The Use of ex vivo Stimulation to Generate a Neoantigen-Specific T-Cell Product for Adoptive T-Cell Therapy

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Background

- Cancer cells contain unique, somatic mutations that result in altered amines and sequences known as neoantigens. These alterations may result in the expression of mutant proteins that are recognized as foreign by the immune system.
- Tumor neoantigens are exclusively expressed in tumors, making them attractive targets for immunotherapy.
- A growing body of evidence supports the role of neoantigens as attractive targets for immunotherapy.

Materials and Methods

- The NEO-PTC-01 induction protocol involves the generation of a neoantigen-specific T-cell product by performing a co-culture of peptide loaded autologous dendritic cells (DCs) with autologous peripheral blood mononuclear cells (PBMCs) (Figure 3).
- The NEO-PTC-01 induction protocol has been optimized to use the healthy donor material.
- The expansion of memory T-cell responses is modeled using peptide multimers that have been reported in the literature to be immunogenic in patients carrying that mutation, referred to as previously identified neoantigens (Table 1).
- Patient material used in this study was collected under the NIEONCE protocol the Netherlands Cancer Institute subsidioed by Neon Therapeutics.

Table 1. Model Used to Optimize System in Healthy Donors

<table>
<thead>
<tr>
<th>DOM (control set)</th>
<th>PIN (test set)</th>
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<tbody>
<tr>
<td>- Pre-existing repertoire is present</td>
<td>- No pre-existing repertoire</td>
</tr>
<tr>
<td>- Memory responses</td>
<td>- Native responses</td>
</tr>
<tr>
<td>- Easy to expand</td>
<td>- Difficult to expand</td>
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Table 2. Results from the ex vivo Induction Protocol

<table>
<thead>
<tr>
<th>T-cell responses</th>
<th>Pre-induction</th>
<th>Post-induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T-cell responses towards MART-1</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>CD8+ T-cell responses towards NY-ESO1</td>
<td>30%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Figure 3. Experimental Setup

DCs + PBMCs + MART-1

Figure 4. Ex vivo Inductions and Functionality of CD8+ T-Cell Responses Toward DOMs in a Healthy Donor

A) Expansion of CD8+ T-cell responses toward DOMs

B) Functionality of CD8+ T-cell Responses to DOMs (Gated on Tetramer Positive Population)

Figure 5. Induced CMV pp65-Specific CD8+ T-Cell Responses Can Upregulate Active Caspase-3 on Antigen-Expressing Tumor Targets

Figure 6. Ex vivo Induction of Naïve CD8+ T-Cell Responses in a Healthy Donor

Figure 7. A) Detection of PIN-Specific CD4+ T-Cell Responses from the Naïve Compartment; B) Induced CD4+ T Cells are Specific for Mutant Neoantigens, but not Wildtype

Figure 8. Stimulation of Patient-Specific CD8+ and CD4+ Responses: Ovarian Patient Sample Example. A) Readout Based on Functionality; B) Readout Based on pMHC Multimers

Conclusions

- The NEO-PTC-01 personal neoantigen-specific T-cell therapy is to induce multiple enriched T-cell populations at high-priority neoantigens unique to each patient, particularly in inductions with a low neoantigen load such as ovarian cancer.
- Technology is in place to substantially characterize the identity, functionality, phenotype and killing capacity of the induced cultures.
- This protocol can successfully:
  - Induce T-cell responses from the naive and memory compartments from healthy donors.
  - Induce T-cell responses to patient-specific neoantigens in an ovarian cancer patient.
  - Generate polyfunctional T-cell responses.
  - Generate T-cell responses that can discriminate mutant from wildtype peptide.
  - Generate T-cell responses capable of killing antigen-expressing tumor targets.

Acknowledgments

- The authors are grateful for the contribution of Yicong Huang, Yevnir Ware, Dominic Kinsella, the Kinship and Industry.
- The authors acknowledge the financial support for this study from Neon Therapeutics.
- The authors acknowledge editorial assistance from MediTech Media (Stephen McDougal) and printing support from MedTech Media, sponsored by Neon Therapeutics.

Disclosures

- JHVD: Research collaboration with BMS and Medimmune.
- B.S., N.S., RB, ABI: Nothing to declare.
- TWM: Scientific founder of Neon Therapeutics
- DASS: Advisory role for Neon Therapeutics.

References