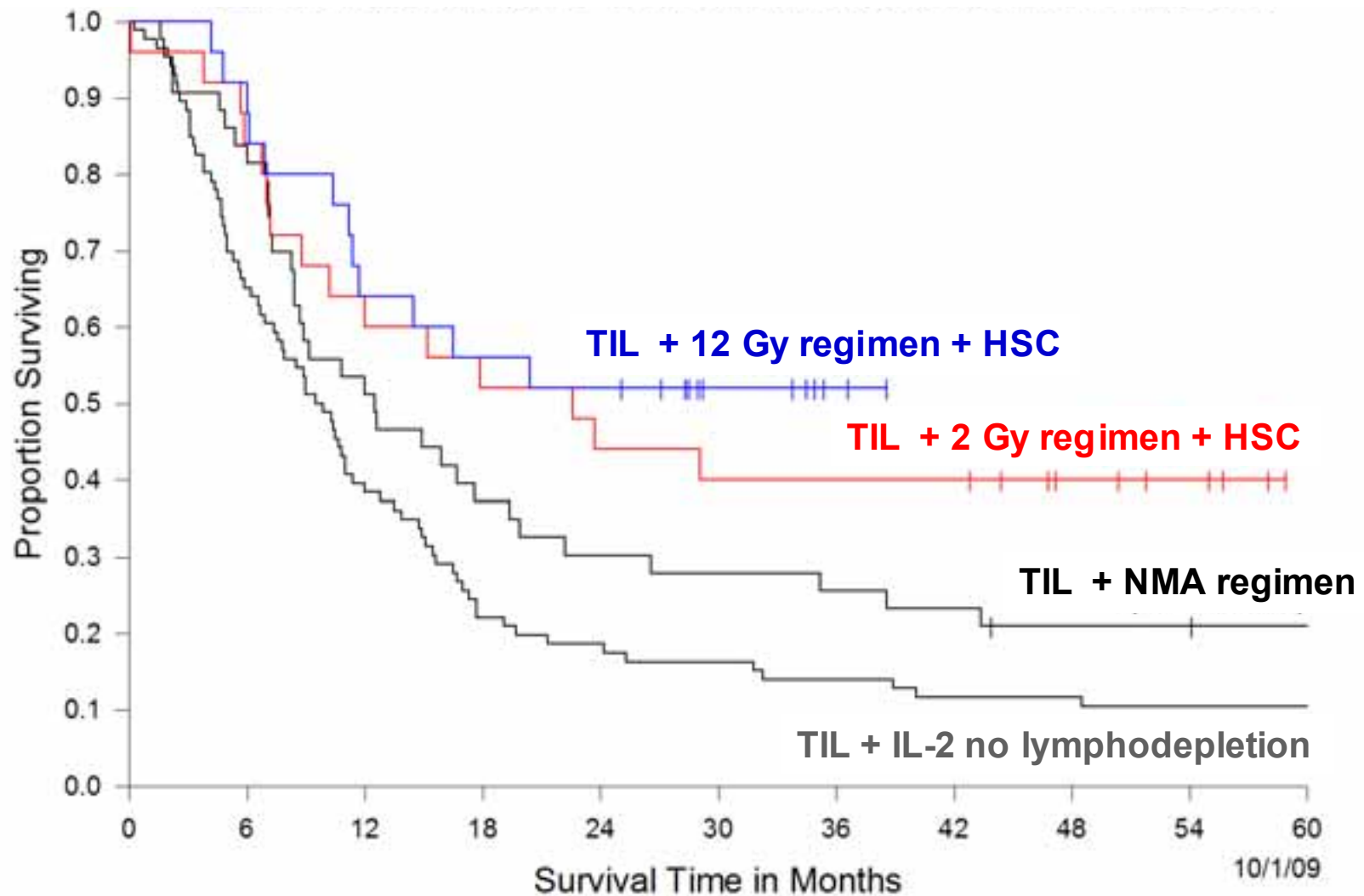
# Increased intensity lymphodepletion enhances treatment efficacy of adoptively transferred tumor-specific pmel-1 T cells

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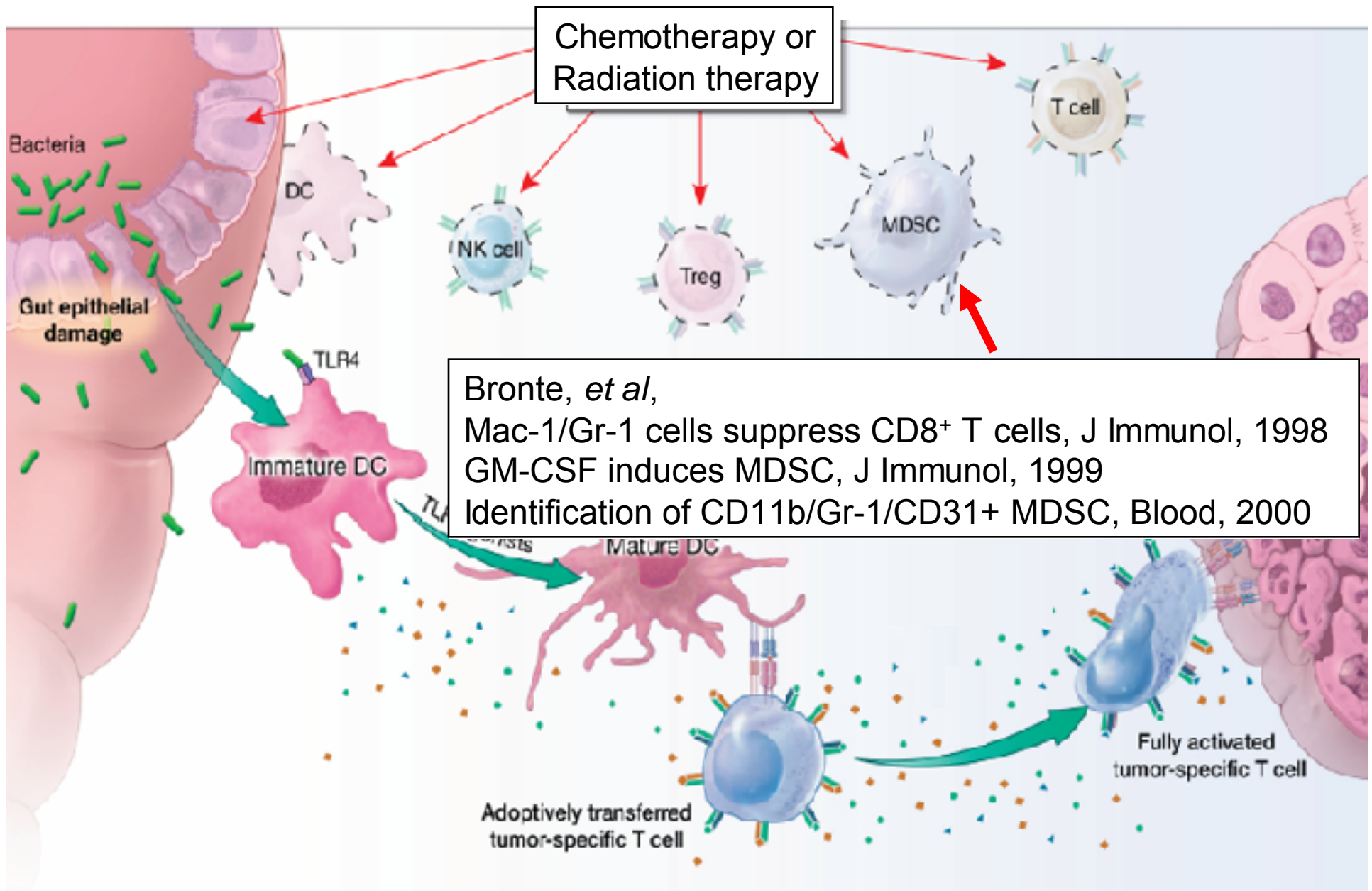


## Survival of patients with metastatic melanoma treated with autologous tumor-infiltrating lymphocytes (TIL) and IL-2: Impact of preparative regimens

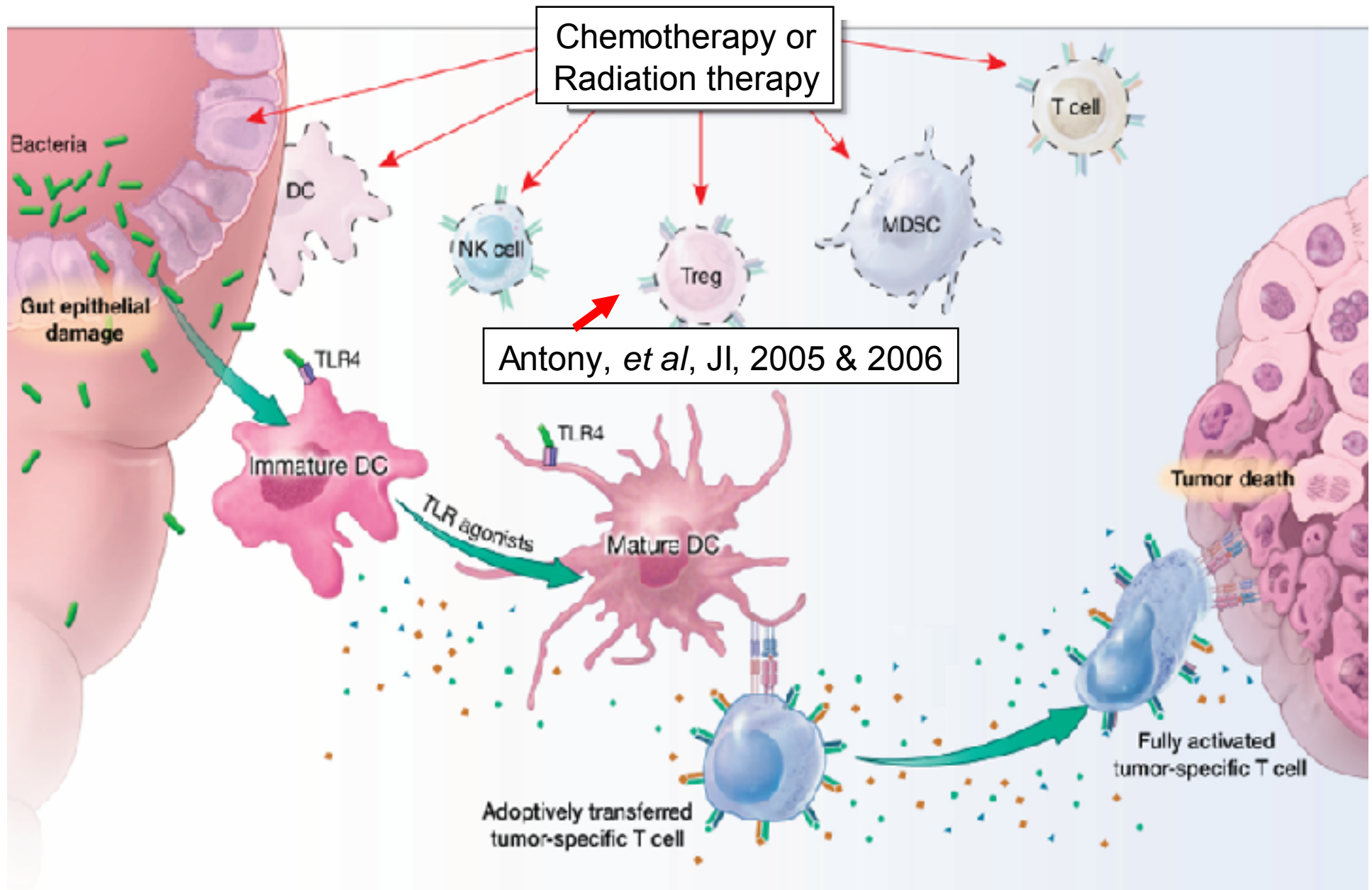
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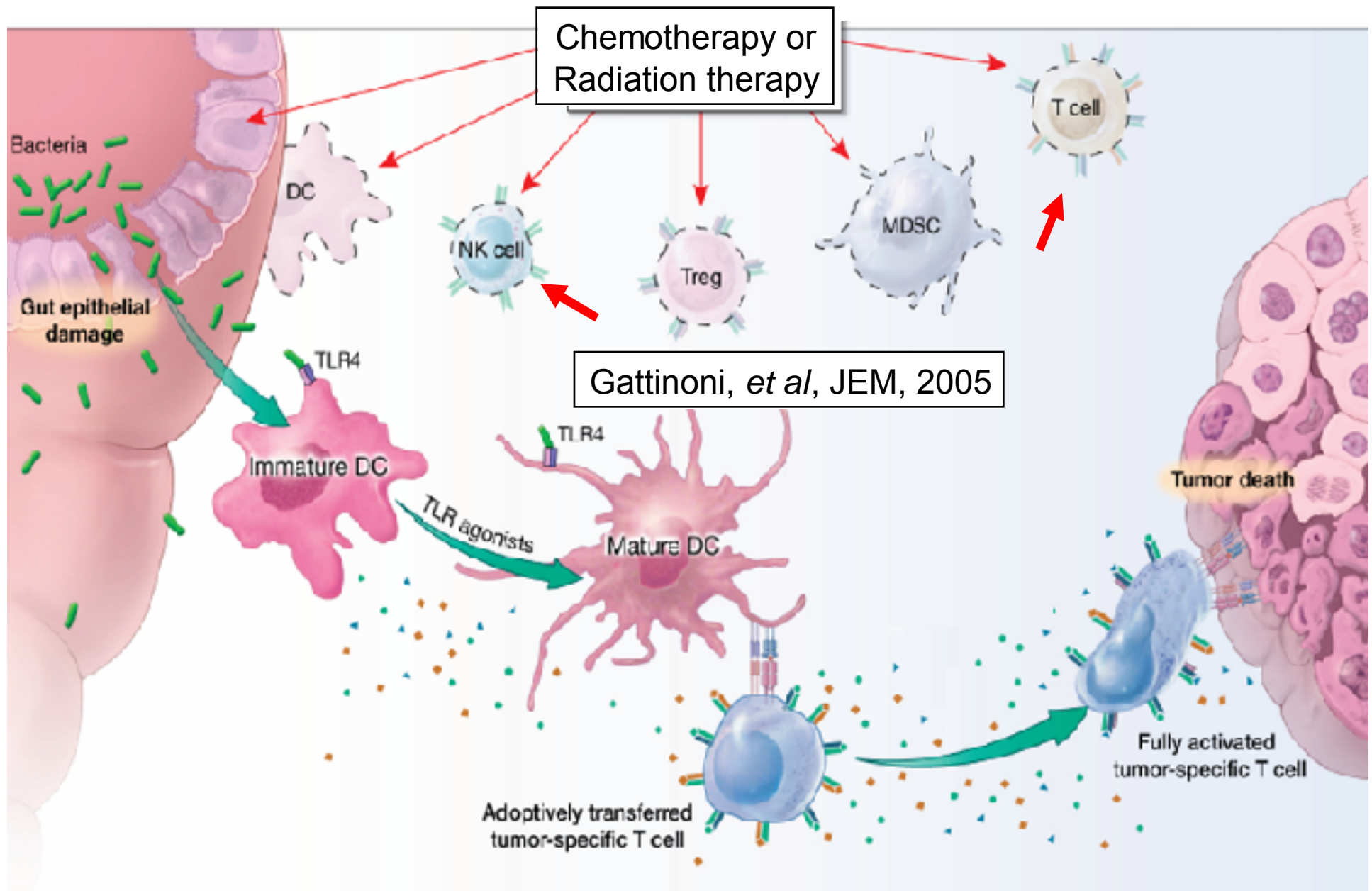
## How lymphodepletion augments the function of adoptively transferred T cells



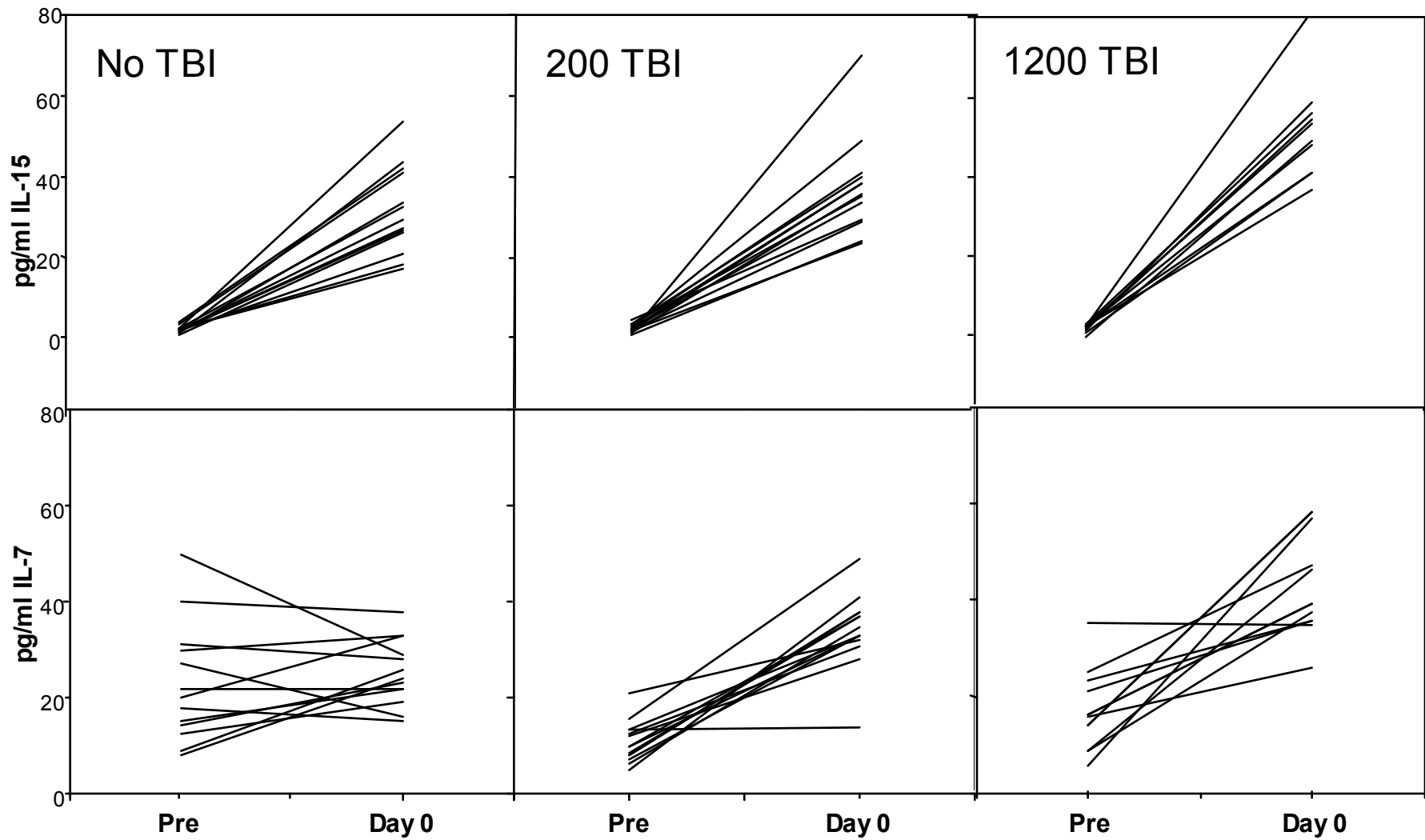
## How lymphodepletion augments the function of adoptively transferred T cells



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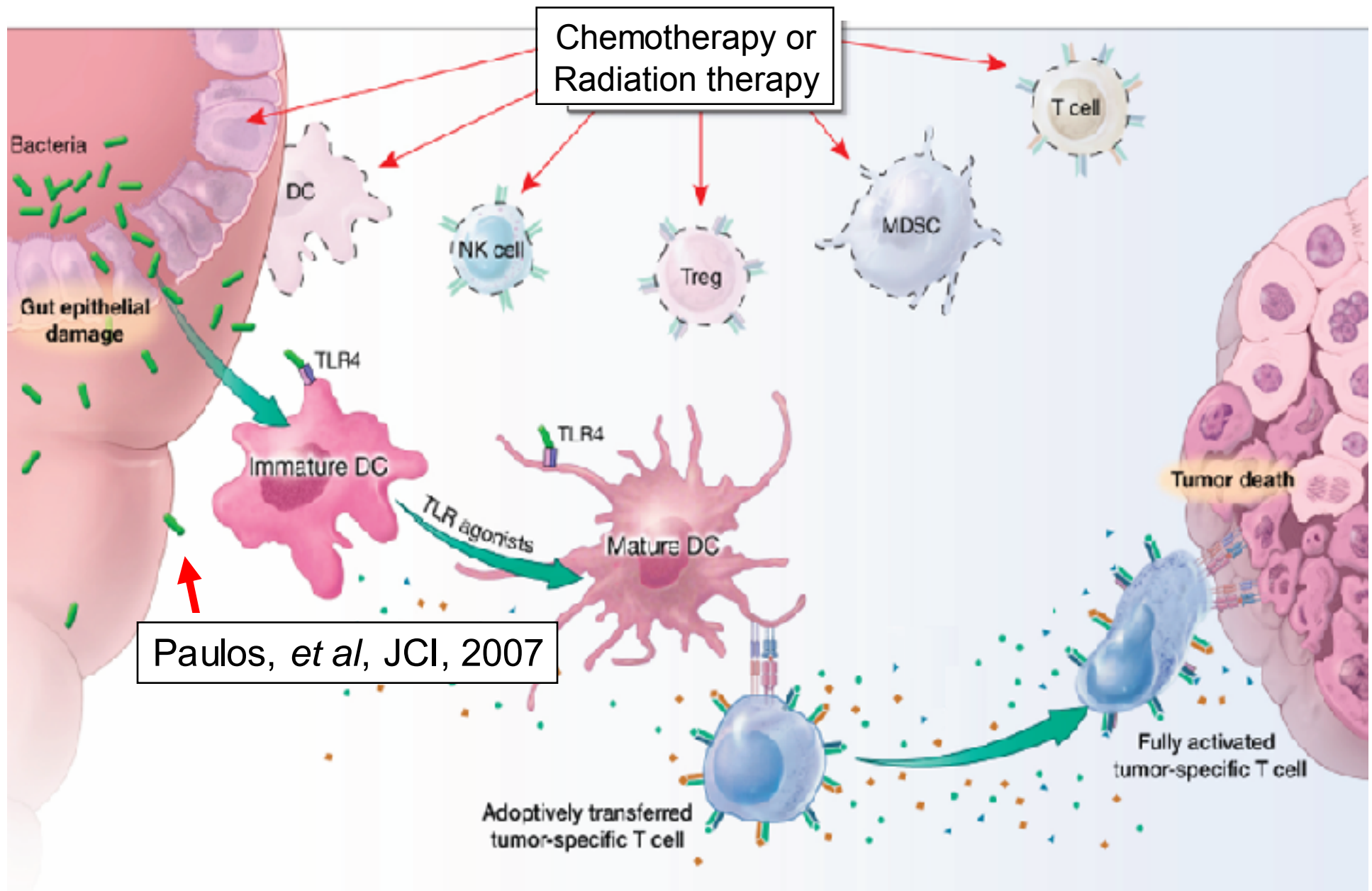


