The Immune Rejection of Human Cancers: Cytokines, Vaccines and T-Cells

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Surgery Branch, NCI
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“Natural” Immunotherapy of Cancer

Rarely, human tumors can spontaneously regress, often after surgery or infection.
The Role of T-Cells

T-lymphocytes were found to be responsible for rejection of transplanted tissue. They can kill cells that they immunologically recognize or they can secrete cytokines.
Immunotherapy for Human Cancers
(“The Dark Ages”)

“It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer.”

W. H. Woglom
Interleukin-2 (IL-2)

"The Dawn"

15,500 m.w. glycoprotein made by CD4 and CD8 lymphocytes

T-cell growth factor

Activates T-cells and NK cells

Essential to the survival and action of regulatory T-cells

Has no direct effects on tumor cells
Interleukin-2

Discovered by Morgan, Ruscetti and Gallo (1976)

Gene for IL-2 cloned by Taniguchi (1983)

Recombinant IL-2 made by the Cetus Corporation and tested in the Surgery Branch (1984)

History of High-Dose IL2

IL-2 was dose escalated to high levels (with significant toxicity) with no responses seen against multiple tumor types.

Lymphokine Activated Killer cells (LAK) were added to HD IL2 based on results in mice.

In the next 25 patients, there was 1 CR and 3 PR in 7 pts with melanoma and 3 PR in 3 patients with RCC.

(Result of selecting tumor types, not LAK)
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1st Codes: ACT CCT CEB FLX HAE HS LS IVP EOE NNT BB DL TOTAL = ______

SOLID-XT 93/07/36 Completed: ______ Entered: ______ Baseline: ______

MAY 01 1987
Metastatic Melanoma
Metastatic Renal Cancer
Interleukin-2 for Metastatic RCC

259 Patients with Metastatic Renal Cell Carcinoma
Treated with High-Dose IL-2

Proportion Responding

Response Duration in Years

23 CR

30 PR

5/07
Interleukin-2 for Metastatic Melanoma

305 Patients Treated with High-Dose IL-2

Survival in Years

Proportion Surviving

12 Complete Responders

27 Partial Responders
Initial Approaches to Improving IL-2

• Understand the T-cells mediating these responses

• Vaccinate patients to generate more tumor-reactive T-cells

• Grow tumor-reactive T-cells in vitro and administer them
Initial Approaches to Improving IL-2

• Understand the T-cells mediating these responses

• Vaccinate patients to generate more tumor-reactive T-cells

• Grow tumor-reactive T-cells in vitro and administer them
The MAGE-1 antigen was the basis of the recognition of a patient’s melanoma by T-cells which had been generated by repeated stimulation with that tumor
Tumor Infiltrating Lymphocytes (TIL)

- Almost all tumors contain lymphocytes that have infiltrated into them from the host

- Placing the entire tumor into culture with IL-2 (T-cell growth factor) will allow the TIL to expand while the tumor cells grow poorly

- TIL grown with IL-2 from human melanomas often show the ability to recognize and kill the tumor they were grown from (other TIL do not)
Melanoma TIL
(Tumor Infiltrating Lymphocytes)

Fresh digest       One week       Two weeks
Figuring Out What Tumor-Reactive Melanoma TIL Are Recognizing

Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor

(tumor antigen/immunotherapy/HLA-A2/melanocyte/MART-1)

YUTAKA KAWAKAMI*, SIONA ELIYAHU*, CYNTHIA H. DELGADO*, PAUL F. ROBBINS*, LICIA RIVOLTINI*, SUZANNE L. TOPALIAN*, TORU MIKI†, AND STEVEN A. ROSENBERG*

*Surgery Branch and †Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

MART-1 (Melanoma Antigen Recognized by T-cells), a protein involved in pigment production, was recognized by tumor-reactive melanoma TIL
Melanoma-Associated Antigens Found Using TIL

- Tissue differentiation antigens (pigment production)*
- Tumor-germline (previously tumor-testis) antigens*
- Tumor-specific mutations

* Normal proteins shared by multiple melanomas
Vaccinations Against Defined Melanoma Antigens

- MART-1, gp100, tyrosinase, NY-ESO1, MAGE family, TRP-2, Her-2 and telomerase were targeted with vaccine protocols

