The Basic Science Behind the Potent New Immunotherapies

iSBT
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Goal: To develop new immunotherapies for patients with cancer

• Strategy: create new animal models that accurately represent the treatment of humans with established tumors.

• Using an iterative process, continually translate bench research into new clinical trials.

• Model clinically generated hypotheses back into the mouse.
A view of translational research

The assumption is that mice and humans are evolutionarily close enough so that findings in one species can be translated into the other.

The challenge is finding realistic mouse models that emulate the human experience.
The challenge: Treat established, vascularized solid tumors in normal mice

The rules:
Tumor must be unmanipulated -- no artificial insertion of costimulatory molecules, alloantigens or neoantigens [like molecules from humans, chickens, bacteria or viruses].

Treatment schema must be realistic -- must be suitable for metastatic (systemic) disease. Cannot involve the manipulation of the mouse prior to its “presentation” to the mouse clinic with a large tumor.
What is adoptive immunotherapy? . . . and how can it be improved?
Lymphodepletion followed by adoptive cell transfer induces objective responses in half of the patients

13 Patients $\rightarrow$ **6 Objective Responses (45%)**
*Dudley, Rosenberg, Science 2002*

35 Patients $\rightarrow$ **18 Objective Responses (51%)**
*Dudley, Rosenberg, JCO 2005*
Modeling the impact of lymphodepletion in the mouse

• It has long been observed that the administration of cyclophosphamide or total body irradiation (TBI) could augment the activities of T cell-based immunotherapies.

• We sought to explore the impact of irradiation and chemotherapy in the induction of a lymphopenic host environment.

• We wanted to understand if these modalities could be used to enhance T cell-based treatment in the pmel-1 model.
Pmel-1 ACT murine melanoma model

Pmel-1 adoptive cell transfer +/- vaccination

0.5 Gy

Tumor implantation

high-dose rIL-2

Blindly assess tumor growth and analyze immune response

Overwijk et al. J exp med 2003
Lymphodepletion enhances the anti-tumor efficacy of adoptively transferred CD8\(^+\) T cells

\[ \text{Absolute lymphocyte count (10}^3/\mu\text{L)} \]

\[ \text{Tumor area (mm}^2) \]

\[ \text{Days post treatment} \]

\[ \text{Days post treatment} \]

\[ \text{No treatment} \]

\[ \text{5Gy TBI+No treatment} \]

\[ \text{Treatment} \]

\[ \text{5Gy TBI+Treatment} \]

Gattinoni et al. J exp med, 2005
Sublethal irradiation acts via indirect mechanisms rather than direct tumor killing.
Lymphodepletion by genetic means recapitulates the effect of sublethal irradiation

Antony et al. JI, 2005
Lymphodepletion does not result in increased numbers of transferred CD8\(^+\) T cells

\[\text{Pmel-1/spleen (10}^6)\]

\[\text{Pmel-1}/\mu\text{L blood}\]

\[\%\text{Pmel-1/all cells at tumor site}\]

\[\text{Days post treatment}\]

\[\triangle \text{ No treatment} \]

\[\blacktriangle \text{ 5Gy TBI+No treatment} \]

\[\text{○ Treatment} \]

\[\bullet \text{ 5Gy TBI+Treatment} \]

\text{Gattinoni et al. J exp med, 2005}
Lymphodepletion augments the effector functions of transferred CD8\(^+\) T cells

-Gattinoni et al. J exp med, 2005
Endogenous CD4^{+} but not CD8^{+} T cells suppress the anti-tumor activity of transferred CD8^{+} T cells

Antony et al. JI, 2005
CD4⁺CD25⁺ regulatory T cells suppress pmel-1 CD8⁺T cells in vitro

Antony et al. JI, 2005
CD4+CD25 + regulatory T cells suppress pmel-1 CD8+T cells *in vivo*

Antony et al. *JI*, 2005
Sublethal irradiation enhances the anti-tumor efficacy of transferred CD8\(^+\) T cells even in the genetic absence of Tregs

![Graph showing tumor area over days post treatment for different conditions](image)

*Gattinoni et al. J exp med, 2005*
Removal of NK cells enhances the anti-tumor efficacy of transferred CD8\(^+\) T cells

![Graph showing tumor area over days post treatment for different treatment groups.](image-url)
IL-15 levels correlate inversely with NK and activated CD8^+ T cell populations

Gress RE, unpublished data
Increased access to endogenous IL-15 and IL-7 into irradiated hosts enhances the anti-tumor efficacy of transferred CD8⁺ T cells

![Graphs showing tumor area over days post treatment for non-irradiated and irradiated hosts with different treatments: WT no treatment, IL15(-/-) no treatment, IL15/IL7(-/-) no treatment, WT + treatment, IL15(-/-) + treatment, IL15/IL7(-/-) + treatment.](Gattinoni et al. J exp med, 2005)
Proliferative responses of transferred CD8$^+$ T cells are impaired in the absence of both IL-7 and IL-15.
Increased access to endogenous IL-15 and IL-7 enhances the effector functions of transferred CD8+ T cells

Gattinoni et al. J exp med, 2005
Damage of the integrity of mucosal barriers by irradiation facilitate translocation of LPS into the blood stream

<table>
<thead>
<tr>
<th>Gy</th>
<th>Score</th>
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<tr>
<td>0</td>
<td>0, 0, 0</td>
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<tr>
<td>5</td>
<td>1, 1, 2</td>
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<tr>
<td>9</td>
<td>2, 3, 3</td>
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Paulos et al. MS in preparation
Block of LPS by *Polymyxin B* partially impairs the effect of sublethal irradiation.