## Impact of Lymphodepletion on Serum Levels Of IL-15 and IL-7

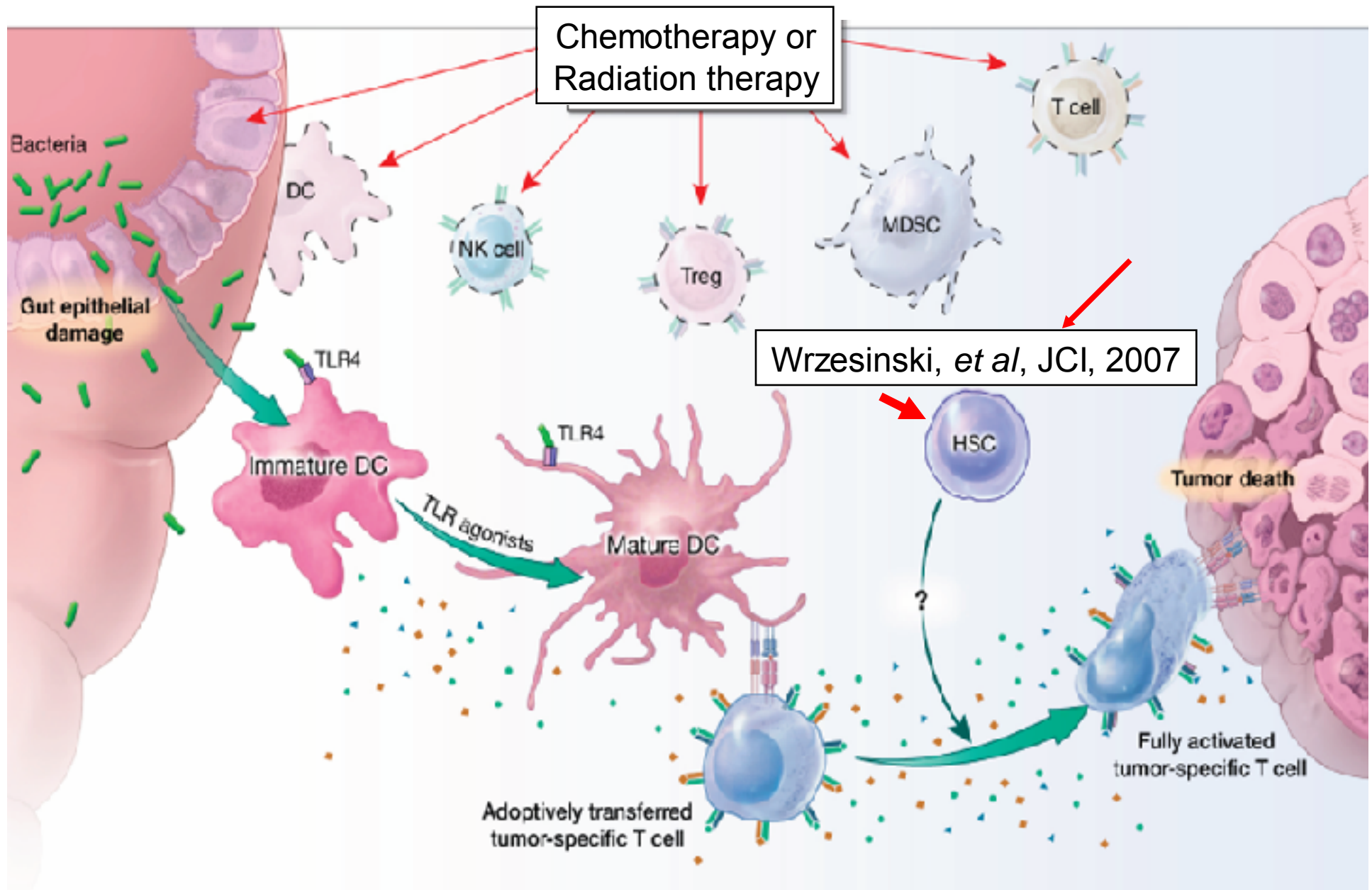


Dudley, *et al*, *J Clin Oncol*, 2008

## How lymphodepletion augments the function of adoptively transferred T cells



## Hematopoietic stem cells augment the function of adoptively transferred CD8<sup>+</sup> T cells



# Major Points

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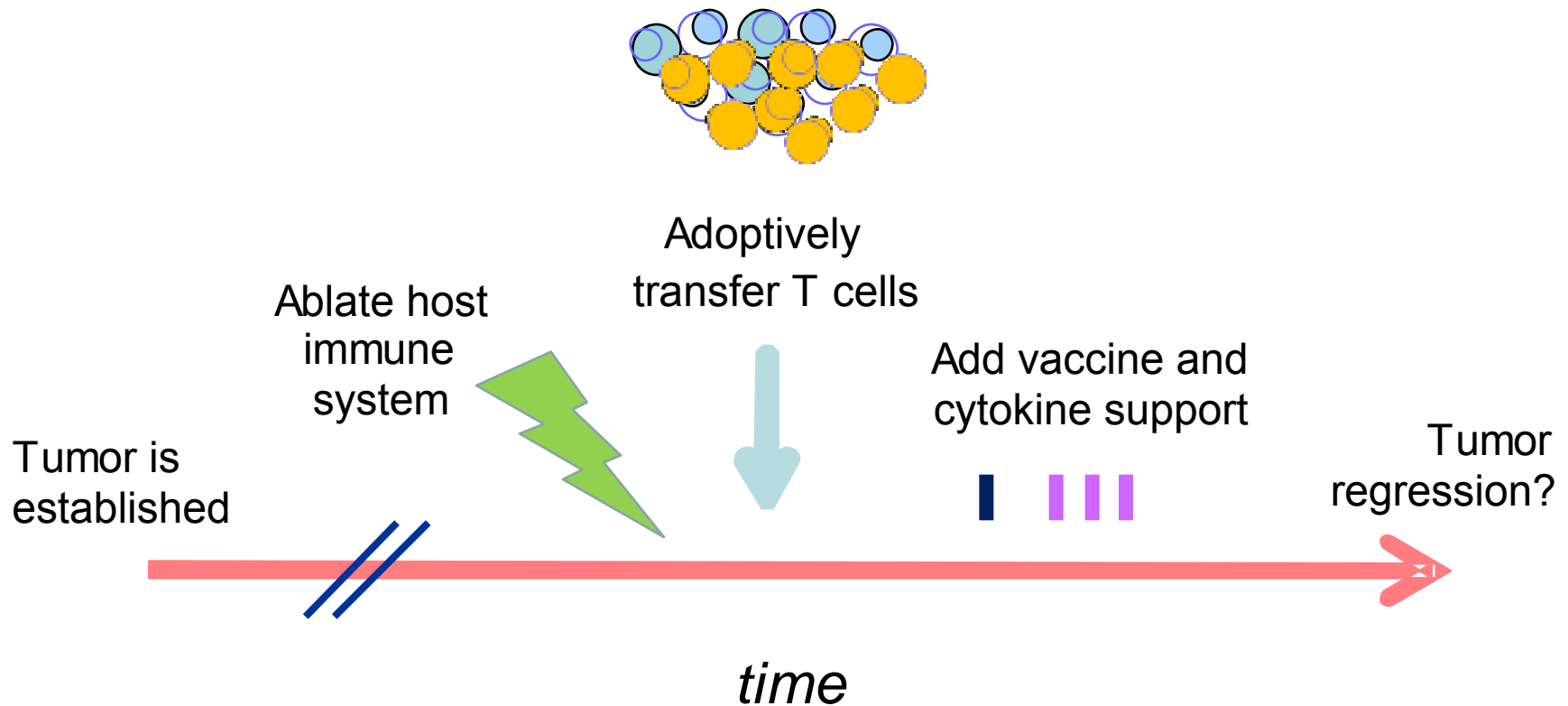
1. Cancer immunotherapies, especially those based on adoptive cell transfer (ACT) of tumor-specific T lymphocytes, can be curative in some patients with metastatic cancer.

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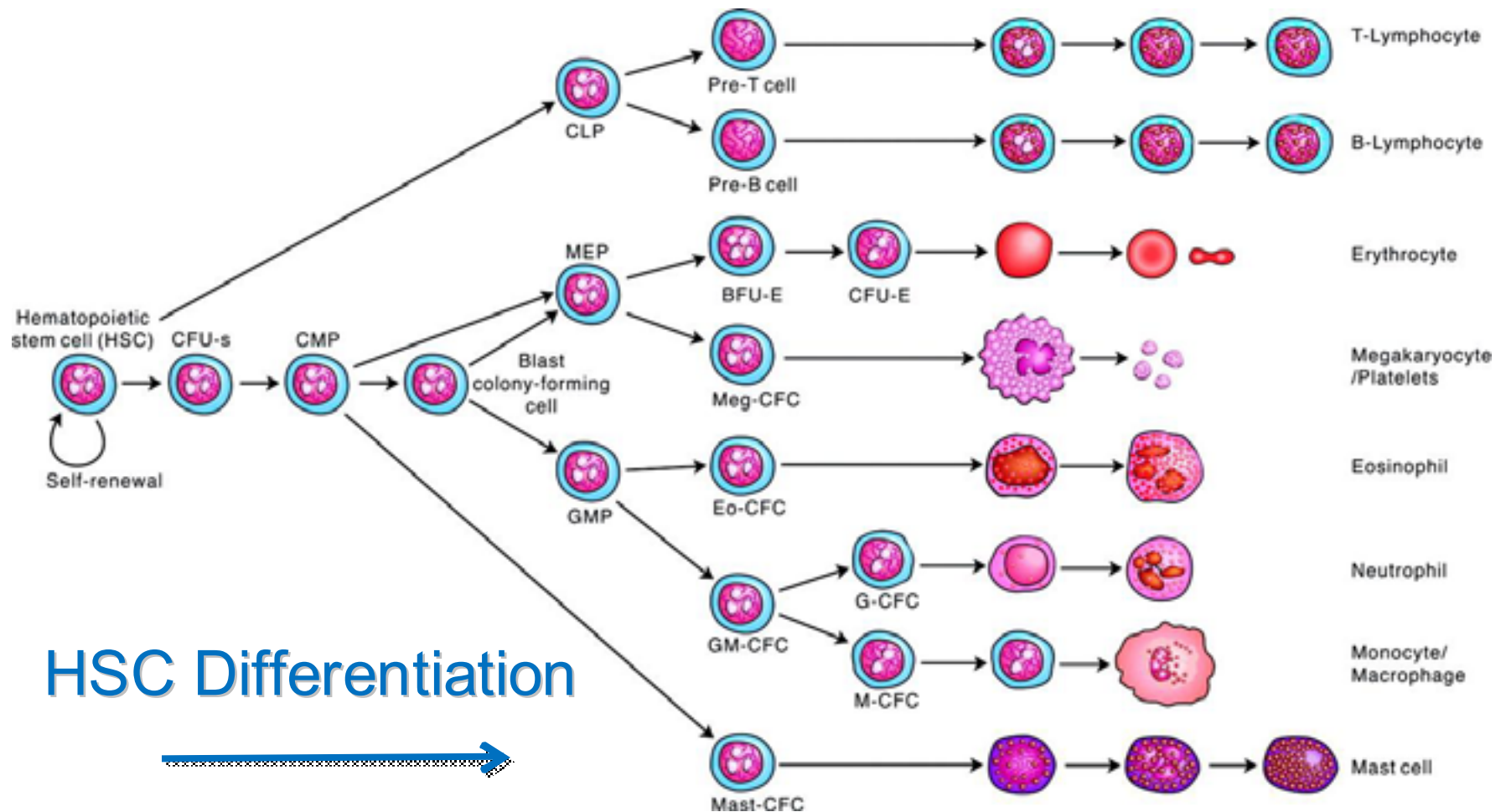
3. The type of tumor-specific T cell that is delivered and its level of differentiation can be manipulated to improve treatment outcomes.

# Adoptive cell transfer (ACT) in mouse and man

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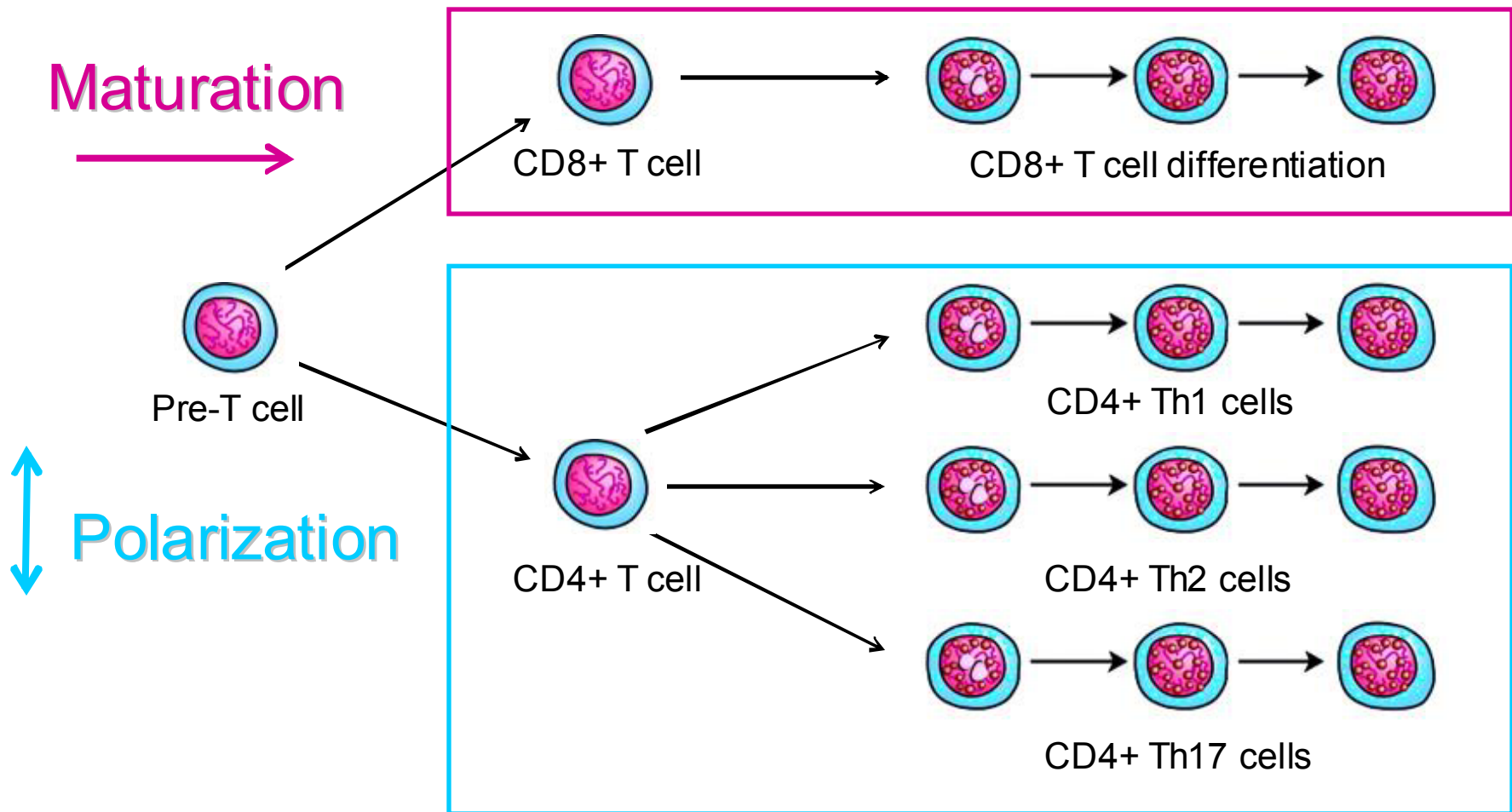


# Differentiation and maturation of mature cells derived from hematopoietic stem cells



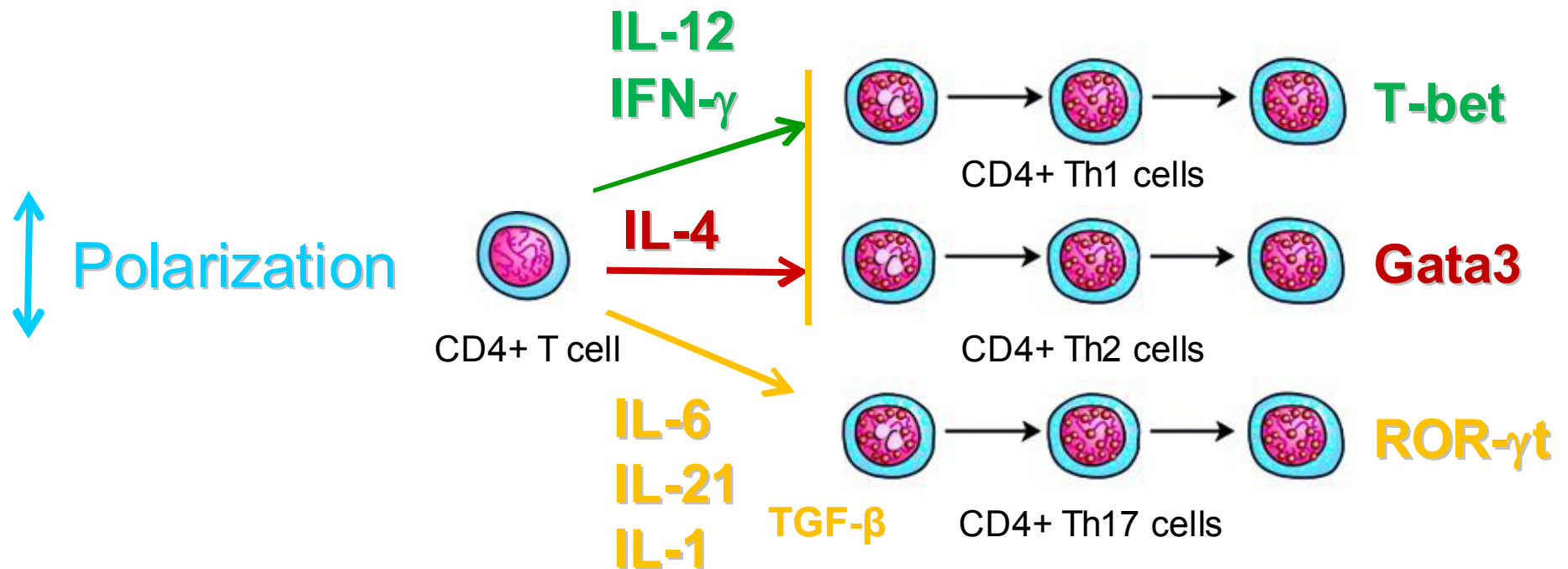
# Differentiation and maturation of post-thymic T cells

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# T cell differentiation and maturation

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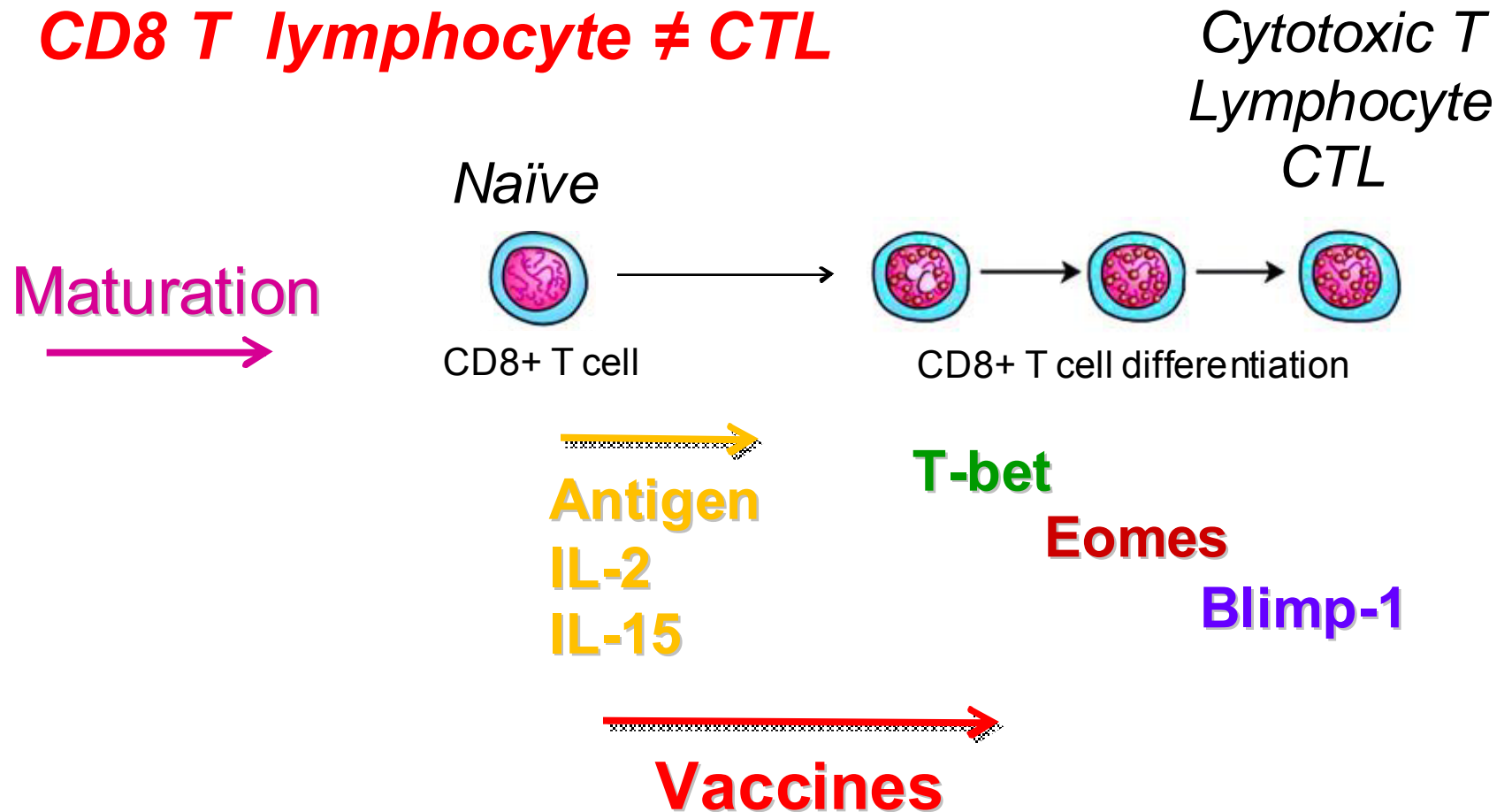


