T Cell Stemness: An Emerging Principle of Successful Adoptive Cell Therapy

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No financial conflicts of interest
All reproducibly effective cancer immunotherapies involve T cells

Checkpoint blockade anti-PD-(L)1, anti-CTLA-4

CAR/TCR/TIL-based treatments
General schema for growing naturally-occurring anti-tumor T cells

- Excise tumor
- Plate fragments
- Culture with 6000 IU/mL IL-2
- Reinfuse post-lymphodepletion
- Select and expand to $10^{10}$ cells
- Assay for specific tumor recognition

Rosenberg & Restifo
Science 2015
Gene-modification of T cells

Complete regression of metastatic breast cancer

Adapted from Zacharakis et al, Nat Med 2018
Why do our efforts focus on adoptive cell transfer (ACT) therapy for treating metastatic cancer?

1. Identify and enrich for qualities associated with anti-tumor efficacy.

2. Pharmacologically or genetically modify T cells to enhance their therapeutic efficacy.

3. Confer new specificity in transferred T cells (eg CAR, TCR).

4. Administer large numbers of tumor antigen-specific cells.

5. ‘Lymphodeplete’ host prior to cell transfer, reducing immunosuppressive cells in the tumor microenvironment.
Cells as Drugs

New paradigms in tissue distribution and pharmacokinetics

Living cells can move ‘against’ concentration gradients and exhibit conditional function after integrating micro-environmental information.
Melanoma-specific survival in patients treated with autologous tumor infiltrating lymphocytes (n=194)

TIL appear capable of eliminating the last cancer cell

Nov 2016
Not all T cells are equal…

More effective cells

Less effective cells
• Cancer cells are generally immortal, but the T cells used to kill tumor cells are themselves programmed to experience aging, senescence and death.
What is T cell stemness and why does it matter?

Stemness: Noun; Etymology stem + -ness

“An essential characteristic of a stem cell”

1. The capacity to **self-new**
2. **Multipotency** (can generate differentiated T cell subsets)
3. **Persistence** and **proliferative potential**.

Gattinoni, Klebanoff & Restifo, Nat Rev Cancer, 2012
Individual T cells (clonotypes) are capable of ‘stemness’

Many organ systems in adult metazoans involve stem cells

Stem cell-like T cells have been identified in mice and in humans

The TCF7 transcription factor is centrally involved in T cell stemness
Stem-like behavior:
“Lymphodepleted” patients can experience clonal repopulation of anti-tumor T cells

Expansion of TIL to $10^{10} - 10^{11}$ cells

Clonal repopulation, Dudley, et al
Science 2002
T cell survival at 1 month is highly correlated with objective clinical response

Patient response status

- Complete Tumor Destruction (CR)
- > 30% Tumor Destruction (PR)
- < 30% Tumor Destruction (NR)

% persistence of the infused cells in peripheral blood

* CR + PR (>30% reduction) vs. NR (<30% reduction) < 0.001
**Tcf7 high T memory stem cells** (T\text{scm}) **cells** are more effective and can be used to treat large established tumors at lower doses

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![Graph showing tumor size and proportion surviving over time](image)

**Time (d) after treatment**

- No treatment
- T\text{SCM}
- T\text{CM}
- T\text{EM}

TCF7*CD8+ T cell frequency in tumor tissue predicts response and better patient survival after checkpoint blockade

Sade-Feldman, et al, Cell, 2018
Each T cell clonotype is a stem cell system

~ 900 Genes are dynamically regulated during post-thymic T cell differentiation
How do we consolidate these advances to make cancer immunotherapy more effective?

- Disrupt the cell death program to keep T cells alive longer (e.g., use CRISPR to remove mediators of senescence and apoptosis).
- Use drugs to uncouple T cell proliferation from differentiation (e.g., inhibitors of cell signaling cascades like PI3K → Akt → mTOR pathway).
- Metabolically or epigenetically alter T cells to improve their longevity.

![Diagram of Senescence and Apoptosis]
Fundamentally reprogram the T cells to make them young and vibrant again.
iPSC-derived T cells treat established solid tumors and prolong survival

Days after cell transfer

Tumor area (mm$^2$)

% Survival

Days after cell transfer

No cells
Pmel-iPSC unprogrammed
CD3T-iRTE
Pmel-iPSC redifferentiated
Naïve T cells

P = 0.028
P = 0.0004
Characterization of human TIL-iPSC

OCT-4
TRA-1-60

NANOG
TRA-1-81

SOX2

Endoderm
Mesoderm
Ectoderm
iPSC derived from human T cells are mutation specific

Mutated peptide pulsed autologous B cells

Bulk TIL-iPSC-derived immature T cells (day 38)

WT: AYRDLQTER
Mut: AYRDLQTRK

WT 10 ug/mL
Mut 10 ug/mL

0 20 40
% of CD3+ 4-1BB+

T cells alone
DMSO
PMA/Iono
WT 10ug/ml
WT 1ug/ml
WT 0.1ug/ml
Mut 10ug/ml
Mut 1ug/ml
Mut 0.1ug/ml

HLA-A*30:01-restricted
> 800 individual iPSC lines derived from mutation-reactive T cells from a variety of cancer histologies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor type</th>
<th>Cell sources</th>
<th>Clonality</th>
<th>Efficiency of iPSC generation</th>
<th>TIL-iPSC lines established</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Colon</td>
<td>Infusion bag</td>
<td>Oligoclonal</td>
<td>1 in 2000</td>
<td>28</td>
</tr>
<tr>
<td>B</td>
<td>Colon</td>
<td>Infusion bag</td>
<td>Oligoclonal</td>
<td>1 in 2500</td>
<td>13</td>
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<tr>
<td>C</td>
<td>Gastric</td>
<td>Limiting dilution</td>
<td>Clonal</td>
<td>1 in 2500</td>
<td>23</td>
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<tr>
<td>D</td>
<td>Pancreatic</td>
<td>Infusion bag</td>
<td>Oligoclonal</td>
<td>1 in 627</td>
<td>87</td>
</tr>
<tr>
<td>E</td>
<td>Melanoma</td>
<td>Tumor Fragment</td>
<td>Polyclonal</td>
<td>1 in 288</td>
<td>178</td>
</tr>
<tr>
<td>F</td>
<td>Melanoma</td>
<td>Tumor Fragment</td>
<td>Polyclonal</td>
<td>1 in 250</td>
<td>353</td>
</tr>
</tbody>
</table>
Summary

• Stemness, the capacity of T cells to self renew, proliferate, persist and form large numbers of more differentiated progeny, may be an emerging concept of cell-based therapy.

• The transcription factor, \textbf{Tcf7}, is central to the stemness phenotype.

• iPSC-derived T cells are clonal (specific for one antigen) and retain a great degree of proliferative capacity and specificity for tumor neo-antigens.
What we need to bring cell-based therapies to the many patients who need them:

1. Concerted and continued commitment to **basic science**.
2. **Clean space** for at or near scale experimental pre-clinical work (eg CRISPR, iPSC).
3. A **robust vector production** laboratory.
4. Large, GMP-quality **cell production laboratories** to produce cells for **patients**.
Restifo Lab: Past and present

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