**Background**

PD-L1 immunohistochemistry (PD-L1 IHC), mutational burden (MuB) and tumor infiltrating lymphocytes (TILs) are the current standard or "primary" immune biomarkers used for selection of immunotherapy today. These markers however, are suboptimal for response prediction to currently approved checkpoint inhibitors (CPIs) across a broad range of tumor types. Machine learning (ML) decision models have great potential for predicting response, however, they risk overfitting data, only performing well in the initial training cohort and lacking generalizability when extended to other cohorts. Alternatively, biology-based decision models can initially underperform due to limited data and a simplified rule set, but often perform equally well when extended to larger similar patient cohorts. We took a combinatorial approach to algorithmic prediction to address these shortcomings.

**Methods**

We evaluated response prediction to currently approved checkpoint inhibitors using both standard primary immune biomarkers and algorithmic approaches. We developed a reference population of 167 patients from Roswell Park Cancer Institute with complete treatment and clinical outcome data for 87. For algorithmic prediction, a ML polynomial regression model based on 54 immune-related genes combined with mutational burden was optimized for prediction of response. A biological 4-gen gene decision tree model was constructed independently based on ML. A second biological decision tree incorporated the weighted average relative rank of the expression of multiple genes in 4 different immune functions including immune cell infiltration, regulation, activation, and cytokine signaling. Bayesian model average (BMA) incorporated all three models’ results into a final prediction.

**Primary Immune Biomarkers**

![Diagram](image)

**Inflamed Tumors**

<table>
<thead>
<tr>
<th>MuB High</th>
<th>MuB Not High</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>78</td>
</tr>
</tbody>
</table>

**Non-Inflamed Tumors**

<table>
<thead>
<tr>
<th>PD-L1 High</th>
<th>PD-L1 Not High</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>60</td>
</tr>
</tbody>
</table>

Inflamed tumors with MuB and PD-L1 in a reference population of 167 tumors

- Tumors with high MuB are as likely to be non-inflamed as inflamed
- Tumors with high PD-L1 are often non-inflamed

**Secondary Immune Biomarkers for Nonresponders**

![Diagram](image)

**Figure 3:** Primary Immune Biomarkers. PD-L1 IHC (Top left), CD8 IHC (Top right) are commonly used biological measurements to predict response to immune checkpoint blockade in a variety of malignancies. An immune response gene expression (GEX) panel was used to measure normalized reads per million (nRPM) for 54 validated genes. nRPM values were then ranked from 0 to 100 based on a reference population of 167 patients to derive GEX interpretation of High and Low. These interpretations were then used to visualize the immune GEX landscape of inflamed vs non-inflamed tumors. DNA Seq was used to estimate mutational burden (MuB), defined as number of nonsynonymous somatic mutations per million exonic bases.

**Testing for Primary and Secondary Immune Biomarkers**

Conclusions:

Prediction of response to checkpoint inhibitors can be improved by combining primary and secondary immune related biomarkers. Our initial study of 87 patients is a framework for expanding to a larger multi-institutional collaboration to prove that combining primary and secondary immune related biomarkers for prediction of response to checkpoint inhibitors has value. The same approach can be used for assigning patients to combination immunotherapies.