Muranski, *et al*, *Blood*, 2008 & 2009; *Curr Opin Immunol*, 2009 and  
Martin-Orozco, *et al*, *Immunity*, E-published, October 29, 2009

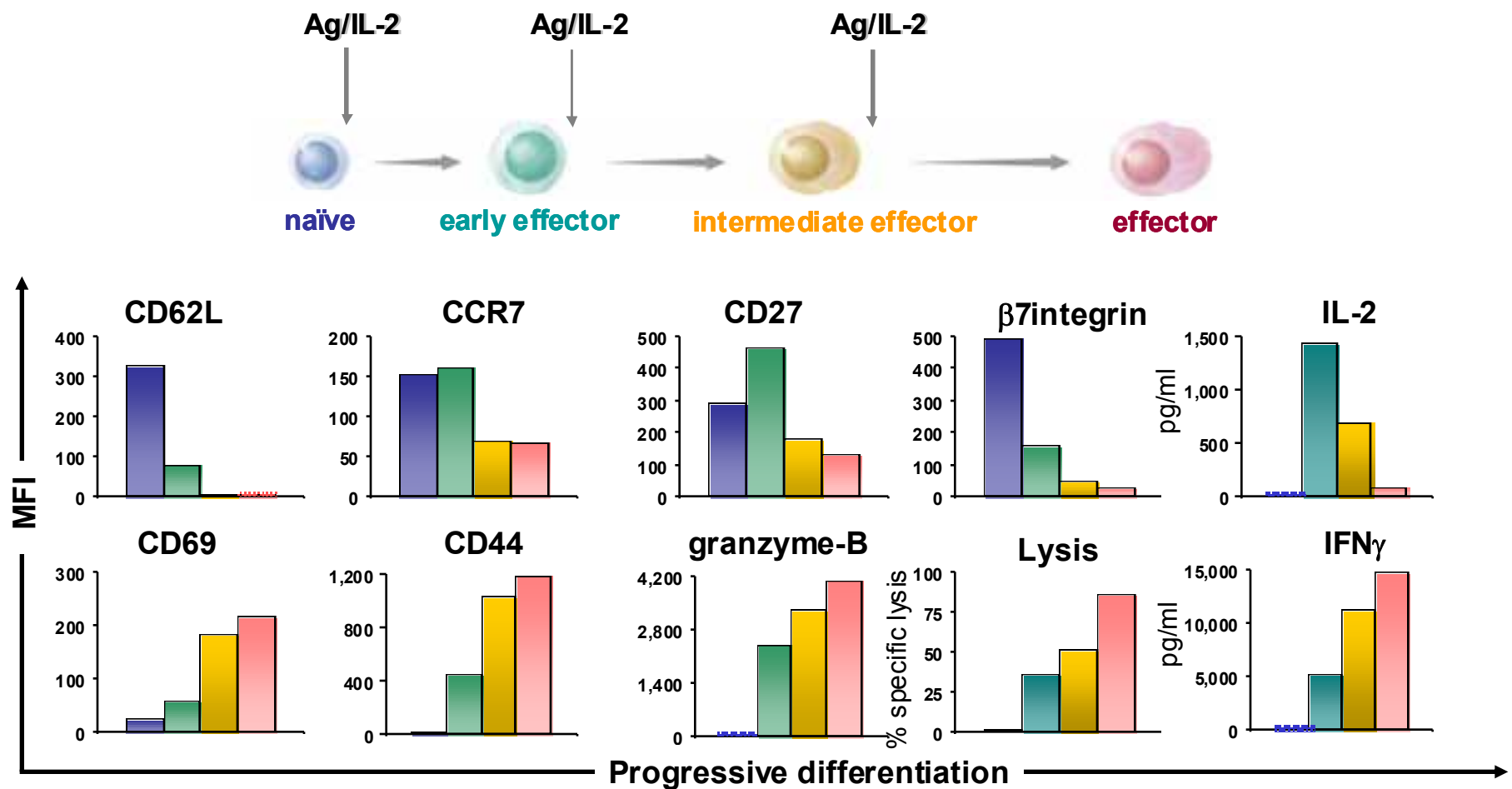
# T cell differentiation and maturation

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***CD8 T lymphocyte  $\neq$  CTL***



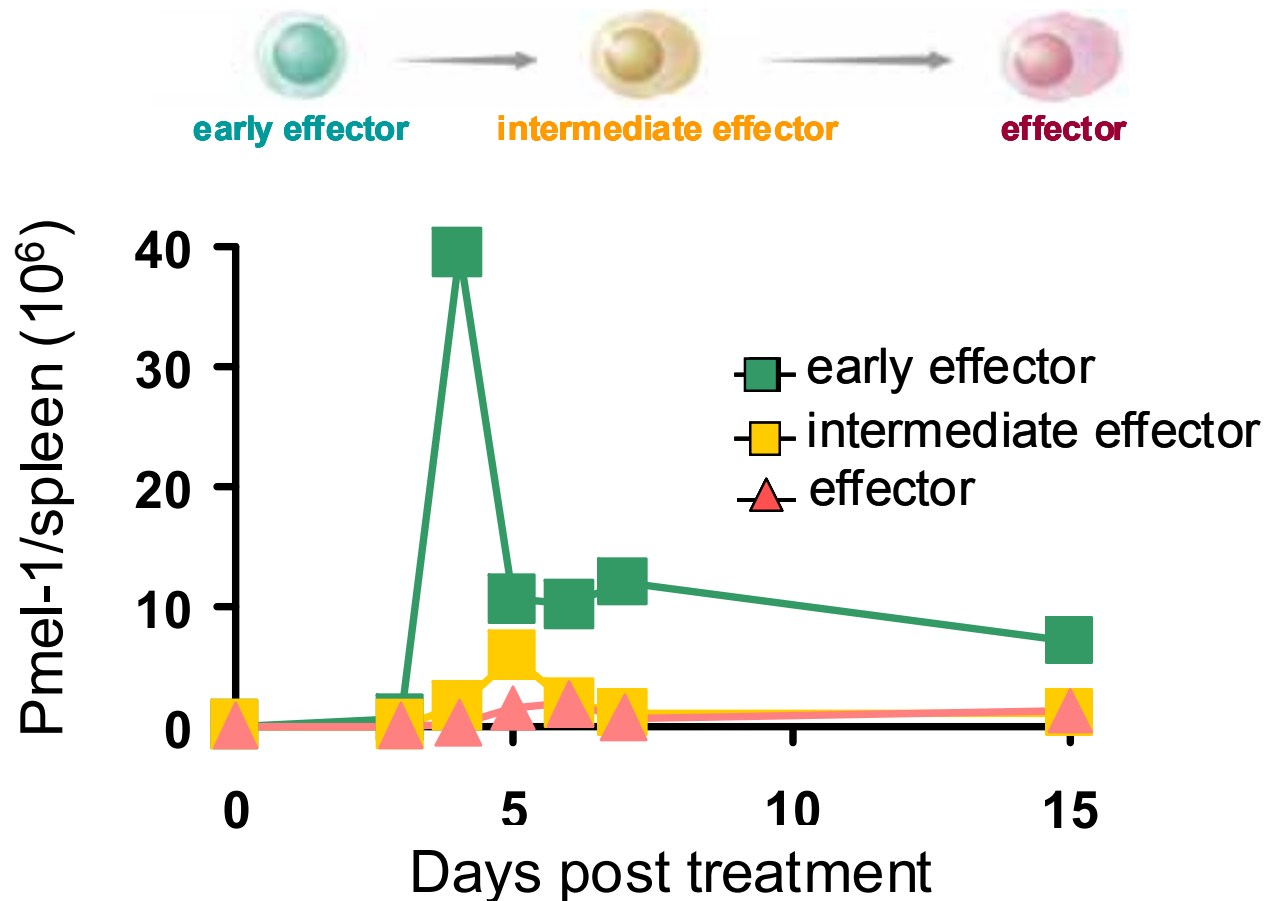
# Phenotypic and functional characteristics of pmel-1 CD8<sup>+</sup> T cells during differentiation



Gattinoni, *et al*, JCI, 2005

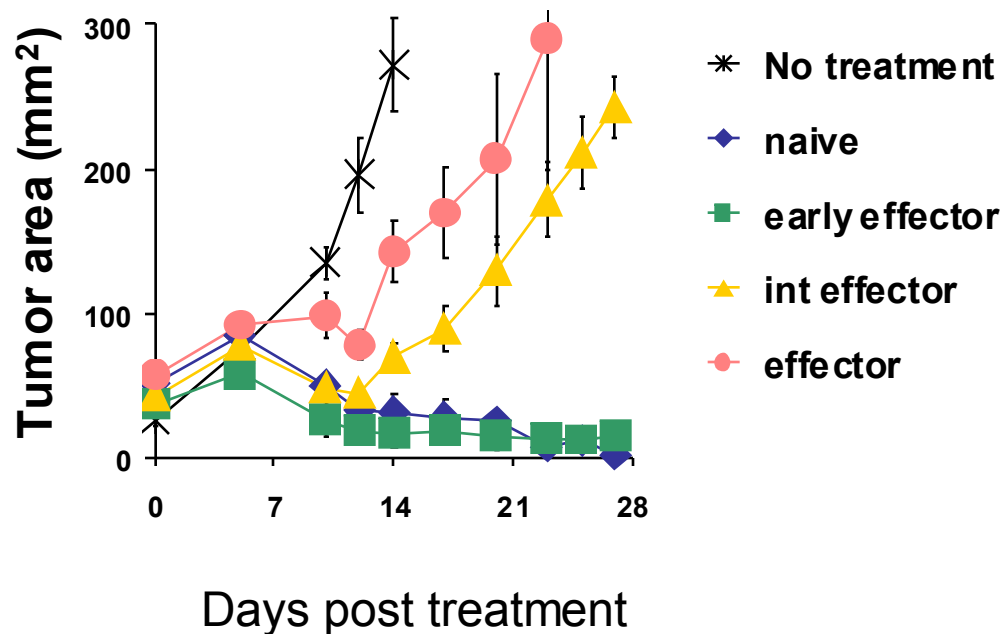
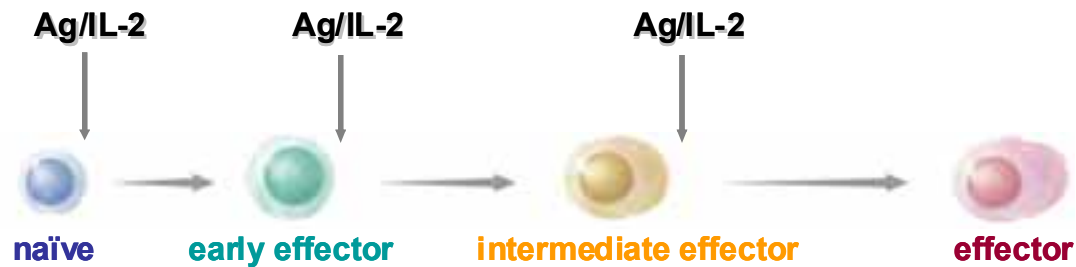
The differentiation state of CD8<sup>+</sup> T cells is inversely related to their proliferative capacity and persistence

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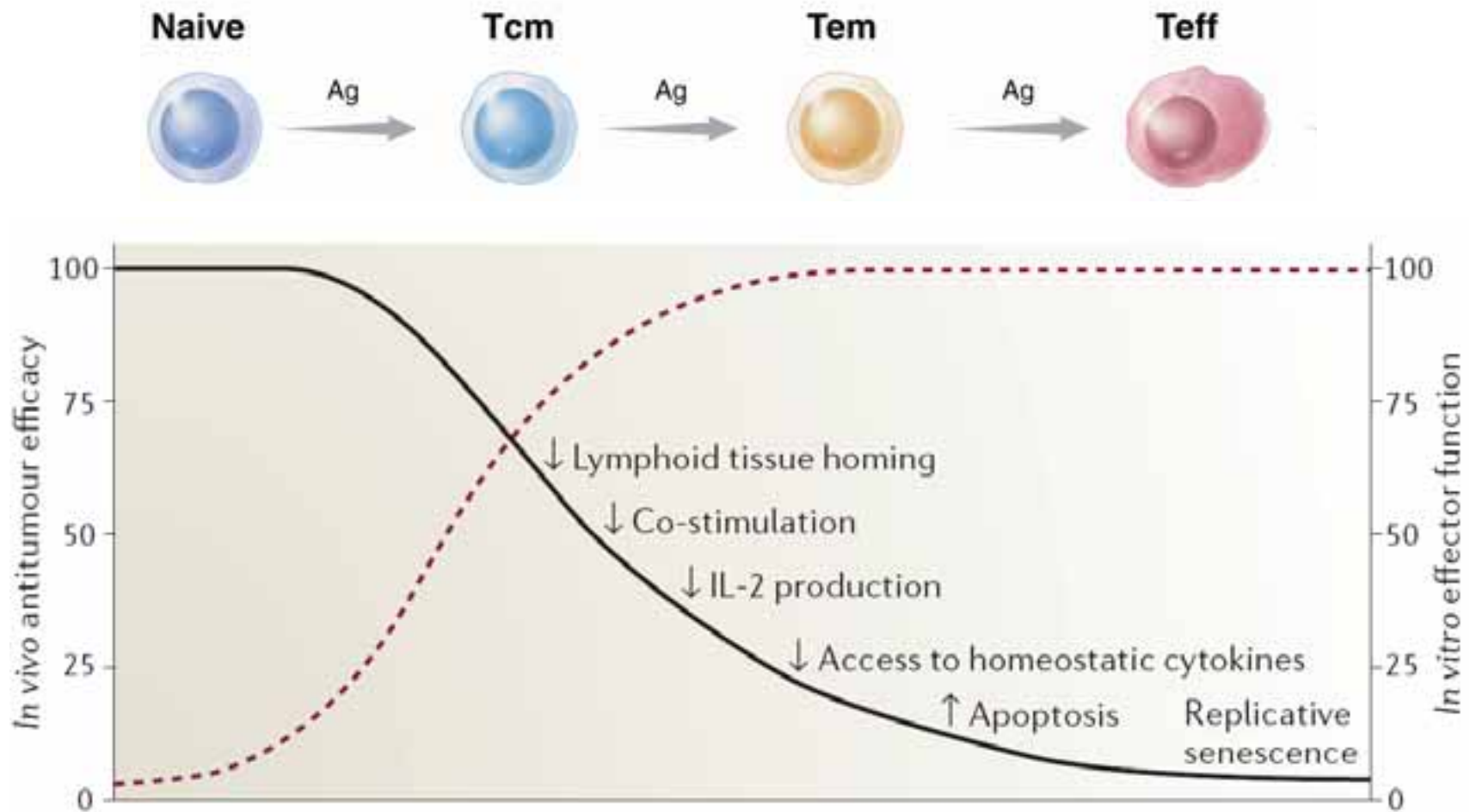
Gattinoni, *et al*, JCI, 2005

# The differentiation state of CD8<sup>+</sup> T cells is inversely related to their antitumor activity



Gattinoni, *et al*, JCI, 2005

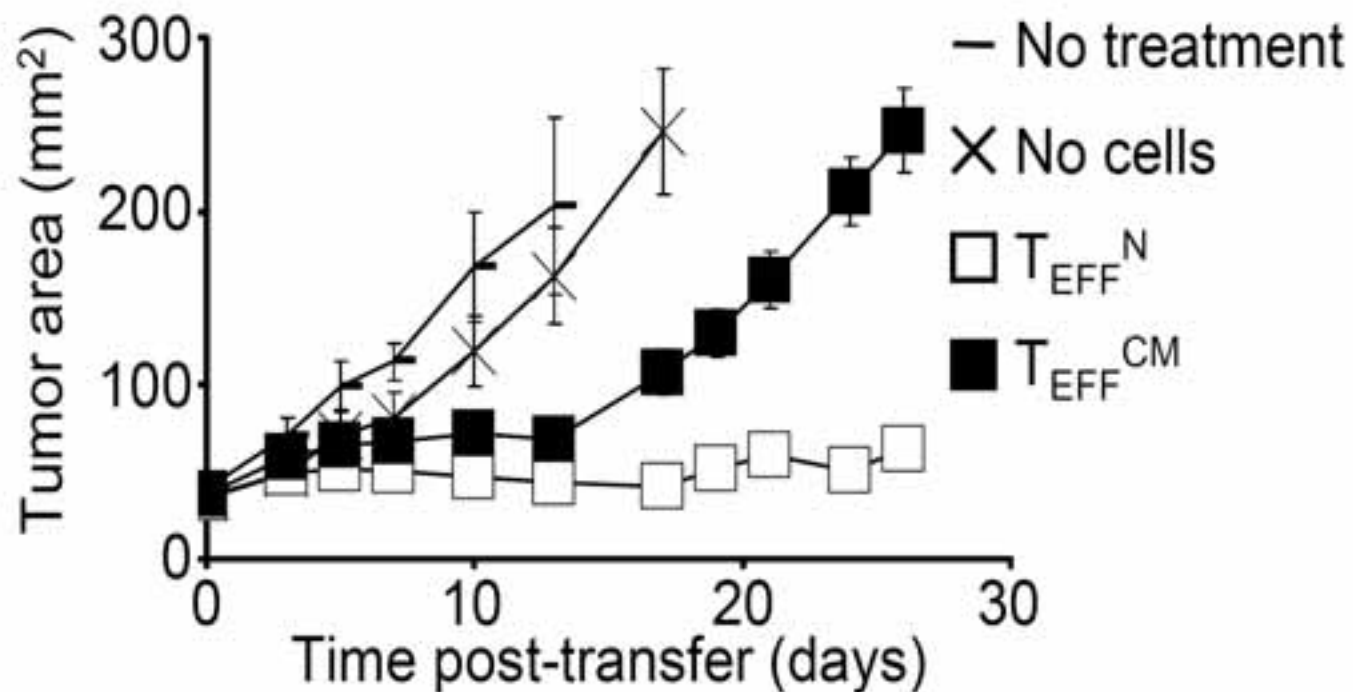
# Acquisition of terminal effector function *in vitro* impairs *in vivo* anti-tumor efficacy



Gattinoni et al. *Nature Rev Immunol* 2006

Effector cells derived from naïve CD8<sup>+</sup> T cells are more effective at treating tumor than those derived from CM cells

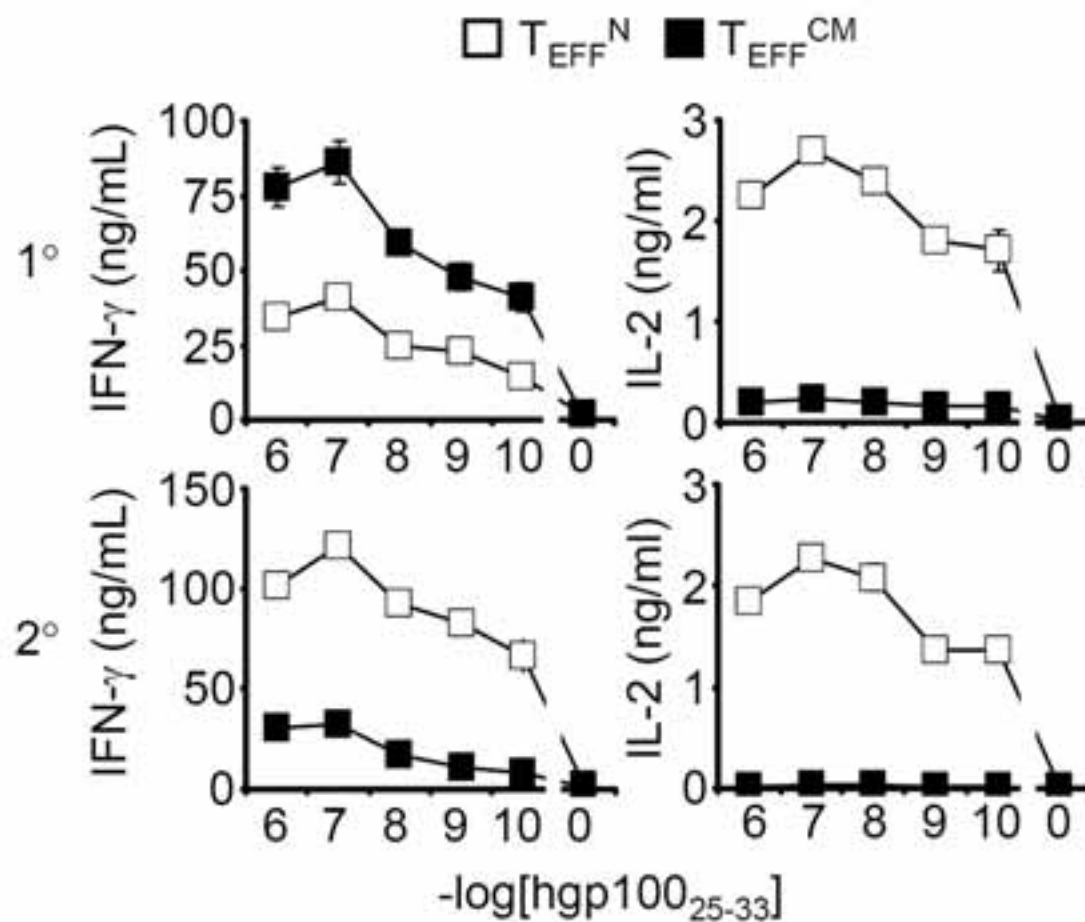
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Hinrichs, *In Press, Proc Natl Acad Sci*, 2009

Effector cells derived from naïve CD8<sup>+</sup> T cells maintain the ability to release IFN- $\gamma$  and IL-2

