T CELL THERAPY: HARNESSING THE IMMUNE SYSTEM TO FIGHT CANCER

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Conflict of Interest

- Financial interest due to a patent in T cell culture systems (Novartis)
Outline

• Immunology 101
• Approaches redirect the immune system
• Trial data in follicular lymphoma
• Trial data in multiple myeloma
• Summary
## Innate vs adaptive immunity

<table>
<thead>
<tr>
<th></th>
<th>innate</th>
<th>adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>self / non-self</td>
<td>present, reaction is against foreign</td>
<td>present, reaction is against foreign</td>
</tr>
<tr>
<td>discrimination</td>
<td></td>
<td></td>
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<tr>
<td>lag phase</td>
<td>absent, response is immediate</td>
<td>present, response takes at least a few days</td>
</tr>
<tr>
<td>specificity</td>
<td>limited, the same response is mounted to a wide variety of agents</td>
<td>high, the response is directed only to the agents that initiated it.</td>
</tr>
<tr>
<td>diversity</td>
<td>limited, hence limited specificity</td>
<td>extensive, and resulting in a wide range of antigen receptors.</td>
</tr>
<tr>
<td>memory</td>
<td>absent, subsequent exposures to agent generate the same response</td>
<td>present, subsequent exposures to the same agent induce amplified responses</td>
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</table>
Innate vs. Adaptive Immunity
CD4 T cell subsets

Mediate antigen specific naïve or memory B cell activation

Humoral immunity
Clearance of certain extracellular pathogens
Allergy

Cellular immunity
Clearance of intracellular pathogens

Tissue inflammation
Autoimmunity
Clearance of certain extracellular pathogens

Tolerance
Immune suppression

O'Shea and Paul. 2010. Science
CD4 T cell subsets
CD8 T cell subsets

CD8⁺ T cells

Naive

CD44 Lo CD62L Hi

Memory stem cell

IL-7 IL-15

CD44 Lo CD62L Hi Sca-1 Hi

Central memory

Effecter memory

CD44 Hi CD62L Hi

CD44 Hi CD62L Lo

Cancer Immunooditig Hypothesis

Evidence to Support Immunosurveillance

- Despite suppressive microenvironment, intratumoral T cells correlate with increased overall survival in multiple human cancers (ovarian, colorectal, melanoma)

- These findings have invigorated attempts to utilize T cells as a treatment against cancer


Sato, et al. PNAS 2005
Approaches to Redirect Immune Response Towards Cancer

Adoptive T Cell therapy
- Site
  - Peripheral blood
  - Tumor site
- Specificity
  - Polyclonal
  - Antigen-specific
  - Engineered

Ideally, will recapitulate the end result of a vaccine to induce T cell-immunity
- Large number of potent antigen specific T cells
- Expansion in vivo in response to antigen encounter
- Potent anti tumor activity
- Contraction and long-term persistence
- Ability to respond to challenge

“Prime Boost” Approach: Combination of Active and Passive Immunity

Hypothesis

“Threshold for regression”

Host Lymphodepletion

% tetrimer positive T cells

0.01

0.1

1

10

100

Cancer vaccine

T cell expansion ex vivo
Utilizing Artificial APCs for T Cell Expansion

*In vivo*

Dendritic cell

Resting T cell

Activated T cell

Dynabeads® activation

Bead

Anti-CD28

Resting T cell

Activated T cell

Anti-CD3

CD28

CD3/TCR

Anti-CD3/TNR
Adoptive Immunotherapy with Autologous CD25-Depleted and CD3/CD28-Costimulated T-Cells (ACTC) Enhances Numeric and Functional Lymphocyte Recovery after Cyclophosphamide - Fludarabine (FC) Chemotherapy in Patients with Low-Grade Follicular Lymphoma

Lymphoma Group
Stephen J. Schuster, PI
ABRAMSON CANCER CENTER of the UNIVERSITY of PENNSYLVANIA
Background and Rationale

• Follicular lymphoma is the second most common form of NHL

• Cyclophosphamide – fludarabine is effective therapy for patients with follicular lymphoma but causes immunosuppression
Hypotheses

• Infusion of autologous T cells may improve the therapy of follicular lymphoma in the setting of minimal residual disease following cytoxan-fludarabine
  • Restoration of cellular immunity
  • Facilitate anti-tumor immunity

• CD25 depletion prior to CD3/CD28-costimulated expansion will reduce the number of CD4+CD25+FOXP3+ regulatory T cells.
Protocol Treatment