- Peptides, DNA, proteins, dendritic cells and recombinant viruses were used as modes of vaccination
• 440 Patients were given 541 vaccines
• 96% had metastatic melanoma
• 765 patients in 35 other vaccine trials were also reviewed
• The overall response rate in the 440 Surgery Branch patients was 2.6% with only 3 patients reaching CR (0.5%) and only 3 responders had visceral involvement
• The 765 reviewed patients had an overall response rate (PR+CR) of 3.8%
Conclusions

- Cancer vaccines alone do not treat patients with metastatic cancer effectively

- The few anecdotal responses are rarely complete and are often against cutaneous or nodal disease

- Better ways to augment the anti-tumor T-cell repertoire were needed
Adoptive Cellular Therapy

• Could cultured T-cells be infused in sufficient numbers to induce tumor rejection?

• What conditions will optimize the effectiveness of these T-cells?

• Where would one consistently obtain T-cells which recognize tumors?

TIL from melanoma frequently have anti-tumor activity when expanded in IL-2
Principles of Adoptive Cellular Therapy

• T-cell transfer is enhanced when the recipient is temporarily immuno-suppressed prior to transfer
  - Deletes host regulatory T-cells
  - Stimulates host T-cell growth factors

• Giving systemic IL-2 with cells may support in vivo expansion and function
Cyclophosphamide + Fludarabine
Preparative Chemotherapy

Infuse T-Cells

- Neutrophils
- Lymphocytes
- Cy
- Flu
- IL-2

Cells/ul (x10E-3)

Day
What Other Factors Affect Tumor Rejection?

- T-cells are turned off by inhibitory receptors (activation “checkpoints”)
  - CTLA4
  - PD1

- Antibodies have been developed to block these “checkpoints” to preserve or sustain T-cell activation
  - Ipilimumab
  - Nivolumab
T-Cell Activation and Inhibition

McDermott et al, Ca Treat Rev
T-Cell Activation and Inhibition

McDermott et al, Ca Treat Rev
T-Cell Activation and Inhibition

- Nivolumab
- Pembrolizumab
- Ipilimumab
Metastatic Melanoma Treated with Ipilimumab (Anti-CTLA4)
Metastatic Melanoma Treated with Ipilimumab (Anti-CTLA4)
Ipilimumab for RCC

Pre-Treatment

24 Months
Randomized Trial with Ipilimumab

**Progression-free Survival**

**Overall Survival**

PR = 6.5%

CR = 0.5%
Anti-PD1 Antibodies for Melanoma

**Nivolumab**
- ORR = 31%
- CR = 3%

Topalian, JCO

**Pembrolizumab**
- ORR = 26%
- CR = 1%

Robert, Lancet
**PD1/PDL1 Blockade for Other Cancers**

### Nivolumab Phase I Long-Term Results

<table>
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<tr>
<th>Tumor Type</th>
<th>ORR (%; no patients)</th>
<th>Response Duration (median; mo)</th>
<th>OS (median; mo)</th>
<th>Survival (%)</th>
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<td><strong>RCC</strong></td>
<td>29 (10/34)</td>
<td>12.9</td>
<td>&gt;22</td>
<td>70</td>
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Topalian, Sznol, Brahmer et al. ASCO 2013
Are the Same Patients Responding to All Immunotherapies?

Impact of Prior Therapy on Response to Adoptive Cell Therapy using Selected Young TIL

- Prior a-CTLA4 (n=11)
- All Patients (n=93)
- Prior IL-2 (n=77)
- Prior Chemotherapy (n=40)
- Prior Interferon (n=52)

Proportion Surviving vs. Survival Time in Months
The Main Obstacle: Getting Tumor-Specific T-Cells

- Not all melanomas have reactive TIL, and some patients still do not respond.
- The TIL from other cancers are rarely tumor-reactive.
- Most cancer cells cannot even be grown in the lab for testing against T-cells.
One Approach: Genetically Engineer Anti-Tumor Receptors into Peripheral Blood Lymphocytes

• If a tumor-reactive T-cell is found, its T-cell receptor can be retrovirally introduced into another patient’s PBL