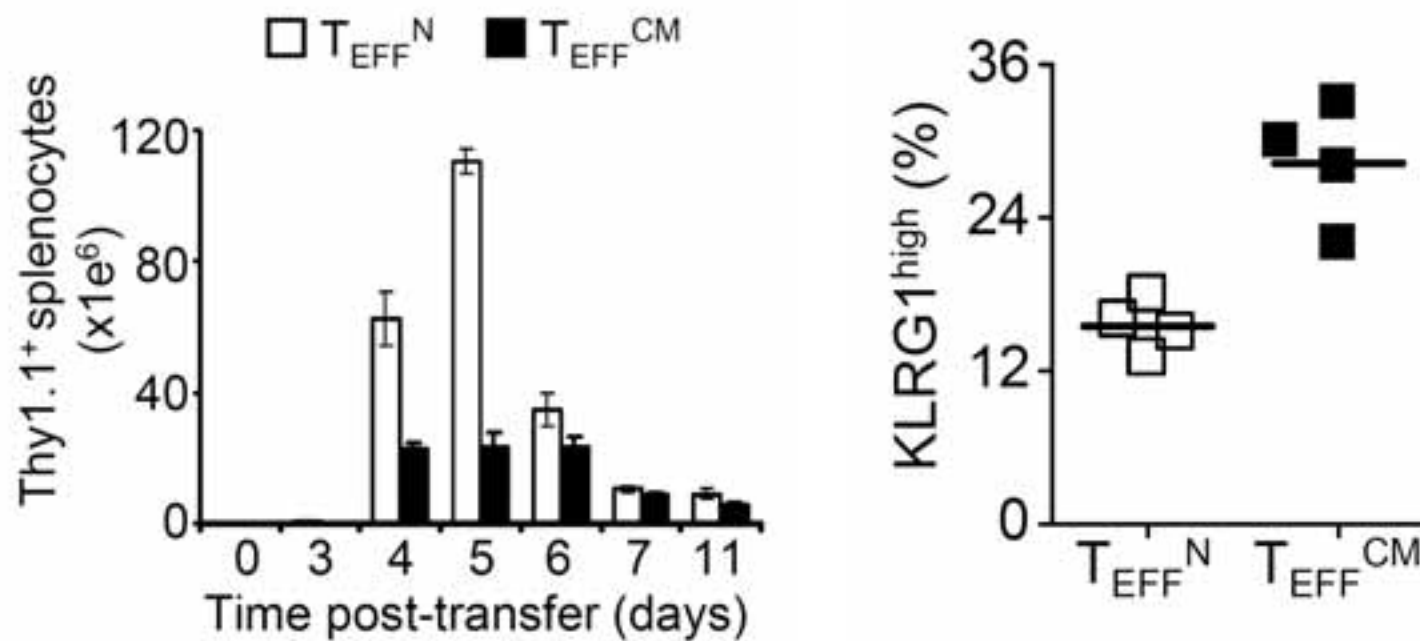
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Hinrichs, *In Press, Proc Natl Acad Sci*, 2009

Effector cells derived from naïve CD8<sup>+</sup> T cells persist longer than those derived from CM *in vivo*

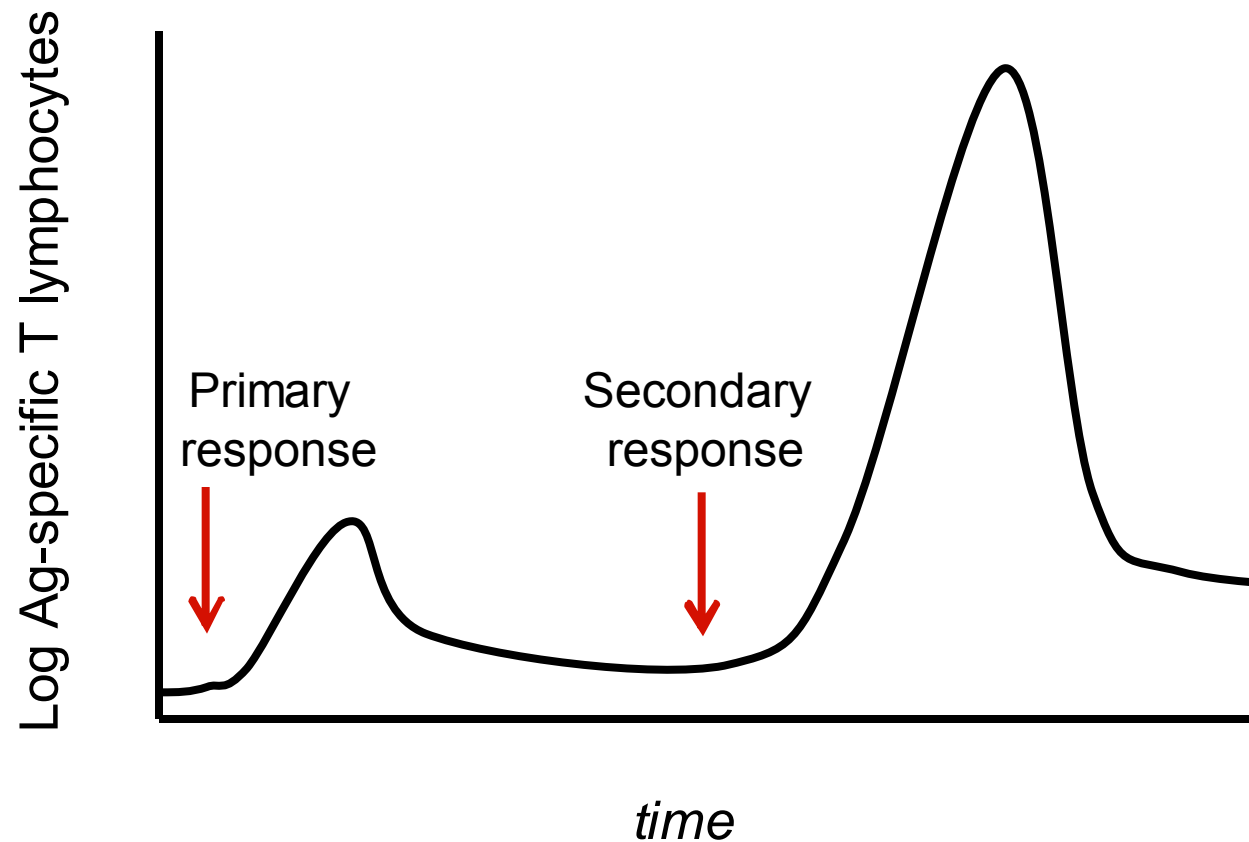
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Hinrichs, *In Press, Proc Natl Acad Sci*, 2009

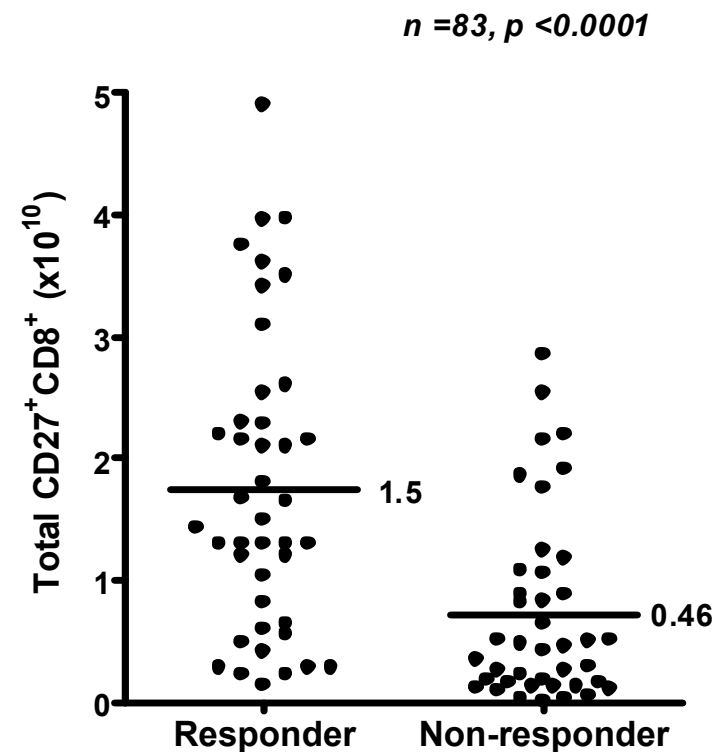
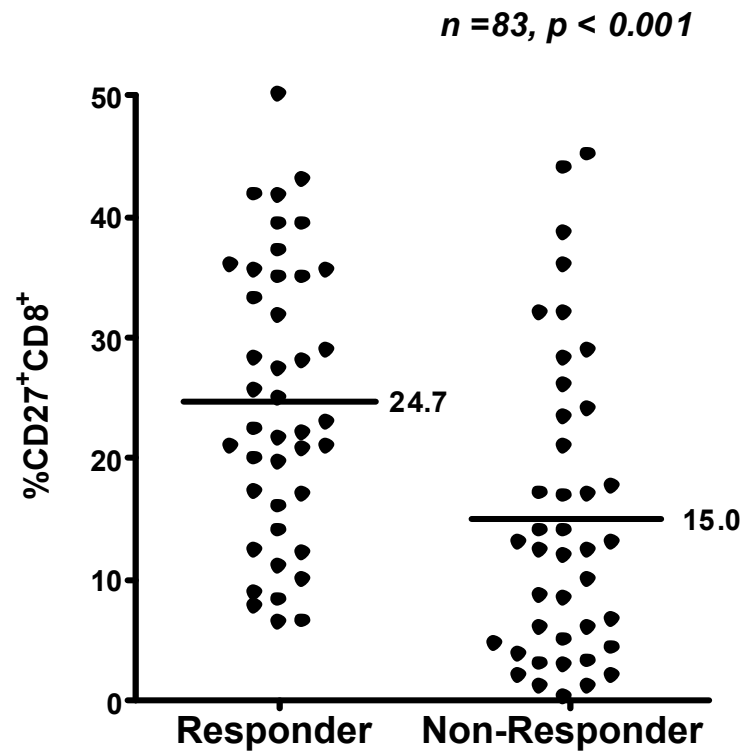
T lymphocyte responses to secondary antigenic stimulation are bigger, faster and stronger

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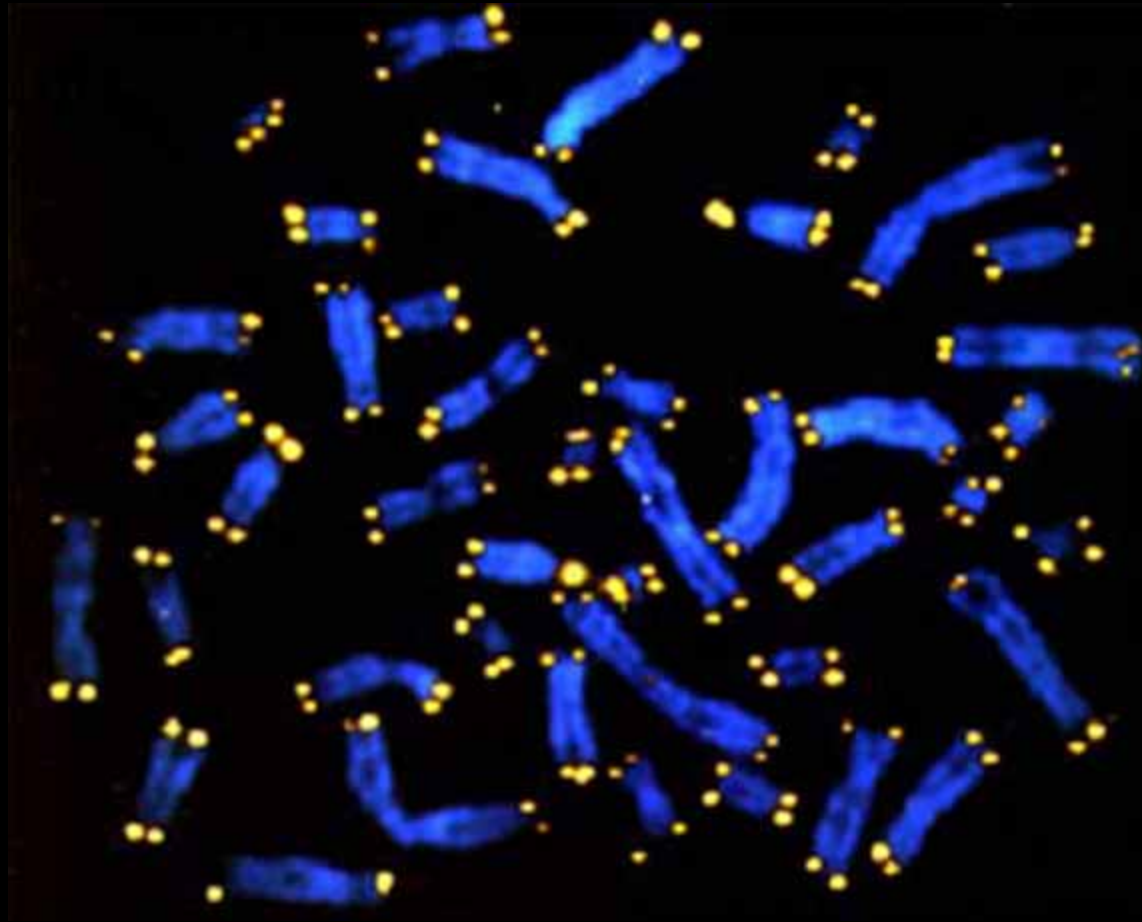
# Cellular differentiation of adoptively transferred T cells is inversely correlated with clinical response

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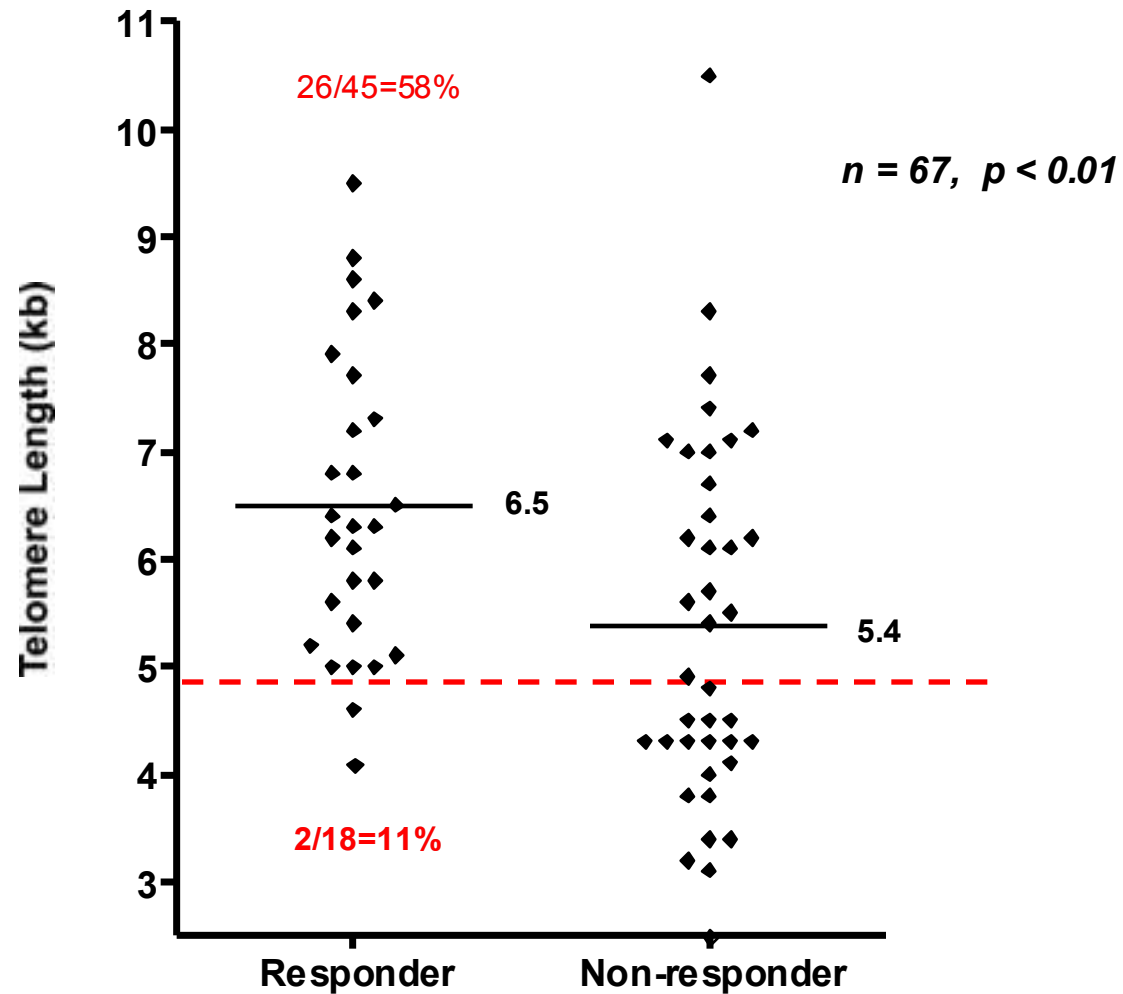


Huang, *et al*, *J Immunother*, 2005

Telomere: The end of a chromosome, a specialized structure involved in the replication and stability of the chromosome.



# Telomere length of the infusion TIL correlates with the response

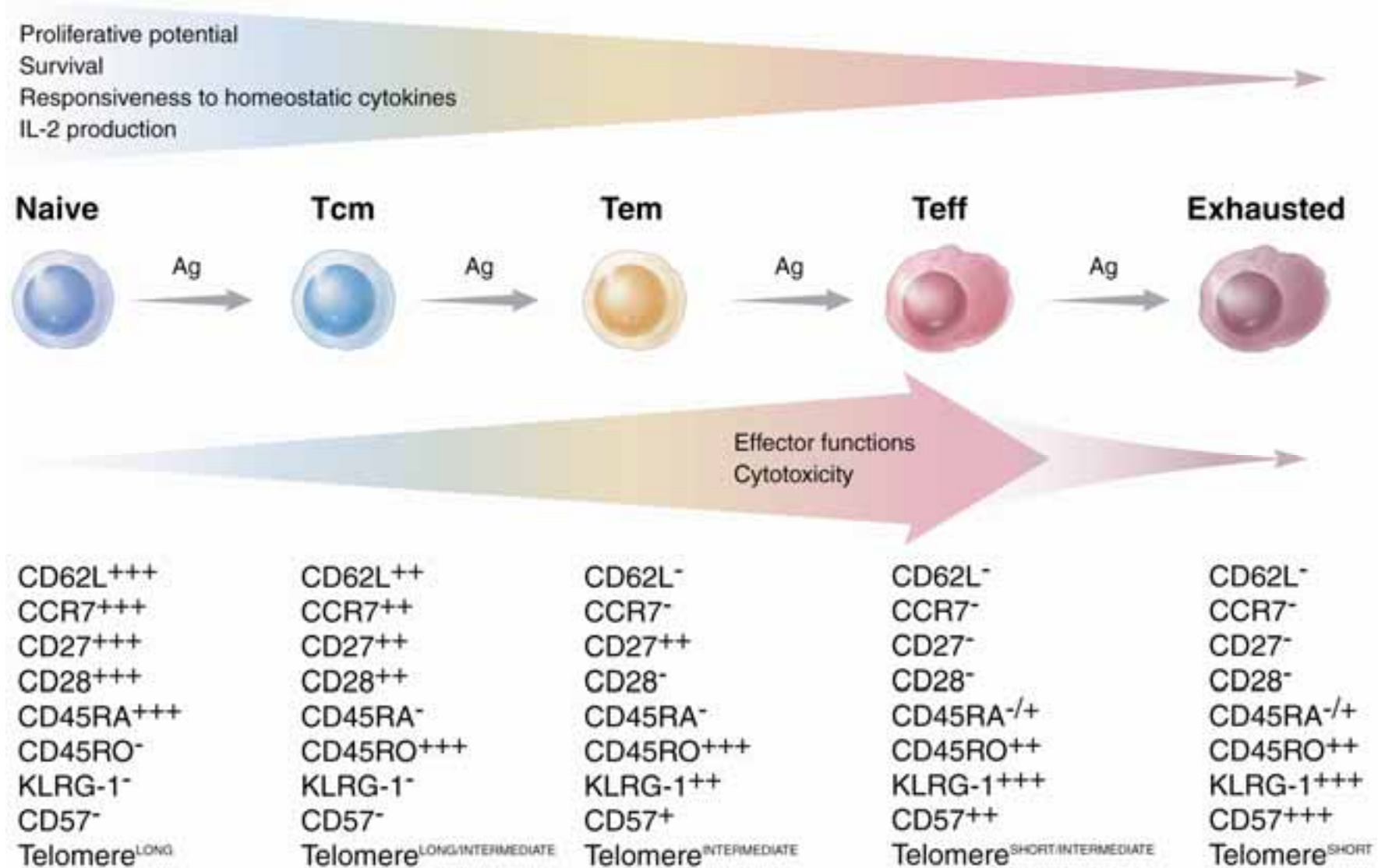


Zhou, et al, *J Immunol*, 2005

Summary: Younger cells are better in mouse  
and in man

How can we generate even younger cells?

# Phenotypic and functional changes in CD8<sup>+</sup> T cells



Klebanoff, *et al*, *Immunol Rev*, 2006

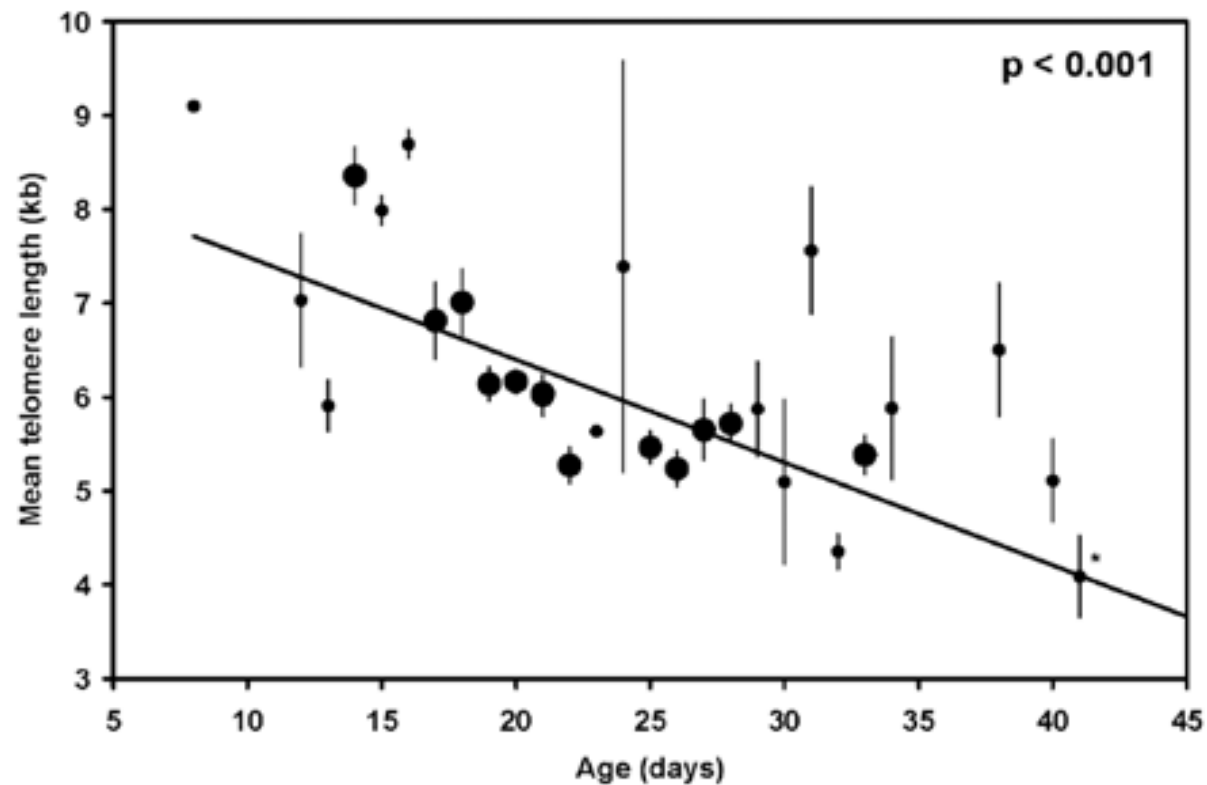
# Patient protocol based on “young TIL”

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Phase II study using **short-term cultured** TIL following a non-myeloablative lymphocyte depleting chemotherapy regimen in metastatic melanoma.”

