Development of *Sleeping Beauty* transposed TCR-T cells for adoptive cell therapy of cancer

Drew C. Deniger

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Development of the non-viral *Sleeping Beauty* transposon/transposase system

Molecular Reconstruction of *Sleeping Beauty*, a Tc1-like Transposon from Fish, and Its Transposition in Human Cells

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T-cell receptor (TCR) and chimeric antigen receptor (CAR)

Integration of tumor-specific receptors into T cells via *Sleeping Beauty* transposition

Integration profile of *Sleeping Beauty* transposition in human T cells was widely distributed

TCR-T cell therapy has exceptionally larger targeting capacity relative to CAR-T cell therapy

Presentation of antigens on human leukocyte antigen (HLA) to T-cell receptor on the T-cell surface

https://www.immunopaedia.org.za/immunology/basics/4-mhc-antigen-presentation/
VDJ recombination as genetic LEGO in thymic development

Germ-line α-chain DNA

Rearranged α-chain DNA

Protein product αβ heterodimer

Rearranged β-chain DNA

Germ-line β-chain DNA

T cell
Diversity of the TCR repertoire is complex on purpose

<table>
<thead>
<tr>
<th>Element</th>
<th>Immunoglobulin</th>
<th>αβ T-cell receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>κ+λ</td>
</tr>
<tr>
<td>Variable segments (V)</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>Diversity segments (D)</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>D segments read in three frames</td>
<td>rarely</td>
<td>–</td>
</tr>
<tr>
<td>Joining segments (J)</td>
<td>6</td>
<td>5(κ) 4(λ)</td>
</tr>
<tr>
<td>Joints with N- and P-nucleotides</td>
<td>2</td>
<td>50% of joints</td>
</tr>
<tr>
<td>Number of V gene pairs</td>
<td>$1.9 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>Junctional diversity</td>
<td>$\sim3 \times 10^7$</td>
<td></td>
</tr>
<tr>
<td>Total diversity</td>
<td>$\sim5 \times 10^{13}$</td>
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</tr>
</tbody>
</table>

Figure 5.9 The Immune System, 3rd (© Garland Science 2009)
Somatic mutations are the blueprint for pan-cancer therapy

• Genomic instability is a hallmark of cancer. Somatic (non-inherited) mutations arising from this instability are largely unique to the patient, but a subset of mutations are shared in “hotspots” of critical cancer genes, e.g., KRAS and TP53.

• Some somatic genetic mutations will be transcribed, translated, processed and presented on the cancer cell surface generating a “neoantigen” which is not in the normal cells.

• T cells, through their TCR, recognize neoantigens in the context of HLA and can kill the cancer cell with mutation.

• Transfer of neoantigen-specific TCRs into naïve T cells with Sleeping Beauty transposition would generate TCR-T cells with unique and highly tumor-specific reactivity.
Presentation of neoantigens to T cells leads to exquisite tumor-specificity of TCR-T cells
Virtually all cancers have somatic mutations which can become neoantigens if processed and presented on the tumor surface.

Most epithelial cancer patients have TILs which recognize at least one autologous neoantigen.

Tumor infiltrating lymphocytes (TIL) therapy in metastatic melanoma: boosting of neoantigen-specific T cell reactivity and long-term follow-up


Detection of neoantigen-specific T cells following a personalized vaccine in a patient with glioblastoma


Immunogenicity of somatic mutations in human gastrointestinal cancers

Eric Tran, Mojgan Ahmadzadeh, Yong-Chen Lu, Alena Gross, Simon Turrotto, Paul F. Robbins, Jared J. Gartner, Zhili Zheng, Yong F. Li, Satyajit Ray, John R. Wunderlich, Robert F. Somervaille, Steven A. Rosenberg


CANCER IMMUNOTHERAPY

T-cell Responses to TP53 "Hotspot" Mutations and Unique Neoantigens Expressed by Human Ovarian Cancers

