We have demonstrated the feasibility of using a single live T-cell capture and multiplexed proteomics profiling platform to determine a clinically relevant biomarker of early CPI response.

**Background**

- Immune checkpoint inhibitors (CPIs) are proven to be effective in treating multiple cancers types. Pembrolizumab and atezolizumab are now approved for 1 line metastatic NSCLC.
- Current predictive biomarkers of CPI response in NSCLC include Programmed-Death Ligand-1 (PD-L1) expression, Tumor mutational burden-High (TMB-H) and Microsatellite Instability-High (MSI-H). However, they appear to be independent of one another, and are all less than adequate [1].
- Moreover, emerging clinical evidence suggested that oncogene-addicted NSCLCs do not respond well to CPIs, regardless of levels of PD-L1 expression status [2].
- This pilot study was performed to evaluate the feasibility and utility of a single live T-cell capture and multiplexed proteomics profiling platform to determine a clinically relevant biomarker of early CPI response.

**Results - Polyfunctionality**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>DxDx/Genotype</th>
<th>Stage</th>
<th>tx Response (s/p 2 Cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0002</td>
<td>M</td>
<td>Adenocarcinoma</td>
<td>IC</td>
<td>Stable Disease</td>
</tr>
<tr>
<td>0008</td>
<td>F</td>
<td>Adenocarcinoma</td>
<td>IC</td>
<td>Stable Disease</td>
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<tr>
<td>0010</td>
<td>M</td>
<td>Squamous Cell</td>
<td>IC</td>
<td>Stable Disease</td>
</tr>
</tbody>
</table>

**Results - Polylfunctional Strength Index (PSI)**

**c-MET Amplified Advanced NSCLC**

- We have demonstrated the feasibility of using a single-cell microfluidics-based platform to longitudinally profile in multiplexed fashion cytokines released by live single T-cells from NSCLC patients.
- Preliminary data nominates PSI as potential predictive biomarker of CPI response, linking single-cell cytokine profiling to clinical outcome, esp. in a MET-amplified lung adenocarcinoma.
- Future studies with larger cohorts are warranted to further validate using PSI as an immune measure of CPI response.

**Conclusions**

**Acknowledgments and References**

**Figure 1.** Single Live T-Cell Capture and Cytokine Analysis Workflow (IsoPlexis) [3]. Each panel profiles for more than 32 cytokines per cell.

**Figure 2.** Overall Polyfunctionality Increases Post-Treatment. Post-treatment CD4+ and CD8+ T-cells demonstrate overall greater percentage of polyfunctional cells (secreting 2+ cytokines) with the exception of patient 0021. The increase in polyfunctionality is heterogeneous among patients and cell types, with the strongest increase in polyfunctionality seen in patient 0008.

**Figure 3.** Overall Increase in Polylfunctional Strength Index (PSI) Post-Treatment. PSI, which is measured as the percentage of polyfunctional cells in the sample multiplied by the intensities of the secreted cytokines, is increased in post-treatment samples compared to pre-treatment samples. CD8+ cells have a more drastic increase in PSI compared to CD4+ cells across all patients. In the CD4+ subset, effector/anti-tumor and regulatory cytokines drive PSI upregulation. In the CD8+ subset, effector/anti-tumor cytokines and, to a lesser extent, regulatory and chemokine/active cytokines drive PSI upregulation.

**Figure 4.** Pretreatment Baseline PET Images of Patient 0008 with Highest Upregulation in Polyfunctionality. In this patient harboring c-MET amplified advanced lung adenocarcinoma with high PD-L1 expression demonstrated excellent near-complete response to the first-line single agent anti-PD-1 CPI pembrolizumab (in combination with local radiation to right adrenal metastasis).

**Figure 5.** CT Images of Patient 0008 with Highest Upregulation in Polyfunctionality. These images of pre-treatment baseline and post 2 cycles of pembrolizumab demonstrate patient response to first-line CPI therapy in the right hilar adenopathy (top) and in the right adrenal metastasis (also locally radiated; bottom).

**Table 1.** Patient Demographics and Clinical Response Data. Patient 0002 was treated with durvalumab, whereas patients 0006, 0010, and 0021 were treated with regimens containing pembrolizumab. PD-L1 expression level: % tumor proportion score (TPS).

**Table 2.** Pretreatment Baseline PET Images of Patient 0008 with Highest Upregulation in Polyfunctionality. In this patient harboring c-MET amplified advanced lung adenocarcinoma with high PD-L1 expression demonstrated excellent near-complete response to the first-line single agent anti-PD-1 CPI pembrolizumab (in combination with local radiation to right adrenal metastasis).

**Table 3.** CT Images of Patient 0008 with Highest Upregulation in Polyfunctionality. These images of pre-treatment baseline and post 2 cycles of pembrolizumab demonstrate patient response to first-line CPI therapy in the right hilar adenopathy (top) and in the right adrenal metastasis (also locally radiated; bottom).