# Telomere length decreased over time as TIL were maintained in culture

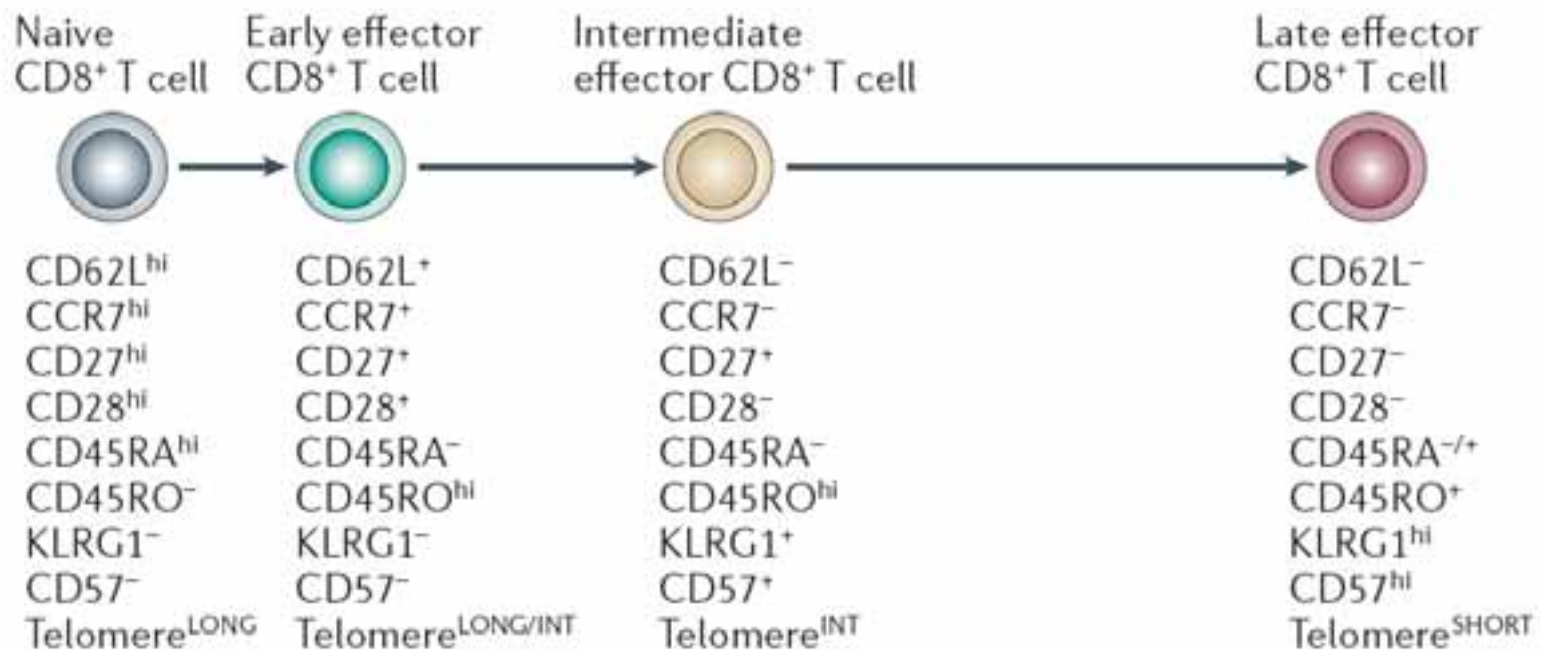
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Tran, *et al*, *J Immunother*, 2008

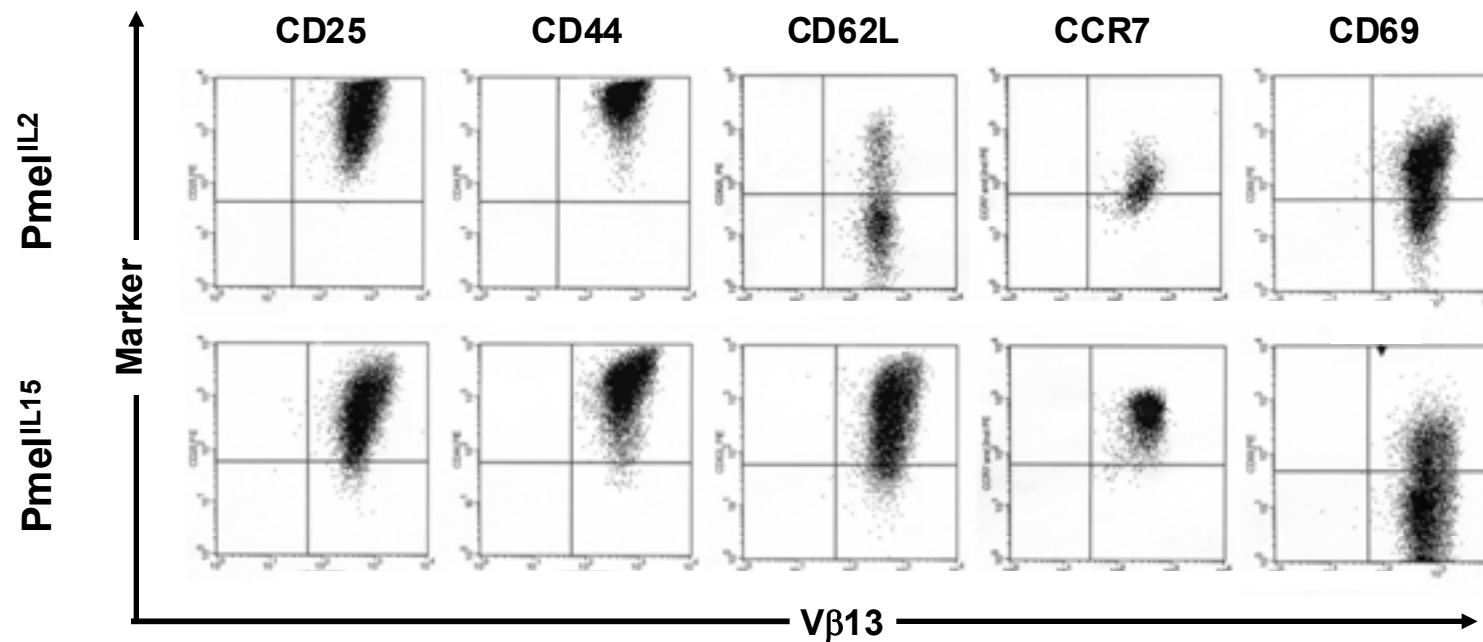
# Modulation of T cell differentiation through $\gamma_C$ -cytokines

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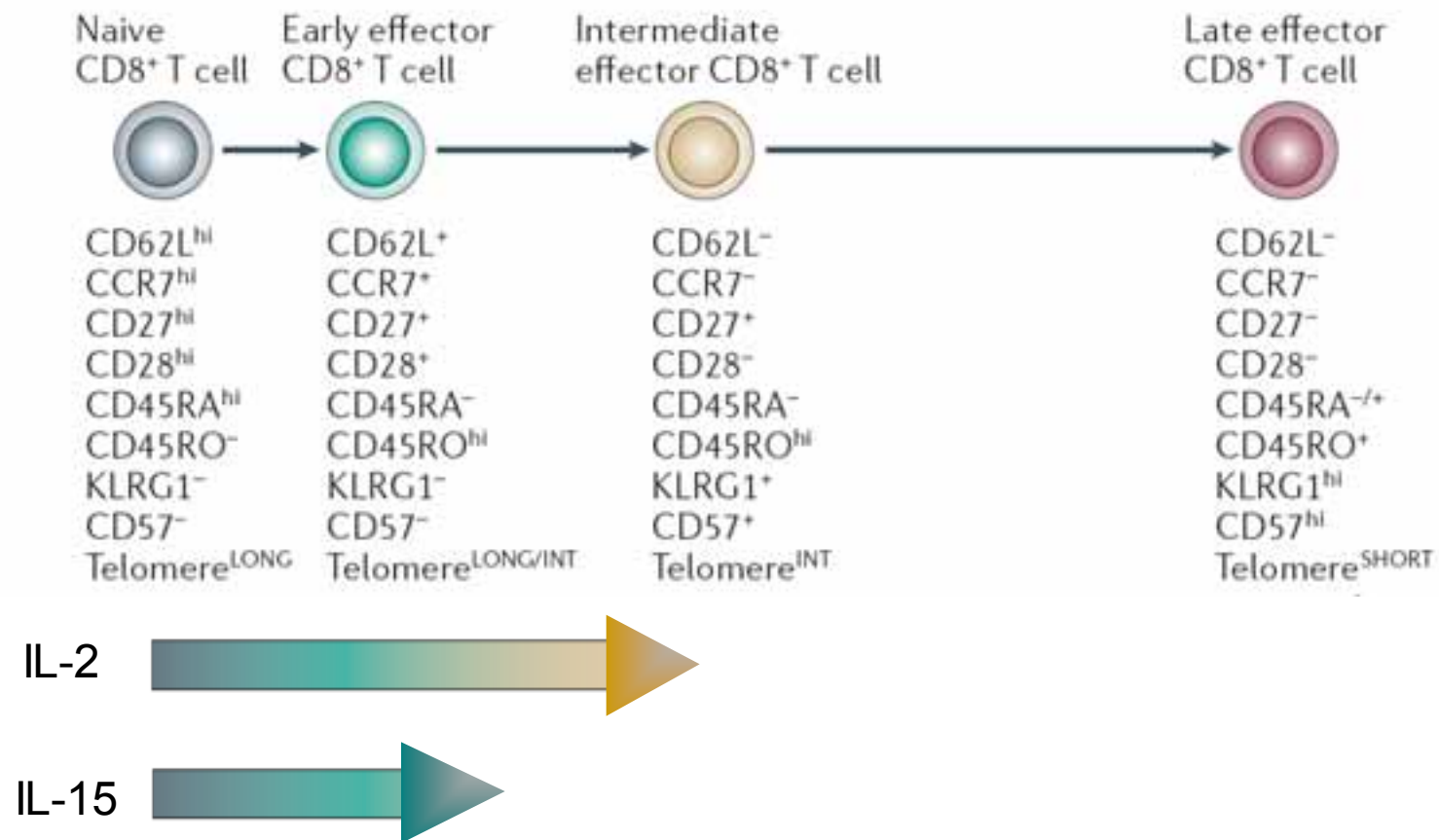
## IL-15 promotes the generation of T<sub>CM</sub>-like CD8<sup>+</sup> T cells

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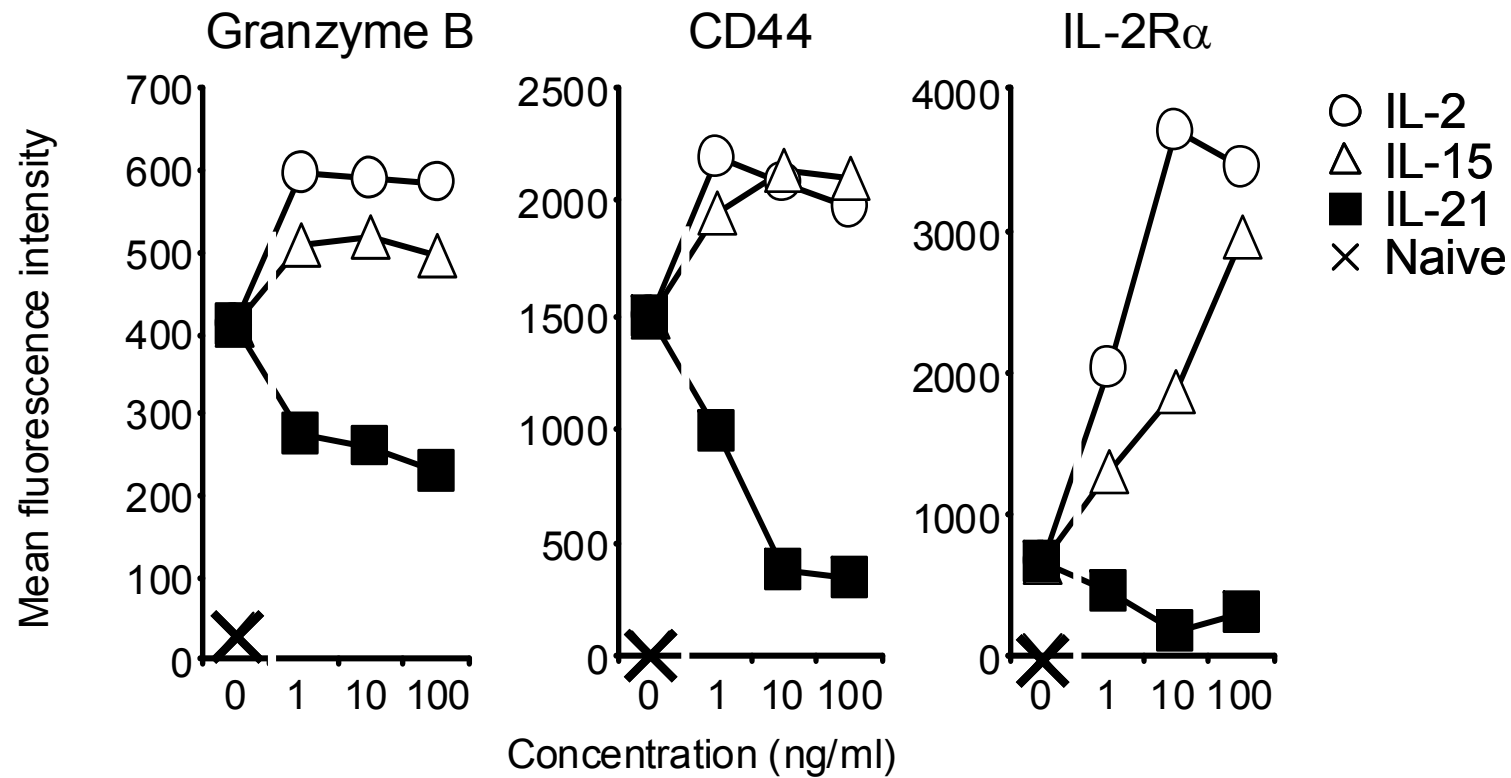
Klebanoff CA et al, PNAS 2004 and 2005

# Modulation of T cell differentiation through $\gamma_C$ -cytokines



# IL-21 inhibits acquisition of effector CD8<sup>+</sup> T-cell phenotype

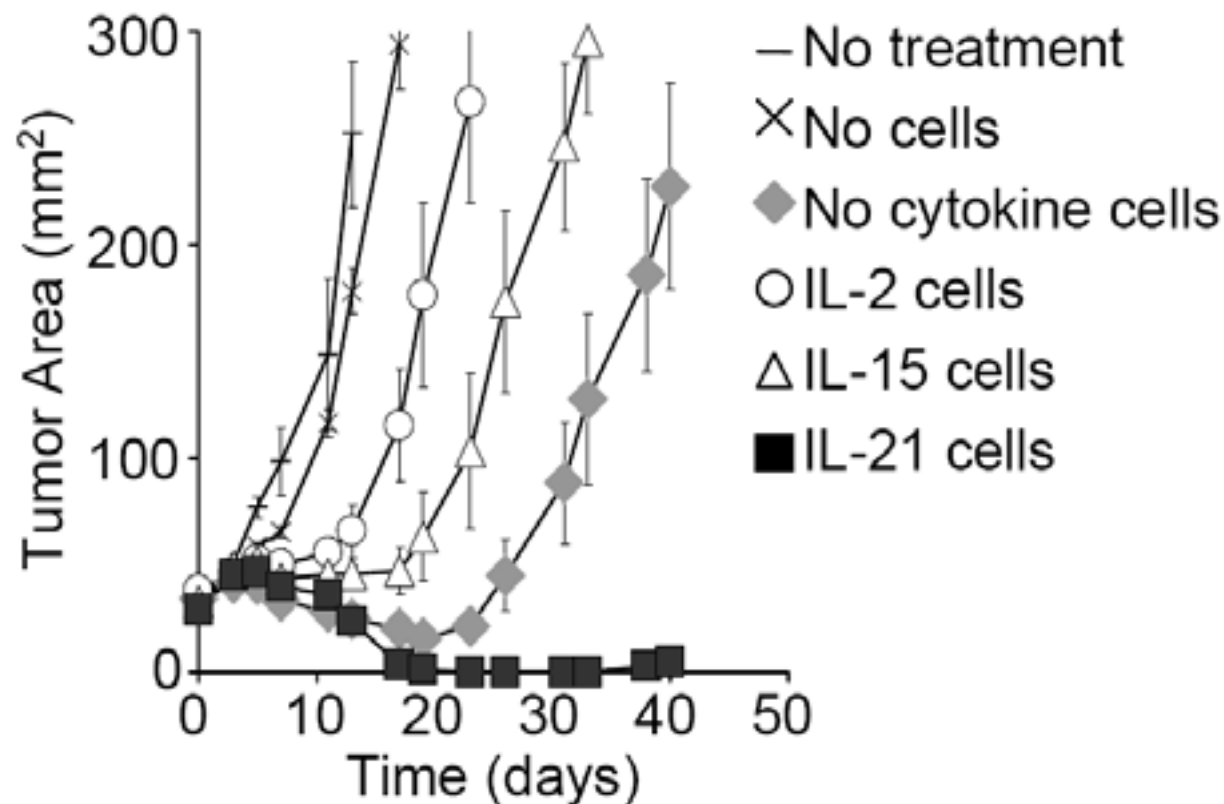
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Hinrichs, et al, Blood, 2008

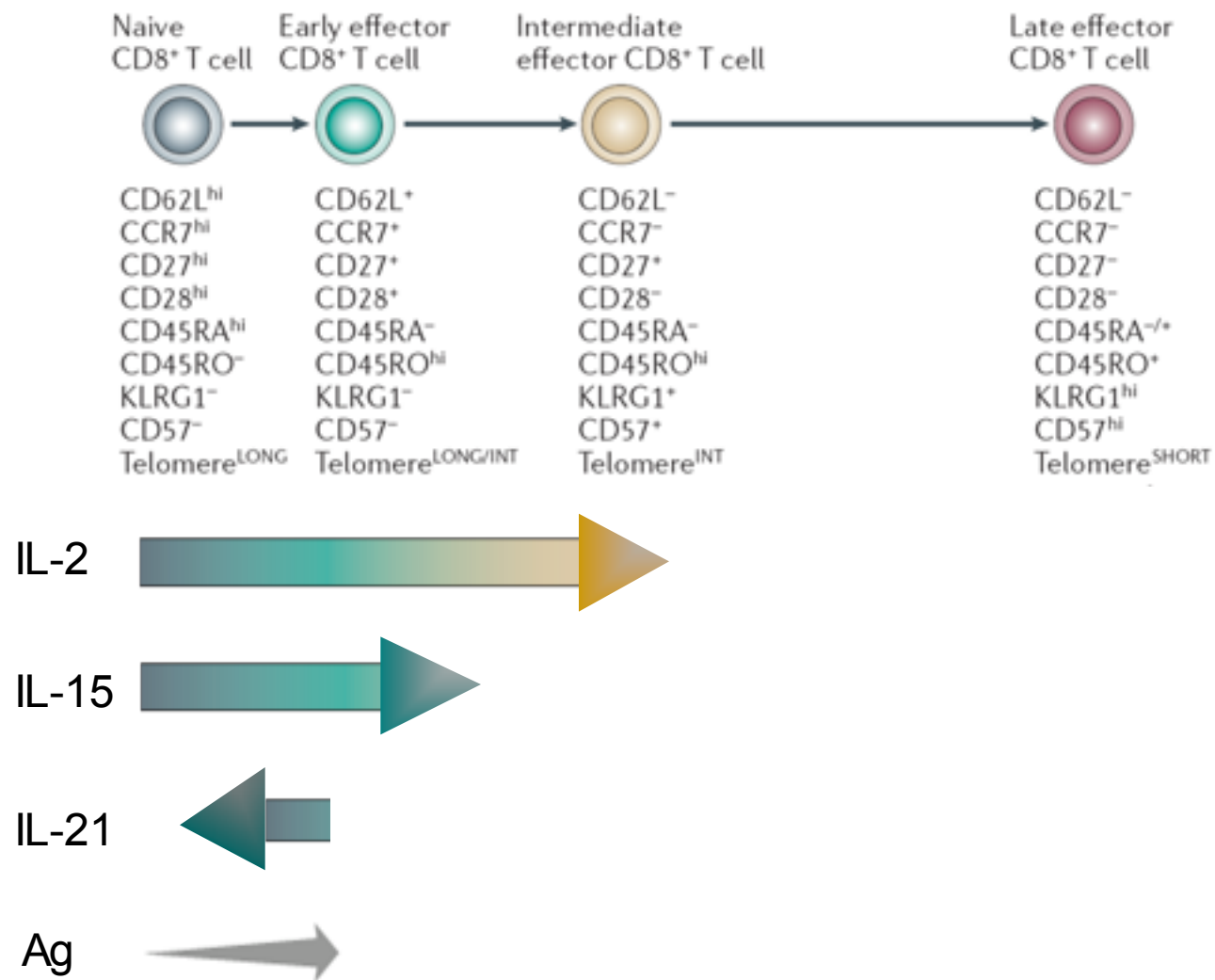
The antitumor efficacy of pmel-1 CD8<sup>+</sup> T cells for adoptive transfer is impaired by IL-2 and IL-15, but enhanced by IL-21