CHEMOTHERAPY

cyclophosphamide (250 mg/m2) days 1 – 3
fludarabine (25 mg/m2) days 1 – 3

T-CELL INFUSION DOSE LEVELS

1 - $\leq 5 \times 10^9$ CD3$^+$ cells
> 5 - 10 $\times 10^9$ CD3$^+$ cells
Study Design

Staging / PBL studies / apheresis → Monodeplete / Cryopreserve

- CF x 4 - 6 cycles
- Response Assessment / PBL Studies
- CR / CRu / PR / SD

T-Cell Infusion: Dose Escalation (Day 0)

PBL Studies / DTH Testing (Day 60)

Response Assessment (Day 120)

Follow-up
## Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count</th>
</tr>
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<tbody>
<tr>
<td>Median age (years)</td>
<td>49 (range: 32–68)</td>
</tr>
<tr>
<td>Sex (male : female)</td>
<td>6 : 9</td>
</tr>
<tr>
<td>Median number of prior therapies</td>
<td>2 (range: 1–3)</td>
</tr>
<tr>
<td>Rituximab refractory</td>
<td>13 (87%)</td>
</tr>
<tr>
<td>Stage II, II\textsubscript{x}, III, IV</td>
<td>1 (6%), 1 (6%), 7 (47%), 6 (40%)</td>
</tr>
<tr>
<td>Bone marrow involved</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>2 (13%)</td>
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**Clinical Outcomes**

**Enrollment (n = 15)**

- **T cell dose: 1 - <5 x 10^9 cells**
  - (n = 5; CR = 3, PR = 2)
  - 3 CR/PR @ 40, 57, 73 months
  - 2 PD @ 2, 14 months

- **T cell dose: 5 - 10 x 10^9 cells**
  - (n = 5; CR = 5)
  - 2 CR @ 35, 40 months
  - 3 PD @ 5, 7, 11 months

**Ineligible for T cell infusion (n = 5)**

- Hematologic toxicity (n = 2)
- Progressive disease (n = 3)
CD4 Counts Pre- and Post-Infusion

- **X = 515** (range 186 – 1153)
- **X = 95** (range 15 – 169)

**T-Cell Dose**
- 1.7E+09
- 3.5E+09
- 3.9E+09
- 4.0E+09
- 4.9E+09
- 5.2E+09
- 9.2E+09
- 10.0E+09

**p = 0.012** (Wilcoxon signed-rank test)
CD8 Counts Pre- and Post-Infusion

- **CD8 Counts**
  - Pre-Infusion: 1.7E+09 to 10.0E+09 CD8 cells/μL
  - Post-Infusion: 5.2E+09 to 10.0E+09 CD8 cells/μL

- **T-Cell Dose**
  - Range: 524 (158 – 1860)
  - X = 82 (19 – 273)

- **Statistical Test**
  - Wilcoxon signed-rank test
  - p = 0.017
Fold Increase in T-Cell Counts by Dose Level

**CD8 cells**

- Dose Level: <5 E+09
- Median: [Graph]

**CD4 cells**

- Dose Level: <5 E+09
- Median: [Graph]
# DTH Candida Skin Test Results

*n = 10*

<table>
<thead>
<tr>
<th></th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRE-CHEMO Rx</strong></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>POST-CF-ACTC</strong></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Increase in T cell function after ACTC

Negative control

PMA/Ionomycin

Enrollment

Day +60

IFN-γ

IL-2

IL-2

IFN-γ

IL-2

IFN-γ

IL-2

IFN-γ

IL-2

IFN-γ
Decrease in the Percentage of CD4+FOXP3+ Peripheral Blood Lymphocytes after T cell Infusion

\[ p = 0.01 \text{ for Prechemo and Day 60} \]
Progression-Free Survival

\[ p = 0.007 \] (log rank test)

- **CF-ACTC**
- **Prior Rx**
Conclusions

• ACTC can be successfully generated *in vitro*, even in previously treated patients with low-grade follicular lymphoma.

• ACTC infusion following fludarabine and cyclophosphamide chemotherapy is well-tolerated and results in a robust increase in CD4+ and CD8+ T-cell counts.

• Overall clinical efficacy is significantly better that of the last chemotherapy received.