• Other “unnatural” receptors such as CAR (chimeric antigen receptors) can also be used

• These cells are then given exactly as native T-cells are administered
Gene-Engineered Anti-Tumor Receptors
Anti-NY-ESO1 TCR
(Synovial Sarcoma)
Anti-CD19 CAR
(Large B-Cell Lymphoma)

Prior Therapy:
R-CHOP
R-ICE
Brentuximab
R-HiDAC
Panobinostat
Lenalidomide
R-GDP
Anti-CD22
MAE
The Problem with Receptors
Targeting Normal Tissue Antigens

Some antigens are highly expressed on tumors but are also expressed by some normal tissues.

*Gene-engineered T-cells can be used to specifically attack these targets hoping to impact the tumor but not the normal tissue.*

Accidental attack on important normal tissues can cause limiting toxicities.
Targeting Melanocytic Proteins: MART-1
Targeting CEA

- Better targets are still needed
The Future of T-Cell Therapy for Melanoma and Other Cancers

• Some melanoma TIL were found which recognized mutated proteins in the patient’s tumor

• All human cancers accumulate genetic mutations as the cause of their transformation

• The mutated proteins that result are completely tumor-specific and are “foreign” proteins to the host
Somatic mutation frequencies observed in exomes from 3,083 tumour-normal pairs.

A Patient with Cholangiocarcinoma

- 43 yo F with metastatic cholangiocarcinoma who had progressed after hepatic and lung resections, cisplatin, gemcitabine and taxotere
- Had TIL grown from a lung metastasis
- Given Cy-Flu, $4 \times 10^{10}$ TIL and 4 doses IL-2
- Had minimal response followed by tumor progression within a year
Best Response to Treatment #1
Continued...

- During that year, her tumor DNA was sequenced
- 26 mutations were found
- “Mini-genes” encoding just these mutated sequences were made and introduced into her own dendritic cells
- These were then tested for the ability to stimulate her TIL
ELISPOT ASSAY FOR TIL RECOGNITION OF ‘MINIGENES’

Co-culture TIL + three pools of Minigenes--
Stain purple for TIL secreting Interferon-gamma
(each done in duplicate)

• Mock  • GFP  • TMG-1  • TMG-2  • TMG-3  • OKT3

Pools of 8-9 minigenes each

Positive Control

The specific mutated gene in pool TMG-1 encoded ERB-B2 interacting protein (ERBB2IP)
Continued...

• Her TIL cultures were examined for T-cells with this reactivity and one culture was found that was 95% pure

• Only these cells were grown in vitro and given in a second treatment

• This second infusion contained 12 times as many of these cells as the first treatment and she received the same chemo and 4 doses of IL-2
Lungs

Treatment #1

Pre-Treatment  
7 Months

Treatment #2

Pre-Treatment  
8 months
Hypotheses and Implications

• Mutated ‘neo-antigens’ drive the native immune response to cancer

• These responses are often weak but can be augmented by T-cell transfer

• Generic immunotherapies such as IL-2 and checkpoint inhibitors will work best in the most mutated tumors

• But any tumor could respond to the right T-cell
Driving Towards Tumor Rejection

T-cell Engine

Checkpoint brakes (CTLA4)

Checkpoint brakes (PD-1)

Cytokine Fuel (IL-2)
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<th>Target Antigen</th>
<th>Type</th>
<th>Cancers</th>
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<td>TIL</td>
<td>Melanoma, bladder and GI cancers</td>
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<tr>
<td>(Native)</td>
<td>TIL</td>
<td>HPV+ cervical and head/neck CA</td>
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<td>(Native)</td>
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<td>Non-small cell lung cancer</td>
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<td>CAR</td>
<td>Large B-cell lymphoma</td>
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<td>EGFRvIII</td>
<td>CAR</td>
<td>Glioblastoma</td>
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<tr>
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<td>CAR</td>
<td>Pancreas, ovary &amp; mesothelioma</td>
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<td>TCR</td>
<td>Melanoma and adeno CA</td>
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<td>Thyroglobulin</td>
<td>TCR</td>
<td>Differentiated thyroid CA</td>
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Immunotherapy for Human Cancers
(“The Golden Age”)

“It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer.”

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