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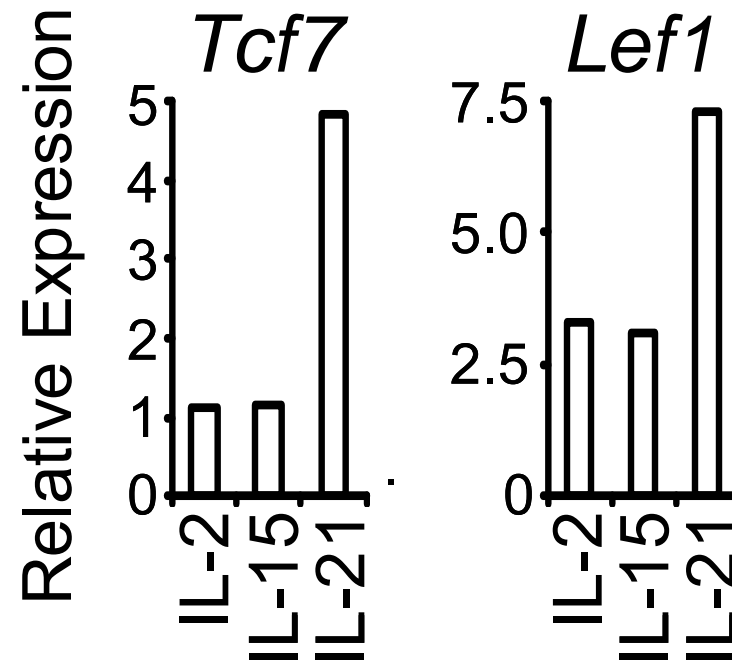
Hinrichs, et al, Blood, 2008

# Modulation of T cell differentiation through $\gamma_C$ -cytokines



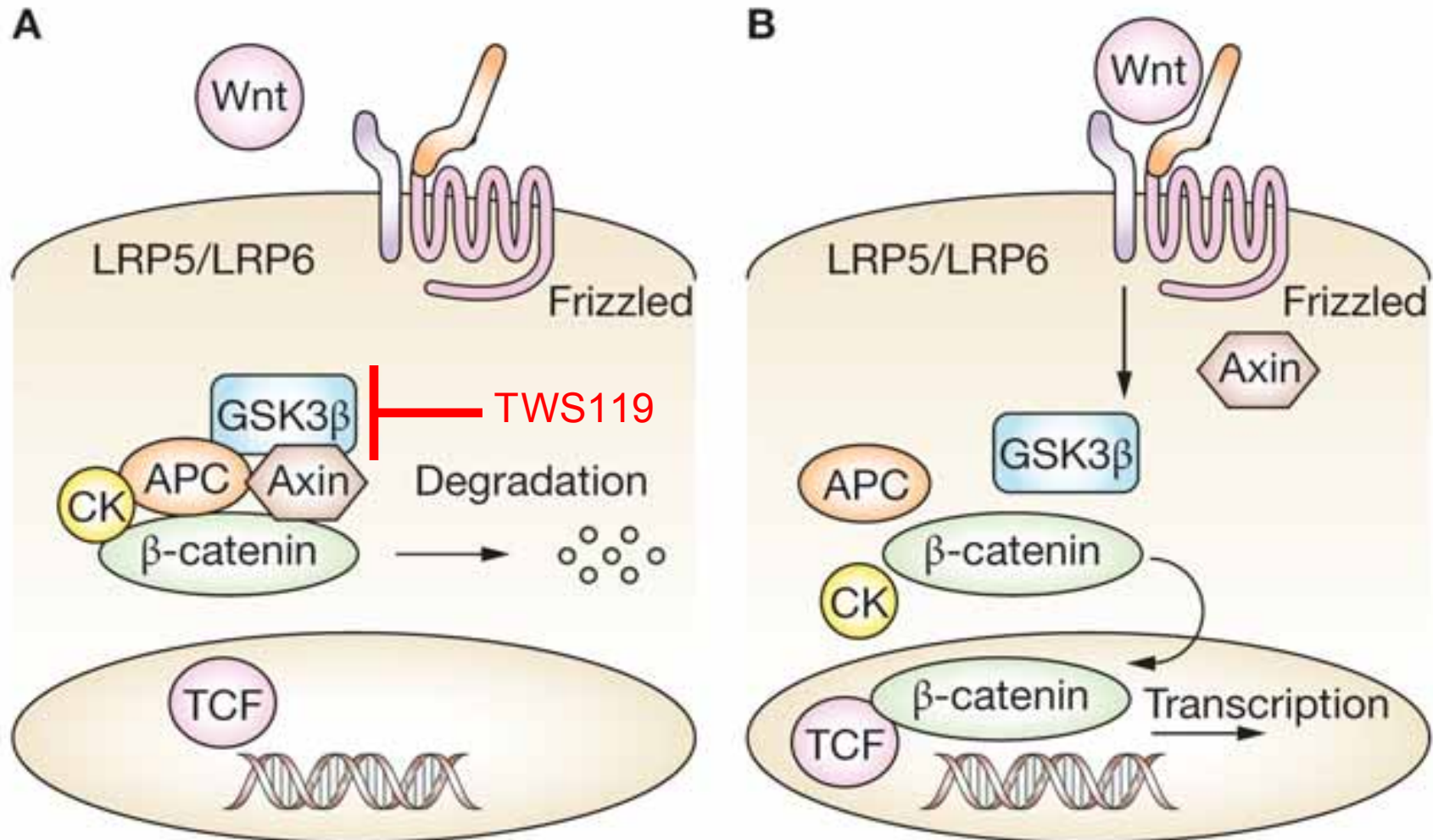
High levels of TCF7 and LEF1 mRNA are detected in CD8<sup>+</sup> T cells programmed in IL-21

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Hinrichs, et al, *Blood*, 2008

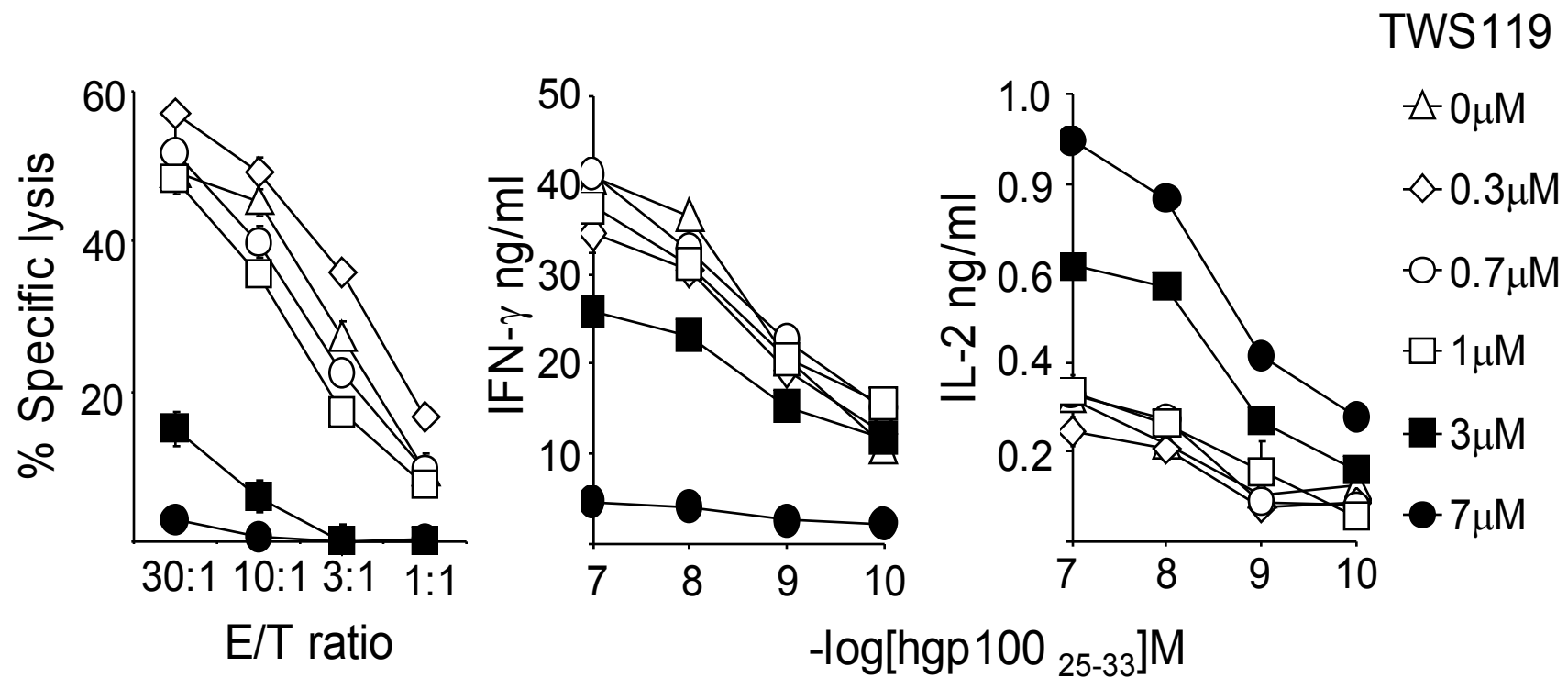
# Wnt- $\beta$ catenin signaling pathway



Willert, K. et al. Nature 2003

# TWS119 inhibits the acquisition of effector function

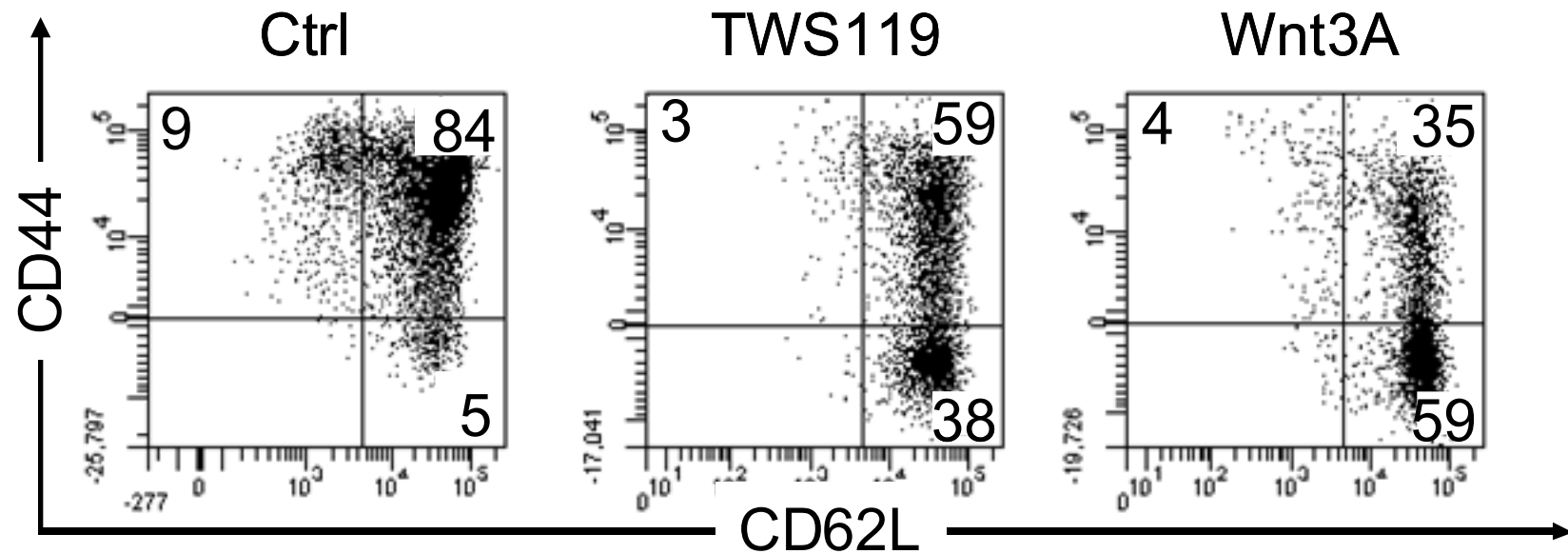
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Gattinoni, *et al*, *Nat Med*, 2009

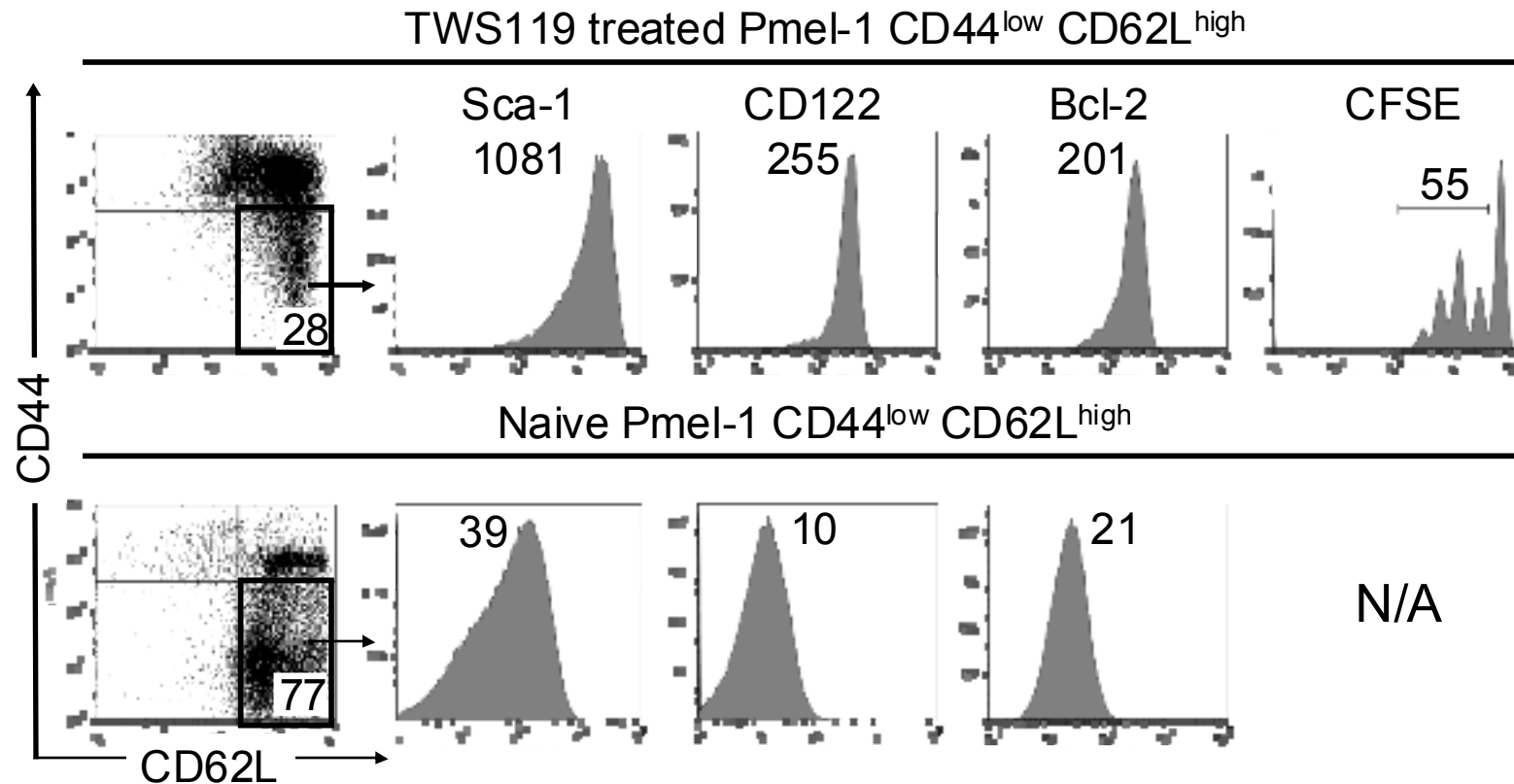
# Wnt3A inhibits T cell proliferation and effector T cell differentiation

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Gattinoni, *et al*, *Nat Med*, 2009

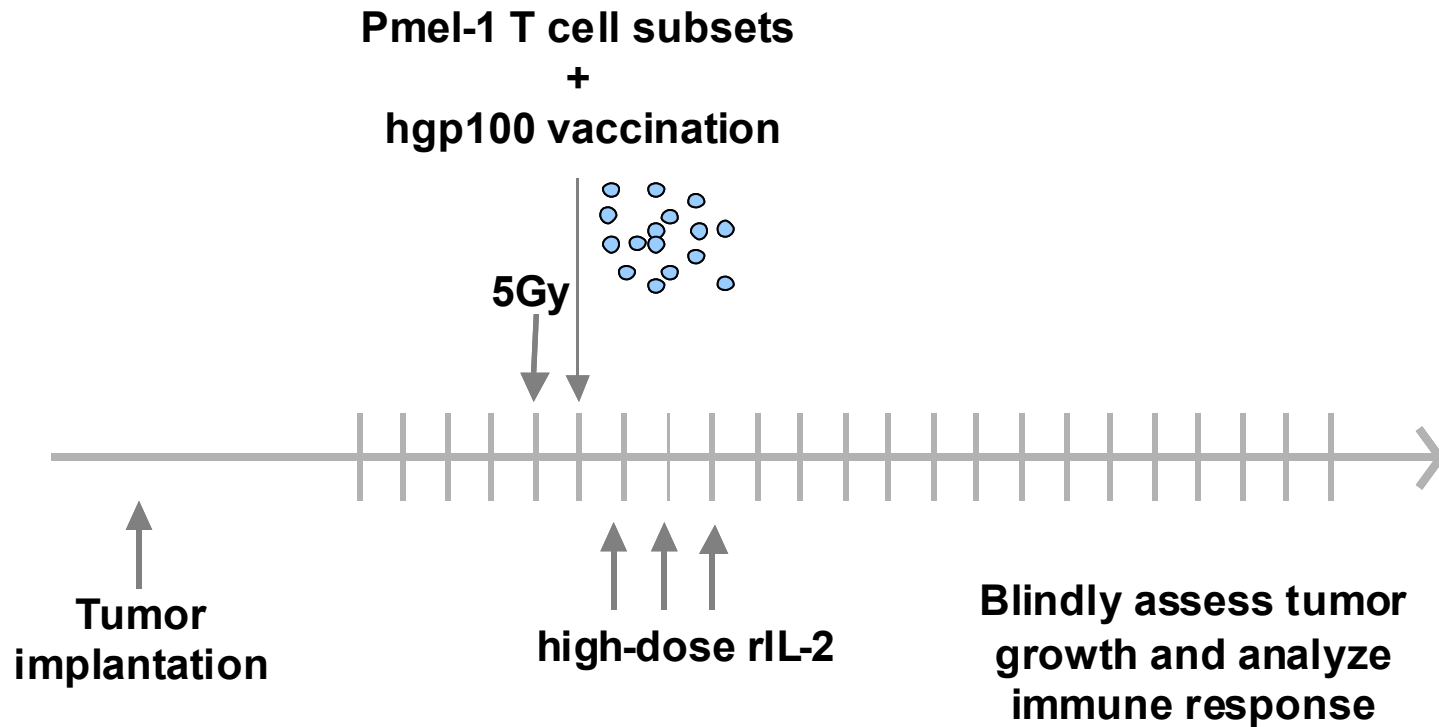
# Identification of CD8<sup>+</sup> memory stem cell



Gattinoni, *et al*, *Nat Med*, 2009

# Pmel-1 ACT murine melanoma model

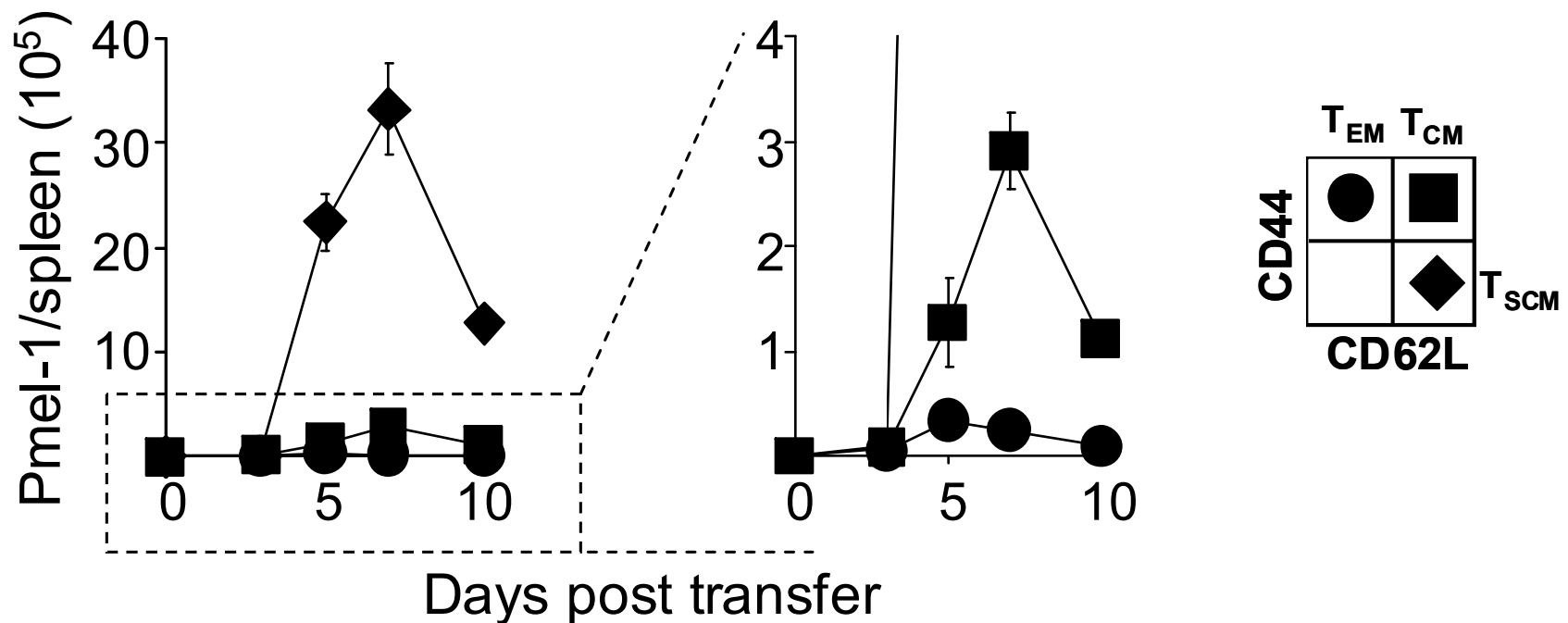
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*Overwijk, et al, J Exp Med 2003*