• Restoration of immune reactivity to *C. albicans* has been demonstrated both *in vitro* and *in vivo*. 
Combination Immunotherapy
After ASCT for Multiple Myeloma (MM)
Using MAGE-A3/Poly-ICLC Immunizations
Followed by Vaccine-Primed and Activated Autologous T-Cells
Background and Rationale

- Myeloma comprises 15% of hematologic malignancies
- Allogeneic transplants can induce cures, but treatment-related risks are high
- Autologous transplants are highly effective for tumor reduction (first line therapy), but cures are infrequent
- Immune reconstitution after hematopoietic stem cell transplant (HSCT) characterized by:
  - Fast (1-2 months) recovery of B cells
  - Impaired lymphocyte recovery (CD4 > CD8)
- Sustained antibody levels post-HSCT require recovery of competent T cells.
- Early recovery of T cells \(\rightarrow\) increased survival post-HSCT
- Post-transplant immunodeiciencies increase the risk for serious infections from microorganisms such as VZV, CMV, and S. pneumoniae.
Research Hypothesis

Adoptive transfer of vaccine-primed, anti-CD3/anti-CD28 costimulated & expanded T cells after autologous transplant may repair immune deficiencies, leading to:

- *Better control of myeloma*
- *Better protection from infection*
Adoptive Transfer of T cells + Prevnar® pneumococcal conjugate vaccine after Transplant for Myeloma (randomized study)

Major Findings: Pre- and Post-transplant immunizations + day +12 infusion of vaccine-primed T cells → robust T + B responses as early as day +30 post-transplant

Adoptive Transfer of T cells + Cancer Vaccine (based on hTERT and survivin peptides) after Transplant for Myeloma

Major Findings: 1) Combination strategy induces T cell responses to tumor antigen peptide vaccine in ~36% of patients; 2) Clinically significant auto-GVHD occurs in ~16% of patients who receive day +2 T cell transfers

Strategies for Augmenting Immune Response and Clinical Impact

• Increase immunogenicity of “priming” and booster vaccinations with novel adjuvants
• Use a vaccine that targets a more relevant/widely expressed myeloma tumor antigen
• Add post-transplant immunomodulator (Lenalidomide)
MAGE-A3

- Member of the cancer-testis antigen family
  - Also includes CT-7, NY-ESO-1
- First described in malignant melanoma
- Detected in ~50% of myelomas
  - More highly expressed in advanced-stage, proliferative, and extramedullary disease
- MAGE-A3 vaccine (GL-0817)
  - Large peptide composed of 3 smaller peptides – two class I, one class II epitopes
  - Class II peptide, HLA-promiscuous
  - Designated an orphan drug by the FDA
Hiltonol® (Poly-ICLC)

- Hiltonol® is a stabilized dsRNA viral-mimic
- TLR-3 agonist ↑ innate and adaptive immunity
- Triggers maturation and activation of APCs
- Enhances co-stimulatory signals and release of cytokines
- Potent inducer of T cell responses in a variety of animal models using microbial and tumor antigens
MAGE-A3 Vaccine Trial Design

**Transplant Stage**

- Myeloma (previously treated or high risk)
- Mobilization
- Stem Cell Collection
- High-dose Melphalan
- Stem Cell Transplant
- T Cell Collection
- T cell in vitro activation and expansion
- T Cell Infusion Day +2
- MAGE-A3, PCV boosters at Day+14, 42, and 90

**Maintenance Stage**

- Restaging (Day 100)
- MAGE-A3 (GL-0817), PCV – D120
- MAGE-A3 (GL-0817), PCV-D150
- Restaging (Day 180)
- Restaging (Day 270)
- Restaging (Day 360)

* Lenalidomide 10 mg daily
Patient Demographics

- 27 patients enrolled to date
- 16 Male / 11 Female
- 17 Caucasian (63%), 6 African American (22%), Asian 4 (15%)
- 19 enrolled at UMD, 8 enrolled at UPENN
- IgG = 19/27(70%), IgA = 4/27(15%), LC = 4/27(15%)
- 12/27 (44%) with abnormal cytogenetics
- Mean T cell dose infused – 3.2 x 10^{10}
  - Range 1.42 x 10^9 – 5.2 x 10^{10}
# Clinical Responses

<table>
<thead>
<tr>
<th>Response</th>
<th>Day 100*</th>
<th>Day 180**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR + nCR + VGPR</td>
<td>15/25 (60%)</td>
<td>14/23 (61%)</td>
</tr>
<tr>
<td>Partial Response</td>
<td>6/25 (24%)</td>
<td>8/23 (35%)</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>3/25 (12%)</td>
<td>1/23 (4%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>1/25 (4%)</td>
<td>0/23 (0%)</td>
</tr>
</tbody>
</table>

