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

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

ACT-based immunotherapies can be enhanced with immunoablation prior to therapy.

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

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.
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\(^{+}\) TCR transgenic model

Anti-tumor activities of α/β T cells
The Trp-1 CD4+ T cell transgenic model

Estimated Available for Sale Date: 14-Dec-09 at www.jax.org
Adoptive cell transfer (ACT) in mouse and man

Tumor is established

Ablate host immune system

Adoptively transfer T cells

Add vaccine and cytokine support

Tumor regression?

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

Wrzesinski, In Press, J Immunother, 2010
Survival of patients with metastatic melanoma treated with autologous tumor-infiltrating lymphocytes (TIL) and IL-2: Impact of preparative regimens
How lymphodepletion augments the function of adoptively transferred T cells

Chemotherapy or Radiation therapy

Bronte, et al,
Mac-1/Gr-1 cells suppress CD8\(^+\) T cells, J Immunol, 1998
GM-CSF induces MDSC, J Immunol, 1999
How lymphodepletion augments the function of adoptively transferred T cells

Chemotherapy or Radiation therapy

How lymphodepletion augments the function of adoptively transferred T cells

Chemotherapy or Radiation therapy

Gattinoni, et al, JEM, 2005
Impact of Lymphodepletion on Serum Levels Of IL-15 and IL-7

How lymphodepletion augments the function of adoptively transferred T cells

Chemotherapy or Radiation therapy

Paulos, et al, JCI, 2007

Fully activated tumor-specific T cell
Hematopoietic stem cells augment the function of adoptively transferred CD8+ T cells

Wrzesinski, et al., JCI, 2007
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Adoptive cell transfer (ACT) in mouse and man

- Tumor is established
- Ablate host immune system
- Adoptively transfer T cells
- Add vaccine and cytokine support
- Tumor regression?
Differentiation and maturation of mature cells derived from hematopoietic stem cells
Differentiation and maturation of post-thymic T cells

Maturation

Pre-T cell → CD8+ T cell → CD8+ T cell differentiation

Polarization

CD4+ T cell → CD4+ Th1 cells → CD4+ Th2 cells → CD4+ Th17 cells
T cell differentiation and maturation

- CD4+ T cell
- IL-12
- IFN-γ
- IL-4
- IL-6
- IL-21
- IL-1
- CD4+ T cell
- CD4+ Th1 cells
- CD4+ Th2 cells
- CD4+ Th17 cells

Polarization

T-bet
Gata3
ROR-γt

T cell differentiation and maturation

CD8 T lymphocyte ≠ CTL

Maturation

Naïve CD8+ T cell → CD8+ T cell differentiation

Antigen T-bet Eomes Blimp-1

IL-2 IL-15

Vaccines

Cytotoxic T Lymphocyte CTL
Phenotypic and functional characteristics of pmel-1 CD8+ T cells during differentiation

[Diagram showing the progression of CD8+ T cells from naïve to effector stages, with markers and cytokines expressed at each stage.

- Ag/IL-2
- CD62L, CD69, CD44, CD27, granzyme-B
- CD27
- β7 integrin
- IL-2
- IFNγ

Progressive differentiation

Gattinoni, et al, JCI, 2005]
The differentiation state of CD8^+ T cells is inversely related to their proliferative capacity and persistence.

Gattinoni, et al, JCI, 2005
The differentiation state of CD8$^+$ T cells is inversely related to their antitumor activity.

Gattinoni, et al, JCI, 2005
Acquisition of terminal effector function \textit{in vitro} impairs \textit{in vivo} anti-tumor efficacy

Effector cells derived from naïve CD8\(^{+}\) T cells are more effective at treating tumor than those derived from CM cells

Effector cells derived from naïve CD8$^+$ T cells maintain the ability to release IFN-\(\gamma\) and IL-2

Effector cells derived from naïve CD8$^+$ T cells persist longer than those derived from CM in vivo

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

![Graph showing primary and secondary responses](image-url)
Cellular differentiation of adoptively transferred T cells is inversely correlated with clinical response.

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+ T cells

Patient protocol based on “young TIL”

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

Modulation of T cell differentiation through $\gamma_C$-cytokines
IL-15 promotes the generation of $T_{CM}$-like CD8$^+$ T cells

Klebanoff CA et al, PNAS 2004 and 2005
Modulation of T cell differentiation through $\gamma_C$-cytokines
IL-21 inhibits acquisition of effector CD8\(^+\) T-cell phenotype

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

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

- Naive CD8$^+$ T cell
- Early effector CD8$^+$ T cell
- Intermediate effector CD8$^+$ T cell
- Late effector CD8$^+$ T cell

- IL-2
- IL-15
- IL-21
- Ag
High levels of TCF7 and LEF1 mRNA are detected in CD8⁺ T cells programmed in IL-21.

Wnt-β-catenin signaling pathway

TWS119 inhibits the acquisition of effector function

Wnt3A inhibits T cell proliferation and effector T cell differentiation

Identification of CD8$^+$ memory stem cell

Pmel-1 ACT murine melanoma model

Pmel-1 T cell subsets + hgp100 vaccination

5 Gy

Tumor implantation

high-dose rIL-2

Blindly assess tumor growth and analyze immune response

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

\( T_{SCM} \) mediate profound anti-tumor immunity upon adoptive transfer

Improving T cell fitness in the Pmel-1 model

Overwijk WW, JEM
Klebanoff CA, PNAS
Hinrichs CS, Blood
Gattinoni L, Nature Med
Summary on Wnt

• Wnt signaling arrests CD8\(^+\) T cell differentiation and promotes the generation of self-renewing, multipotent \(T_{SCM}\)

• \(T_{SCM}\) have enhanced proliferative capacity and mediate profound anti-tumor immunity at low numbers (4 \(\times\) 10\(^4\) cells)
Differentiation and maturation of post-thymic T cells

Maturation

Pre-T cell → CD8+ T cell → CD8+ T cell differentiation

Polarization

CD4+ T cell → CD4+ Th1 cells
CD4+ Th2 cells
CD4+ Th17 cells
Differentiation and maturation of post-thymic T cells

Pre-T cell → CD8+ T cell → CD8+ T cell differentiation

Pre-T cell → CD4+ T cell

CD4+ T cell → CD4+ Th1 cells

CD4+ T cell → CD4+ Th2 cells

CD4+ T cell → CD4+ Th17 cells

Gene expression characteristics of Tc17-polarized Pmel-1 CD8$^+$ T cells

Pmel-1 CD8^+ T cells can be crisply polarized like their CD4^+ counterparts.
Tc17-polarized Pmel-1 CD8$^+$ T cells are not lytic

Hinrichs, et al, Blood, Epub, 2009
Tc17-polarized Pmel-1 CD8^+ T cells are more effective than IL-2 polarized cells

Hinrichs, et al, Blood, Epub, 2009
Type 17–polarized CD8\(^+\) T cells demonstrate greater persistence after transfer \textit{in vivo}. 
Populations of Tc17-polarized Pmel-1 CD8⁺ T cells evolve in vivo

Hinrichs, et al, Blood, Epub, 2009
Plasticity of IL-17-polarized anti-tumor responses

Muranski & Restifo, Current Opin Immunol, 2009
Conclusions

- Both CD4\(^+\) and CD8\(^+\) T cells experience maturation and polarization.
- The state of T lymphocyte differentiation profoundly affects their anti-tumor activities *in vivo*.
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