$T_{SCM}$  exhibit greater secondary responses compared to the other T cell memory subsets

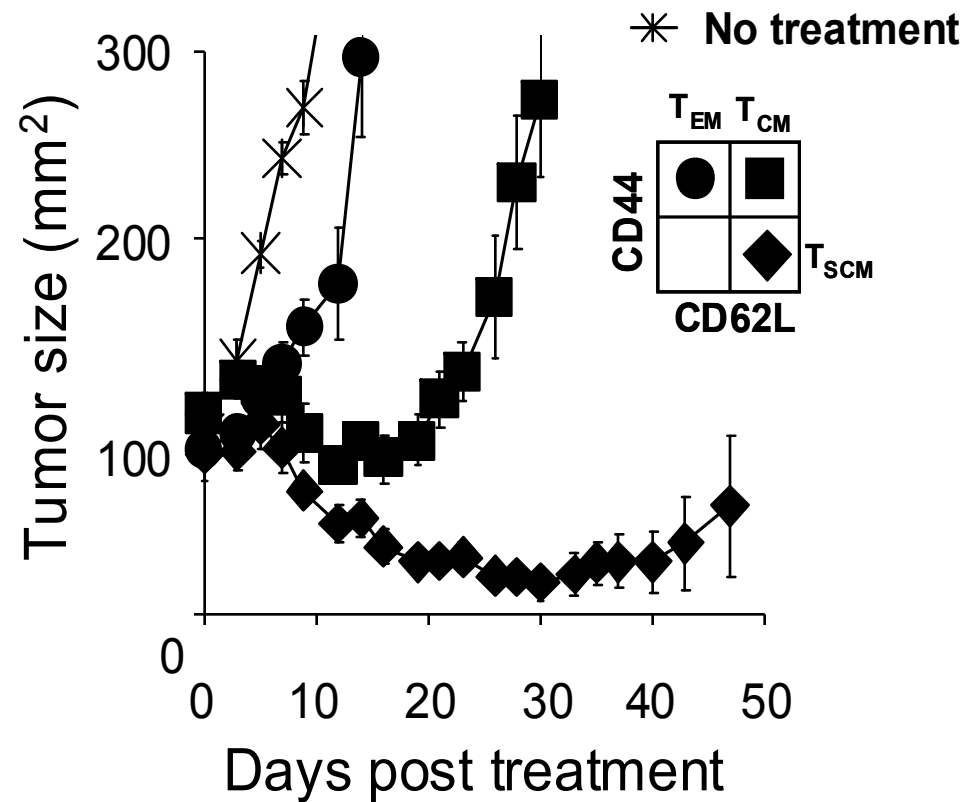
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Gattinoni, *et al*, *Nat Med*, 2009

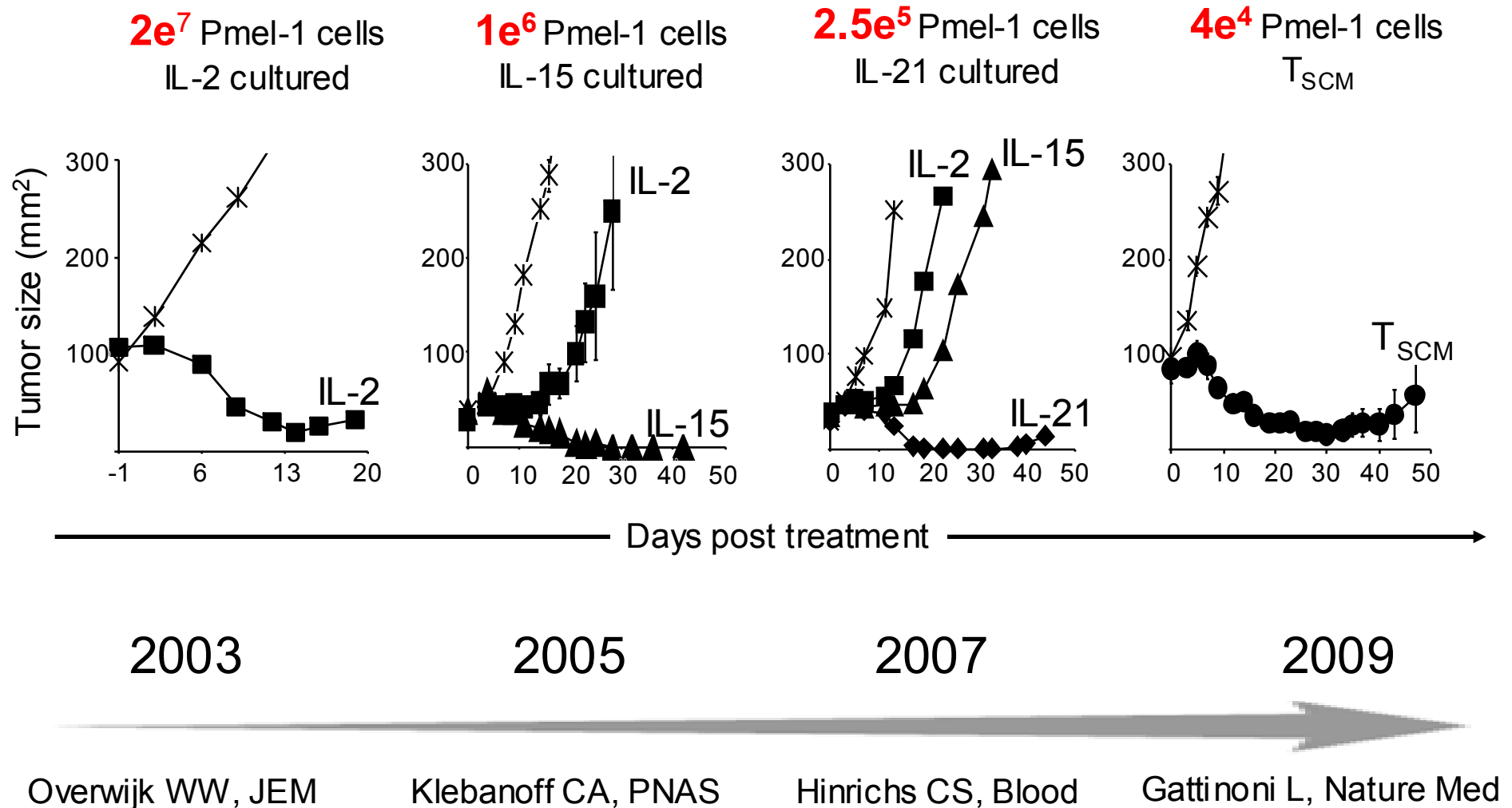
# $T_{SCM}$ mediate profound anti-tumor immunity upon adoptive transfer

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Gattinoni, *et al*, *Nat Med*, 2009

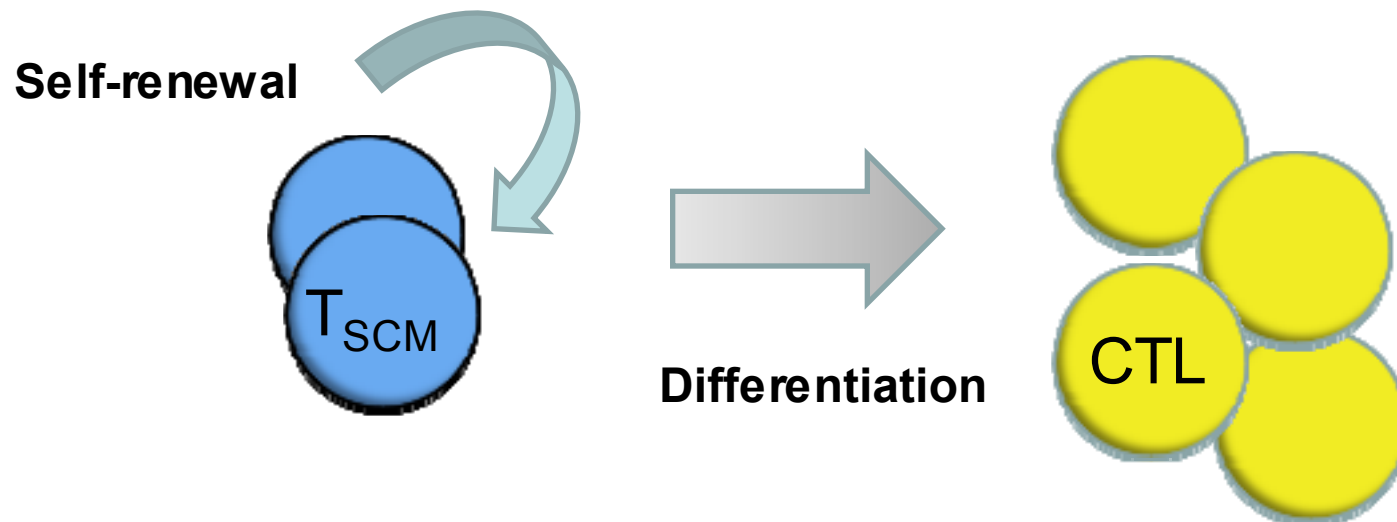
# Improving T cell fitness in the Pmel-1 model



# Summary on Wnt

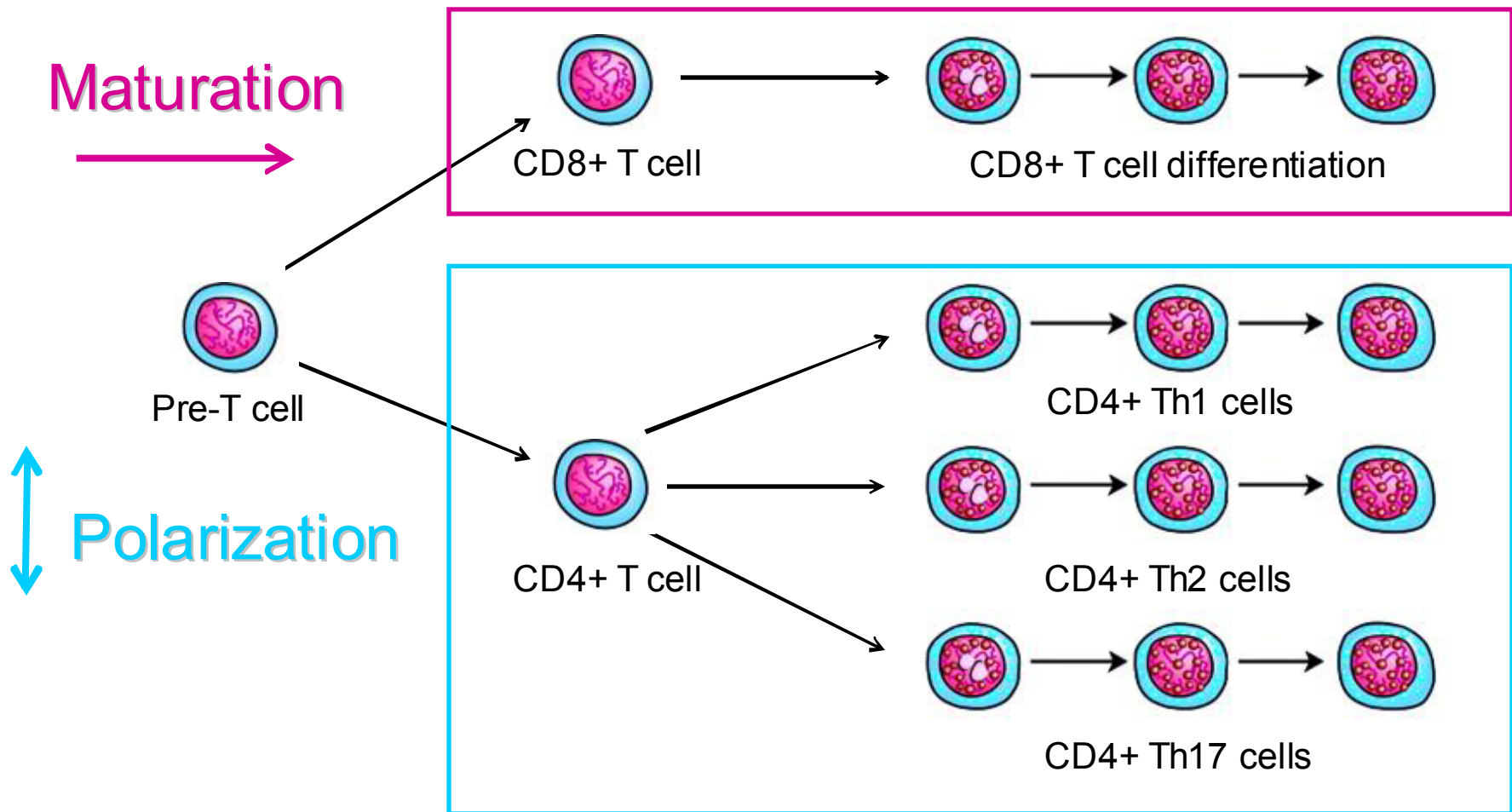
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- Wnt signaling arrests CD8<sup>+</sup> T cell differentiation and promotes the generation of self-renewing, multipotent T<sub>SCM</sub>
- T<sub>SCM</sub> have enhanced proliferative capacity and mediate profound anti-tumor immunity at low numbers (4 X 10<sup>4</sup> cells)

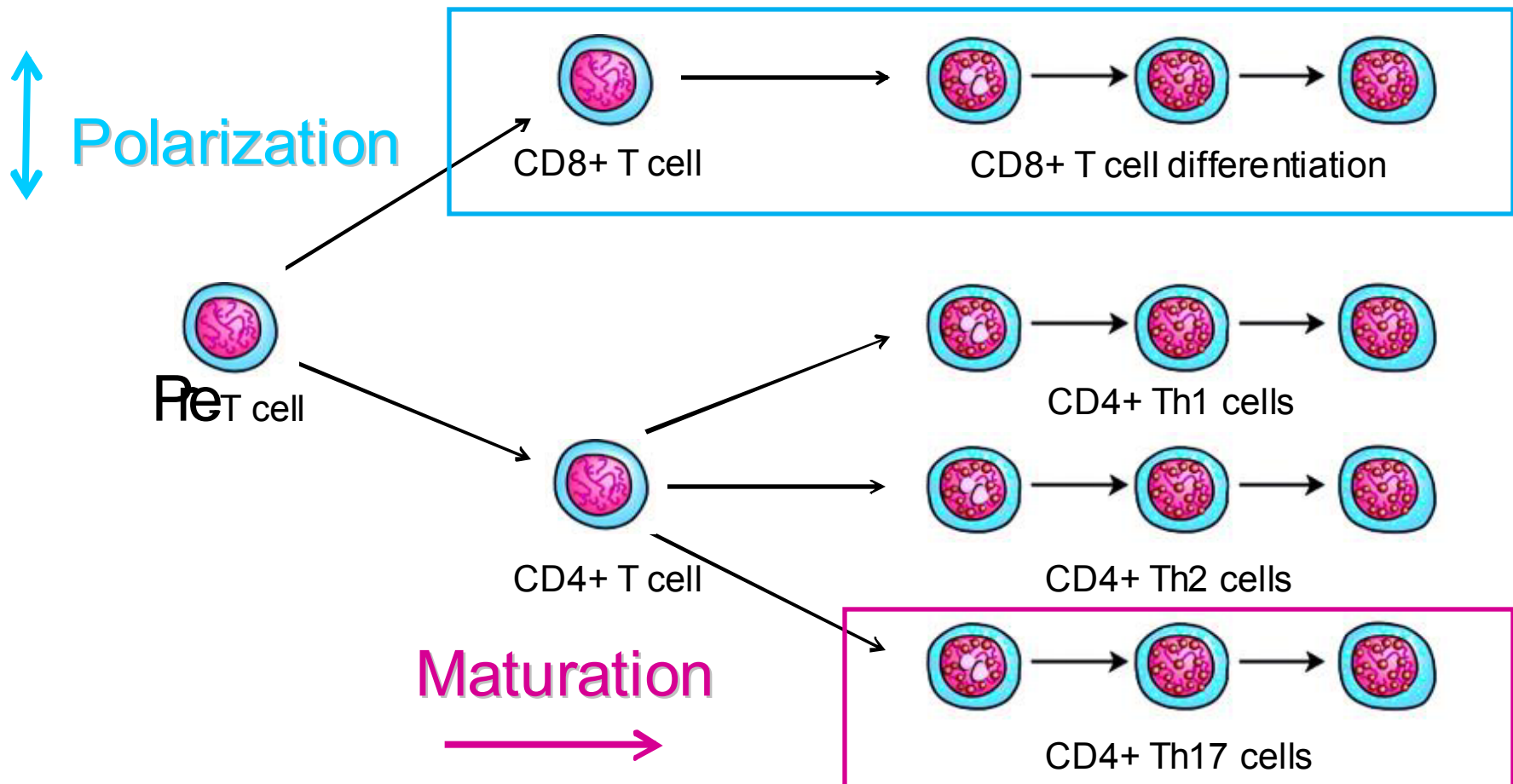


# Differentiation and maturation of post-thymic T cells

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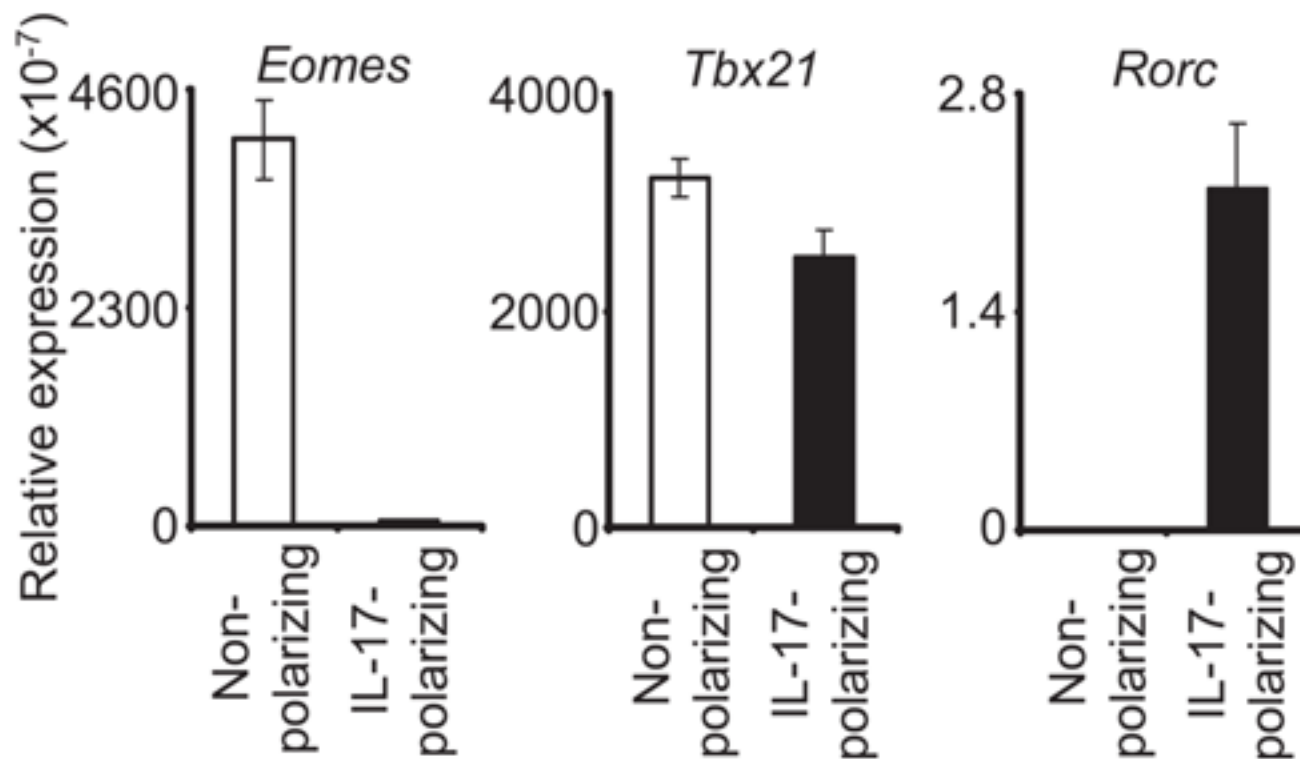
# Differentiation and maturation of post-thymic T cells



Muranski, et al, *Blood*, 2008; *Curr Opin Immunol*, 2009

## Gene expression characteristics of Tc17-polarized Pmel-1 CD8<sup>+</sup> T cells

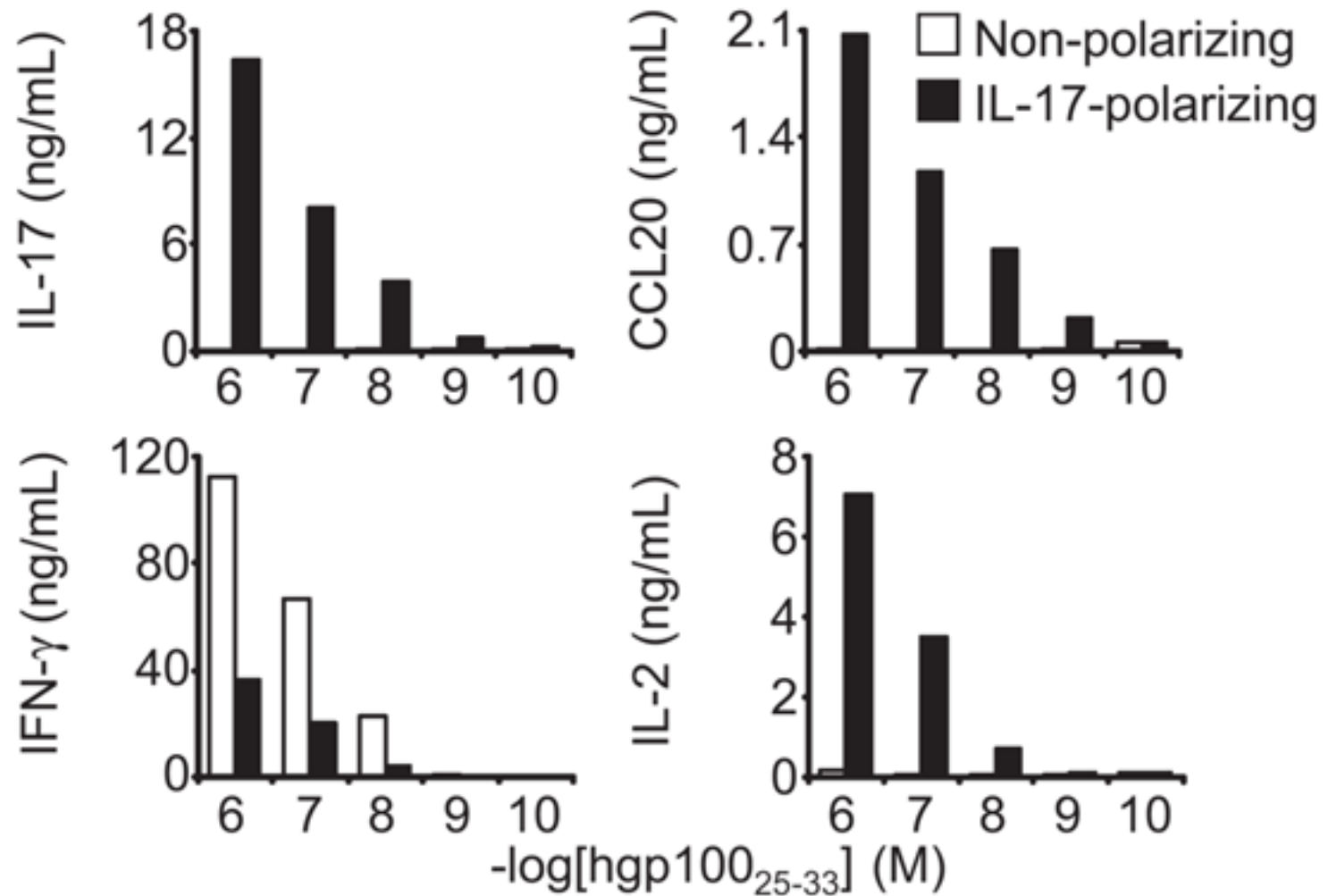
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Hinrichs, *et al*, *Blood*, Epub, 2009