* 2 pts too early  
** 4 pts too early
Event-free survival

21/27 censored
6/27 events
Overall Survival

25/27 censored
2/27 events
Injection site reaction 3 days after 2\textsuperscript{nd} post-transplant (day 42) MAGE-A3 immunization (02710-203, RT thigh)
T cell recovery after ASCT

![Graph showing T cell recovery over days post-transplant for CD3, CD4, and CD8 cells.](image-url)
Vaccine-specific T cells post-immunization (02710-217)

**Cultured with**
- CTL-1 peptide
  - Enrollment: 0
  - D2: 0.0167
  - D14: 0.0867
  - D100: 3.27e-3
  - D180: 0.025
- CTL-2 peptide
  - Enrollment: 0
  - D2: 0.0873
  - D14: 0.306
  - D100: 0.472
  - D180: 0.291
- Mage A3 Whole vaccine
  - Enrollment: 0.229
  - D2: 1.64
  - D14: 0.577
  - D100: 0.579
  - D180: 0.816

**Stained with**
- CTL-1 Dextramer
- CTL-2 Dextramer
- CTL-1 Dextramer
- CTL-2 Dextramer

**CD8**
Summary of Patient Dextramer Responses to Vaccine Peptides

**CTL-1 (Class I) peptide**
- Pt. Dextramer (CTL-1) +: 40%
- Pt. Dextramer (CTL-1) -: 60%

**Mage A3 whole vaccine**
- Pt. Dextramer (CTL-1) +: 40%
- Pt. Dextramer (CTL-1) -: 60%

**CTL-2 (Class I) peptide**
- Pt. Dextramer (CTL-2) +: 100%

**Mage A3 whole vaccine**
- Pt. Dextramer (CTL-2) +: 80%
- Pt. Dextramer (CTL-2) -: 20%
Vaccine-induced MAGE-specific T cells are functional

HTL peptide

Enrollment  T cell collection  D100  D180

Mage A3 Whole vaccine

IFN-γ

CD4+

0  0.0218  0.699  1.58

0  0.121  0.275  3.1
Summary of Patient Interferon-γ Responses to Vaccine Peptides

**Mage A3 whole vaccine IFN-γ+ patient**
- IFN-γ- 29%
- CD4+ 52%
- CD8+ 33%
- CD4+CD8+ 24%
- N=21

**HTL(Class II) peptide**
- Pt. IFN-γ+ 29%
- Pt. IFN-γ- 71%

**Mage A3 whole vaccine IFN-γ+ patient**
- IFN-γ- 29%
- CD4+ 52%
- CD8+ 33%
- CD4+CD8+ 24%
- N=21
GL-0817 induces MAGE-A3-specific antibody formation
GL-0817 induces MAGE-A3-specific antibody formation

![Graph showing Mage A3 ELISA titer over time with and without Montanide.](image-url)
T cell function may correlate with clinical response
Conclusions

• The addition of Hiltonol®/Poly-ICLC appears to significantly increase the frequency of both CD8+ and CD4+ T-cell responses to a tumor antigen vaccine based on MAGE-A3 peptides.

• When given with montanide, Hiltonol®/Poly-ICLC regularly elicits large and long-lasting skin reactivities to the vaccine, but the elimination of montanide nearly abolished the B-cell (but not T-cell) responses to the tumor antigen vaccine.

• Early clinical myeloma responses were encouraging and preliminary studies suggest that enhanced T-cell function may correlate to better clinical outcomes.

• A study of combination immunotherapy using a MAGE-A3 vaccine + vaccine primed T-cells for patients with MAGE-A3 positive myeloma is warranted.
Summary

• Immune system plays a dual role in cancer
  • Suppress tumor growth by either destroying cancer cells or preventing outgrowth
  • Select tumor cells that are more fit to survive
  • T cells are important in both these functions
• T cell immunotherapy can mediate tumor regression in patients with cancer
• Recent advances include more accurate targeting of antigens expressed by tumors and the associated vasculature, and the successful use of gene engineering to re-target T cells before their transfer.
• New research has helped to identify the particular T cell subsets that can most effectively promote tumor eradication
• Biomarkers for clinical efficacy are needed
  • Immune response vs clinical response
Acknowledgments

Courageous and selfless patients who willingly participated in this and other similar studies

Jim and Frannie Maguire

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