Continuous administration of Polymyxin B (10 µg/day for three weeks)

*Paulos et al. MS in preparation*
Exogenous administration of LPS augments the anti-tumor efficacy of transferred CD8⁺ T cells

Paulos et al. MS in preparation
LPS induces qualitative rather than quantitative improvement on transferred CD8$^+$ T cells

Graph 1: Pmel-1 cells (10$^3$)/spleen over Day post transfer for PFI, PFI+LPS, and 5 Gy_PFI.

Graph 2: IFN-γ (pg/ml) over Day post transfer for PFI, PFI+LPS, and 5 Gy_PFI.
An interactive model for the host mechanisms underlying the impact of lymphodepletion on adoptively-transferred-T cells

Wrezinski, Current Opinion In Immunology, 2005
Klebanoff, Trends in Immunology, 2005
Paulos, MS in Preparation
TLR-mediated DC activation?

A. Lymphoreplete host

- Competing lymphocyte
- CTL
- Fully activated CTL
- Treg
- TGF-β
- IL-10
- Mature DC

B. Lymphodepleted host

- IL-2
- IL-7
- IL-15
- Immature DC

Wrzesinski, Current Opinion In Immunology, 2005
Klebanoff, Trends in Immunology, 2005
Antony, J Immunol, 2005
Paulos, MS in Preparation
In vitro generation of pmel-1 CD8+ T cells at different stages of differentiation

 naïve → early effector → intermediate effector → effector
T cells differentiation stage was validated by FACS………

[Graph showing the expression levels of various markers across different T cell differentiation stages.]
...... and functional analyses

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

![Graph showing tumor area over days post treatment](image)

Gattinoni et al. JCI, 2005
Further characterization of effective and impaired T cells

Genes highly expressed in effective cells
- IL-7Rα
- CD62L
- CCR7
- CD27
- GZM-B
- IFN-γ
- BID
- EOMES
- MRGX

Genes highly expressed in impaired cells
- ITGAE
- CD27
- GZM-B
- BID
- MIP-1α
- KLRG-1
- MRGX

X-axis: S36C_SF_S1_v_2 (All Samples): S36C_SF_S1_v...
Y-axis: S36C_SF_S1_v_2 (All Samples): S36C_SF_S1_v...
Gene List: all genes (21921), RIKEN cDNA 4930529122...
<table>
<thead>
<tr>
<th>Genes highly expressed in effective cells</th>
<th>Genes highly expressed in impaired cells</th>
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<tr>
<td><strong>Lymphoid-homing</strong></td>
<td><strong>Effector functions</strong></td>
</tr>
<tr>
<td>CD62L</td>
<td>Granzyme A</td>
</tr>
<tr>
<td>CCR7</td>
<td>Granzyme B</td>
</tr>
<tr>
<td>Integrin $\alpha_E$</td>
<td>Granzyme C</td>
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<td>Granzyme D</td>
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<td>Perforin</td>
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<td>FASL</td>
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<td>IFN-(\gamma)</td>
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<td>Eomesodermin</td>
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| **Co-stimulatory molecules**              | **I:E fold changes**                    |
| CD27                                     | 2.9                                     |

| **T cell survival / memory generation**   |                                          |
| IL-7R-$\alpha$                           | 7.4                                     |
| CD27                                     | 2.9                                     |

| **Apoptosis**                             |                                          |
| BID                                      | 10.7                                    |
| BAD                                      | 4.4                                     |
| FASL                                     | 2.4                                     |

| **Replicative senescence**                |                                          |
| KLRG-1                                   | 2.5                                     |
| MRG1X                                    | 2.3                                     |

E, effective cells (early effector)
I, impaired cells (intermediate effector)

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

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

Gattinoni et al. JCI, 2005
Can we generate and select more effective CD8+ T cells?

**Graph:**
- X-axis: Days (0, 7, 14, 21)
- Y-axis: # viable cells
- Two lines:
  - Pmel-1^{IL-2}
  - Pmel-1^{IL-15}

**FluorescenceActivatedCellSorting (FACS) plots:**
- 14-d: Pmel-1^{IL-2} (0.2%), Pmel-1^{IL-15} (48%)
- 21-d: Pmel-1^{IL-2} (0.02%), Pmel-1^{IL-15} (16%)

*Gattinoni et al. JCI, 2005*
Programming T cells for adoptive immunotherapy with IL-15

Klebanoff, et al, PNAS, 2005
Explosive growth of central memory tumor-specific T cells

Klebanoff, et al, PNAS, 2005
Programming T cells with IL-15 improves their efficacy in adoptive Immunotherapy

Klebanoff, et al, PNAS, 2005
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