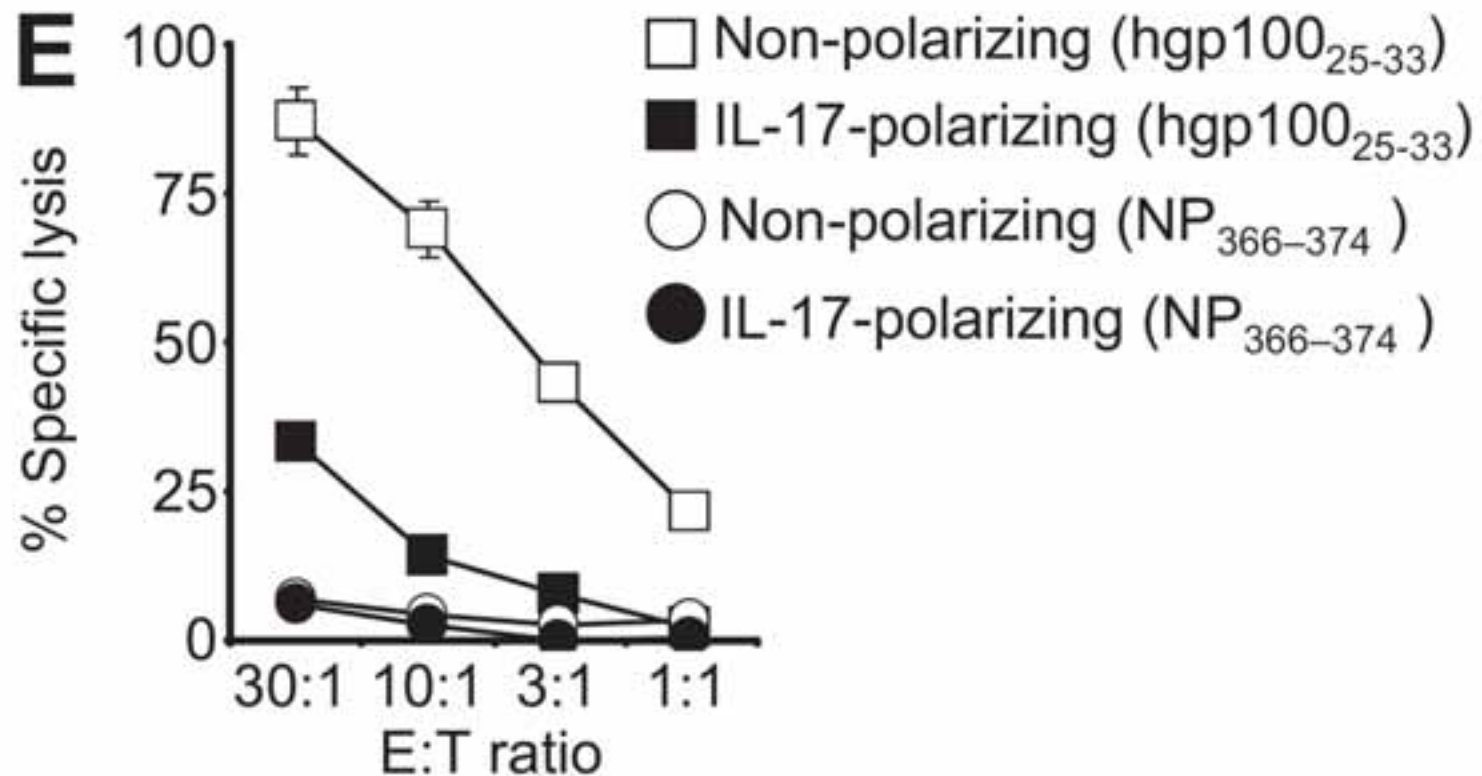
Pmel-1 CD8<sup>+</sup> T cells can be crisply polarized like their CD4<sup>+</sup> counterparts

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## Tc17-polarized Pmel-1 CD8<sup>+</sup> T cells are not lytic

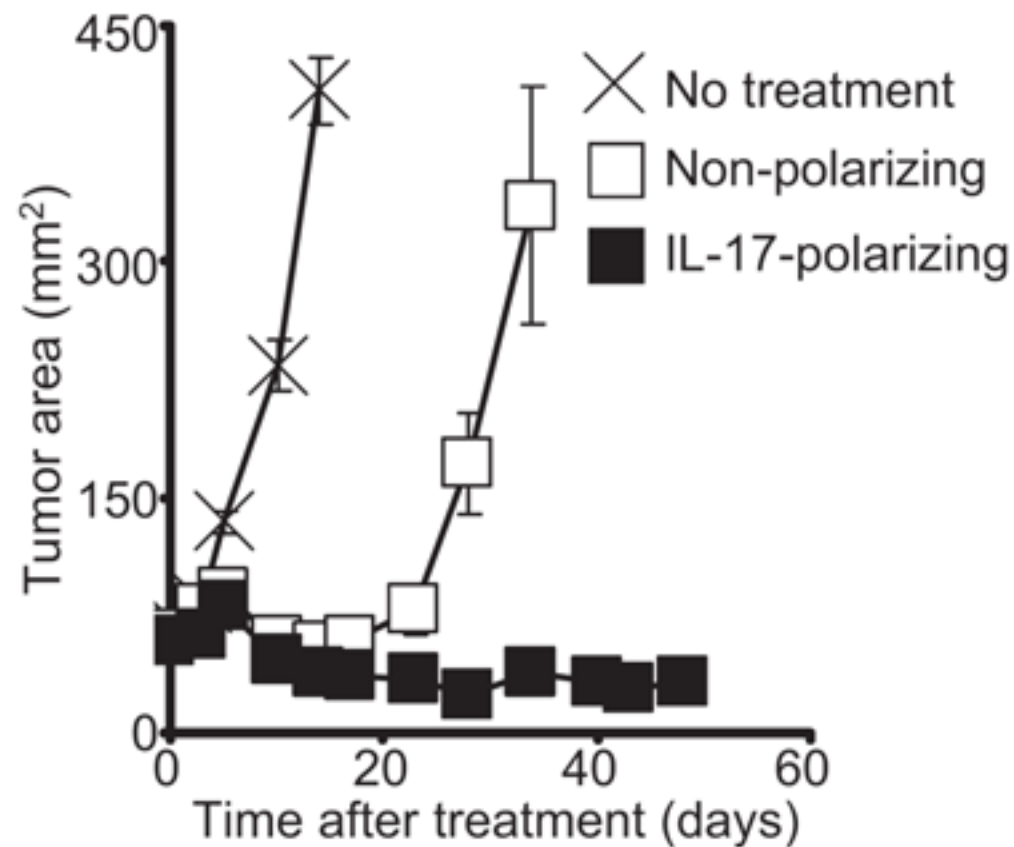
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Hinrichs, *et al*, *Blood*, Epub, 2009

Tc17-polarized Pmel-1 CD8<sup>+</sup> T cells are more effective than IL-2 polarized cells

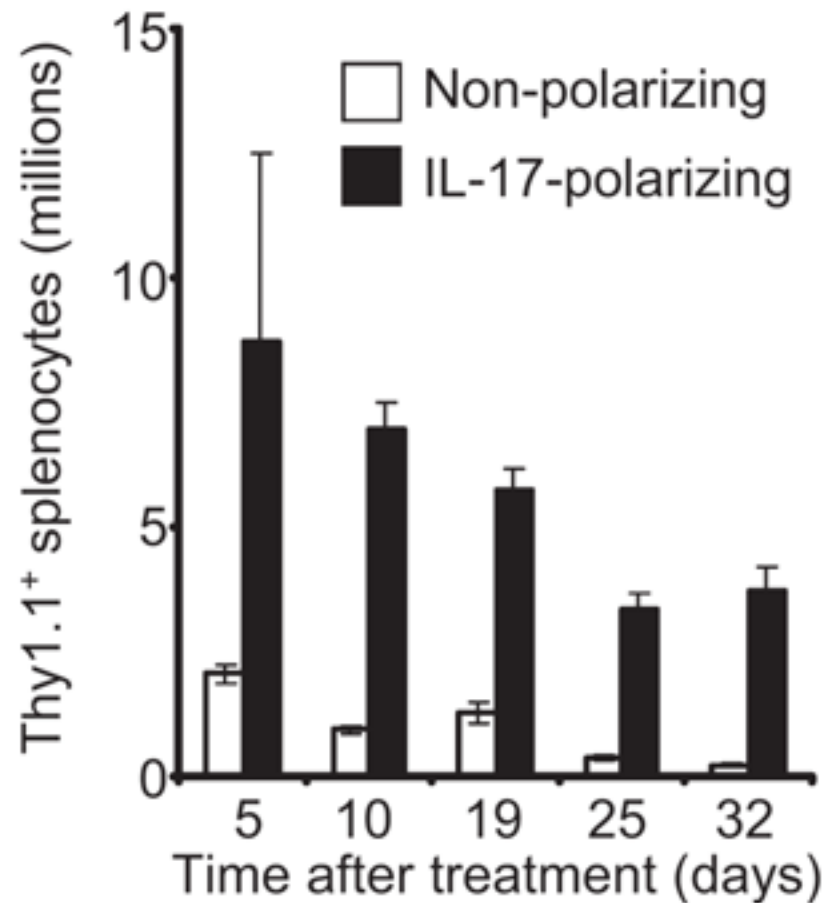
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Hinrichs, *et al*, *Blood*, Epub, 2009

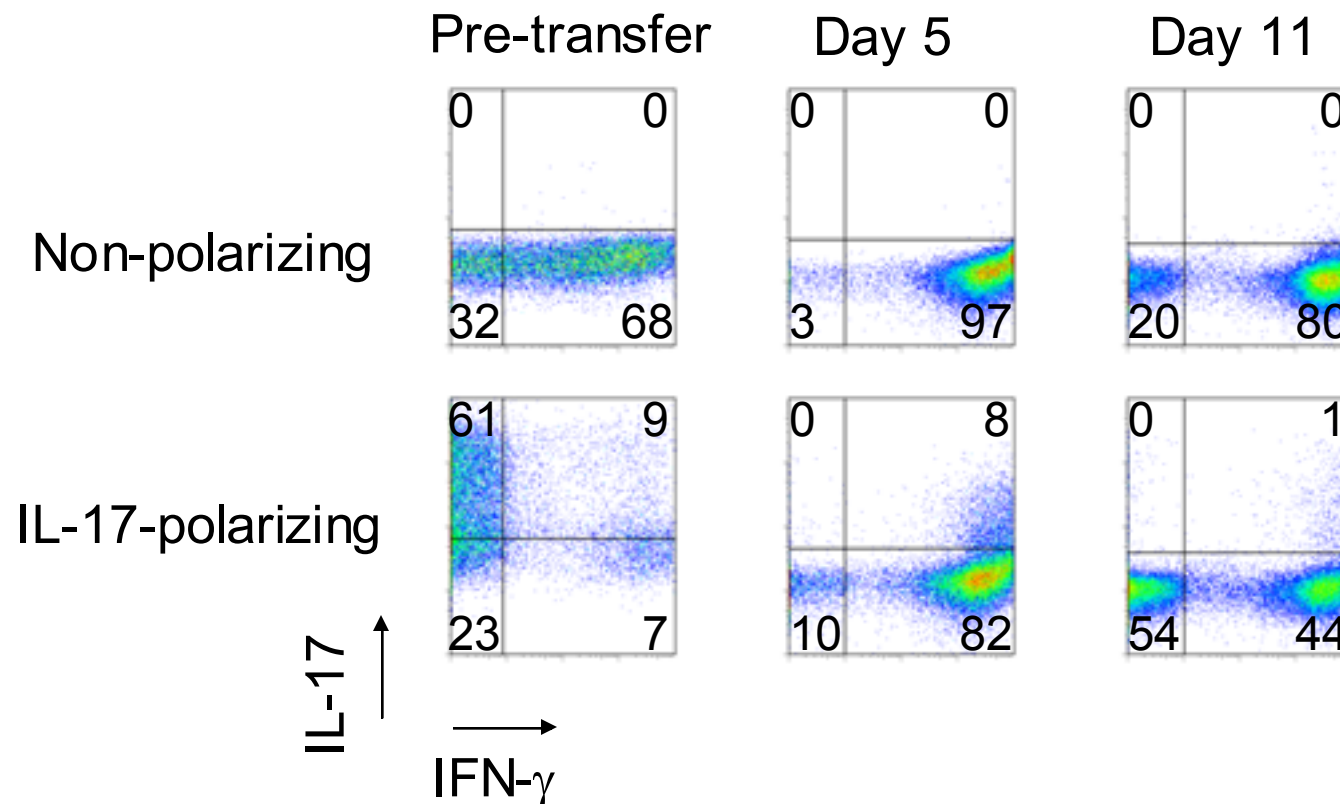
## Type 17–polarized CD8<sup>+</sup> T cells demonstrate greater persistence after transfer *in vivo*

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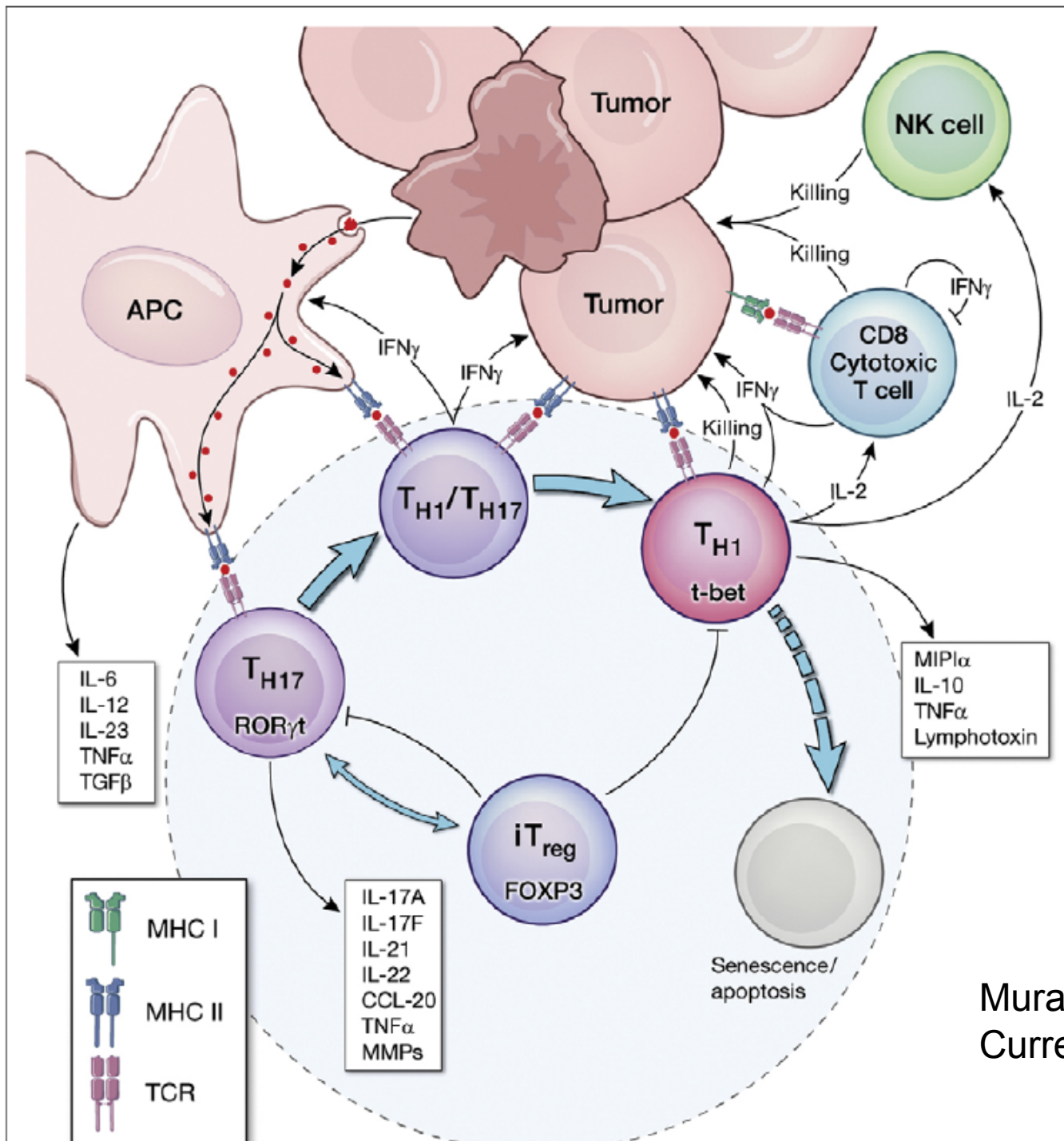


# Populations of Tc17-polarized Pmel-1 CD8<sup>+</sup> T cells evolve in vivo

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Hinrichs, *et al*, *Blood*, *Epub*, 2009



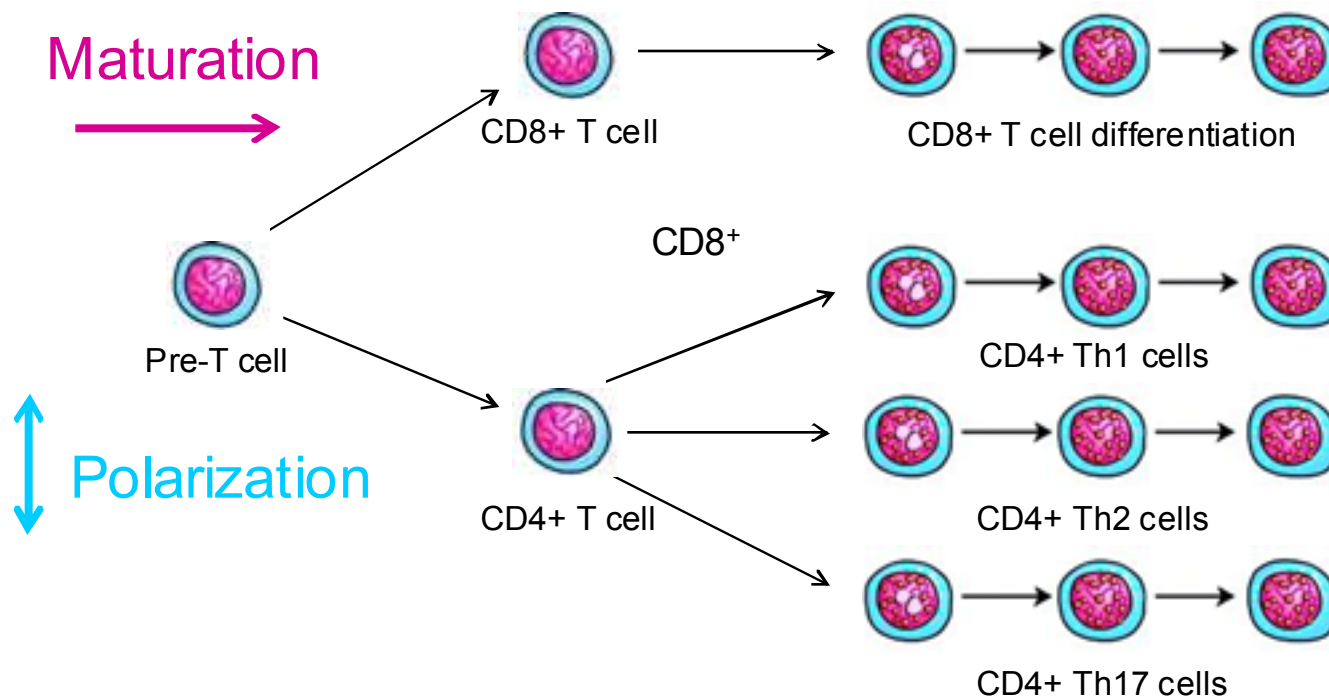
## Plasticity of IL-17-polarized anti-tumor responses

Muranski & Restifo,  
Current Opin Immunol, 2009

## Conclusions

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- Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells experience maturation and polarization.
- The state of T lymphocyte differentiation profoundly affects their anti-tumor activities *in vivo*.



# Special Thanks to

## **Restifo Lab**

**Luca Gattinoni**

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Christian Hinrichs

Pawel Muranski

Christopher Klebanoff

Yun Ji

Sid Kerkar

Madhu Sukumar

Zhiya Yu

Robert Reger

Dorina Frasheri

## **Some Star Graduates**

Vincenzo Bronte

Paul Antony

Christopher Touloukian

Willem Overwijk

Chrystal Paulos

Christian Hinrichs

**Steve Rosenberg**

**James Yang**

**Mark Dudley**

**Paul Robbins**

**Rick Morgan**

**Franco Marincola**

**Ena Wang**

**Bob Danner Lab**

Carol Logun

**restifo@nih.gov**