Drew C. Deniger, Anna Pesetto, Paul F. Robbins, Jared J. Gartner, Todd D. Pritchett, Biman C. Paria, Paria Malekzadeh, Li Ja, Ran Yossel, Michelle M. Langhans, John R. Wunderlich, David N. Danforth, Robert P.T. Somervaille, and Steven A. Rosenberg


Identification of Neoantigen-Reactive Tumor-Infiltrating Lymphocytes in Primary Bladder Cancer

Vid Leko, Lucas A. McDuffie, Zhili Zheng, Jared J. Gartner, Todd D. Pritchett, Andrea B. Apoulo, Priysh K. Agarwal, Steven A. Rosenberg, and Yong-Chen Lu

Shared “hotspot” mutations in \textit{KRAS} and \textit{TP53} genes are immunogenic and can be used for off-the-shelf TCR-T cells


Enhanced detection of neoantigen-reactive T cells targeting unique and shared oncogenes for personalized cancer immunotherapy

Rami Yossif,\textsuperscript{3} Eric Tran,\textsuperscript{2} Drew C. Deniger,\textsuperscript{2} Alena Gros,\textsuperscript{1} Anna Pasetto,\textsuperscript{2} Maria R. Parkhurst,\textsuperscript{1} Jared J. Gartner,\textsuperscript{1} Todd D. Prickett,\textsuperscript{1} Gal Cafri,\textsuperscript{2} Paul F. Robbins,\textsuperscript{2} and Steven A. Rosenberg\textsuperscript{2}


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Unbiased identification of neoantigen-reactive T cells

Neoantigen-reactive T cells (non-gene modified) resulted in objective regressions of metastatic epithelial cancers

1 unique driver neoantigen from 26 total mutations

Multiple unique neoantigen specificities
Application of neoantigen-specific TCR-T cell therapy

- Prior TCR-T cell therapy established that metastatic cancers can be effectively treated in some patients when targeting non-neoantigen targets, but the application was limited by target expression and cancer type.

- Non-gene modified adoptive cell therapy (TIL) resulted in objective clinical regressions in some patients but was ineffective for most people likely due to infrequent and/or terminal differentiation of neoantigen-specific T cells.

- The ability to translate a library or personalized neoantigen-specific TCR-T cell approach is complex and will likely require a rapid, mobile and cost-effective solution.

*Sleeping Beauty* transposition is an ideal candidate for this because it uses plasmid DNA, which is inexpensive to manufacture and allows for rapid personalization.
Sleeping Beauty transposition has been established for TCR-T cell and translated for CAR-T cell to the clinic

Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells


Stable, Nonviral Expression of Mutated Tumor Neoantigen-specific T-cell Receptors Using the Sleeping Beauty Transposon/Transposase System

Drew C Deniger1, Anna Patetto, Eric Tran1, Maria R Parkhurst1, Cyrille I Cohen2, Paul F Robbins3, Laurence JN Cooper4,5 and Steven A Rosenberg1

1Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; 2Department of Immunology and Immunotherapy, Ben-Gurion University, Beer-Sheva, Israel; 3Division of Pediatics, University of Texas MD Anderson Cancer Center, Houston, Texas, USA; 4Ziopharm Oncology, Inc., Boston, Massachusetts, USA


To the Editor:

Long-term outcomes of Sleeping Beauty–generated CD19-specific CAR T-cell therapy for relapsed-refractory B-cell lymphomas


Ziopharm is the first and currently only commercial group evaluating library TCR-T cells targeting shared “hotspot” neoantigens in the non-viral setting.
Ziopharm’s *Sleeping Beauty* library TCR-T cells were neoantigen-specific and led to tumor cell lysis.
Conclusions

• Neoantigens are the blueprint and “Achilles heel” for effective targeting of all cancers.

• TCR-T cell therapy is the answer to targeting neoantigens, which will require a rapid, flexible, cost-effective gene transfer platform, of which *Sleeping Beauty* transposition is the most advanced and commercially appealing.

• Ziopharm is the world leader of *Sleeping Beauty*-transposed TCR-T cell therapy and is positioned for clinical and commercial success treating solid tumors.