In-Depth Characterization of Immune Responses Induced Against Patient-Specific Neoantigens using NEO-STIM™

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Background

- Emerging data shows the importance of CD4 T cell recognition of epitopes derived from mutant protein products, neoantigens, in clinically effective cancer immunotherapies.
- Neoantigens are tumor-specific antigens that are important in eliciting and directing effective anti-tumor immune responses. These tumor-specific neoantigens are not subject to central immune tolerance and are therefore potentially more immunogenic than shared, tumor-associated antigens.
- Accurate prediction and validation of therapeutically relevant neoantigens will be key in advancing immunotherapies to the clinic that target neoantigens.
- Our objective is to better understand the rules governing neoantigen immunogenicity and, with that, improve our ability to select high-quality neoantigen targets for the development of patient-specific immunotherapies.

In this study, we used NEO-STIM®. Neoantigen T cell induction protocol, to test immunogenicity of specific neoantigen targets. NEO-STIM is used across Neo's diverse neoantigen pipelines which include our personalized adoptive T cell therapy (NEO-PTC-0).”

Materials and Methods

- Patient-specific neoantigens were predicted using our bioinformatics engine, RECON®.
- Synthetic long peptides covering the predicted neoantigens were used as immunogens in NEO-STIM to assess the immunogenic capacity.
- The NEO-STIM protocol involves feeding these neoantigen-encoding peptides to patient-derived APCs, which are then co-cultured with patient-derived T cells to prime neoantigen-specific T cells (Figure 1).

Results

- NEO-STIM was successful in the expansion of pre-existing CD8 T cell responses, as well as the induction of de novo CD8 T cell responses (Table 1).
- Using PBMCs from melanoma patient NV01, expansion of a pre-existing CD8 T cell response was observed from 4.5% of CD8 T cells to 72.1% of CD8 T cells (SRSF1 E>K). Moreover, NEO-STIM was effective in inducing two presumed de novo CD8 T cell responses towards patient-specific neoantigens (ARAP1 Y>H, PKDREJ G>R) and CD8 T cells, respectively. (Figure 2A).
- NEO-STIM successfully induced seven de novo CD8 T cell responses, as well as two previously described and novel model neoantigens using PBMCs from another melanoma patient, NV6, up to varying magnitudes (ACTN4 S>L, CREBBP S>L, GLI3 P>L, QARS R>W, FAM178B P>L, RPS26 P>L). (Figure 2B).

![Figure 2: NEO-STIM Can Induce CD8 T Cell Responses Towards Patient-specific Neoantigens (A) and Patient-Specific and Model Neoantigens (B) in Patient Material](image)

- When re-challenged with different concentrations of neoantigen peptides, the induced CD8 T cells responded significantly to mutant neoantigen peptide but not to the wildtype peptide (Figure 4).
- In patient NV01, CD8 T cell responses were identified using a recall response assay with mutant neoantigen loaded DCs (Figures 5A & 5B).
- Three CD8 T cell responses were identified (MKRN1 CREBBP, and TPCH4, respectively) based on the reactivity to DCs loaded with mutant neoantigen peptide.
- These CD8 T cell responses also showed a polyfunctional profile when re-challenged with mutant neoantigen peptide: 31.3%, 34.5% and 41.9% of CD8 T cells exhibited one, two, or three functions; MKRN1 CREBBP, and TPCH4, respectively (Figure 5).

![Figure 5: Induced CD8 T Cell Responses Show Response to Mutant Neoantigen Peptide and Show a Polyfunctional Profile](image)

- Finally, the cytotoxic capacity of the induced CD8 T cell responses from patient NV01 was also assessed (Figure 6).
- Both SRSF1 E>K and ARAP1 Y>H responses showed a significant upregulation of CD107a on the CD8 T cells and active Caspase3 on the tumor cells transduced with the mutant construct after co-culture.

![Figure 6: Induced CD8 T Cell Responses Have a Cytotoxic Phenotype and Can Kill Antigen-Expressing Targets](image)

Conclusions

- Using NEO-STIM, patient-specific neoantigens predicted by RECON, as well as the model neoantigens, were confirmed to be immunogenic by the induced and multi-functional CD8 and CD4 T cell responses in patient material.
- Our ability to induce polyfunctional and mutant-specific CD8 and CD4 T cell responses proves our capability of predicting high-quality neoantigens and generating potent T cell responses.
- The presence of multiple enriched neoantigen-specific T cell populations (memory and de novo) at the end of the NEO-STIM process demonstrates our potential to raise new T cell responses and generate effective cancer immunotherapies to treat cancer patients.

Table 1: Gene Information of Induced T Cell Responses

<table>
<thead>
<tr>
<th>Patient</th>
<th>HUGO Symbol</th>
<th>Full Gene Name</th>
<th>Type</th>
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<tbody>
<tr>
<td>NV01</td>
<td>SRSF1</td>
<td>Serine and Arginine Rich Splicing Factor 1</td>
<td>CD8</td>
</tr>
<tr>
<td></td>
<td>ARAP1</td>
<td>Aromatic Richness Aromatic Protein 1</td>
<td>CD4</td>
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<tr>
<td></td>
<td>MKRN1</td>
<td>Myokinin</td>
<td>CD8</td>
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<td></td>
<td>CREBBP</td>
<td>Casein Kinase 1 Alpha 1</td>
<td>CD4</td>
</tr>
<tr>
<td></td>
<td>TPCH4</td>
<td>DEAH-Box Helicase 40 (TCEH)</td>
<td>CD8</td>
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<td>ASH1</td>
<td>Antioxidant-Sensitive Protein 1</td>
<td>CD8</td>
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<tr>
<td></td>
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A: pmHC multimer plots showing CD8 T cell responses induced with DCs loaded with mutant or wildtype neoantigen peptides at different concentrations (0 μM, 0.05 μM, 0.2 μM, 0.8 μM, and 3.2 μM) and measured IFN-γ and/or TNFα and/or CD107a (% of CD8+). Percentage in pie charts represent percentage functional CD8+ T cells: 1, 2, or 3 functions. Data generated from CD8 T cell responses post NEO-STIM, patient NV01.

B: pmHC multimer plots of SRSF1 E>K and ARAP1 Y>H pre and post NEO-STIM (left panel), pie charts depicting the functionality of neoantigen-specific T cells upon re-challenge with neoantigen-loaded DCs, gated on pmHC multimer-CD8 T cells, and percent of CD8 T cells with 1, 2, or 3 functions. Data generated from CD8 T cell responses post NEO-STIM, patient NV01.

Abbreviations: APC = antigen-presenting cell; CD107a = Cluster of Differentiation 107a; DC = dendritic cell; FDR = false discovery rate; HLA = human leukocyte antigen; Ig = immunoglobulin; IFN-γ = interferon-γ; IFN-γ/R = interferon-γ receptor; IL = interleukin; MHC = major histocompatibility complex; PBMC = peripheral blood mononuclear cell; T cell = T lymphocyte; TNFα = tumor necrosis factor alpha.


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