Tumor mutational burden (TMB) ring study: Comparison of multiple targeted next-generation sequencing (NGS) platforms

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Introduction

Tumor mutational burden (TMB), a measurement of the frequency of mutations in tumor cells, is currently being evaluated as a biomarker to predict response to immune checkpoint inhibitors (Figure 1). Whole exome sequencing is considered the gold standard assay, but is inefficient and too costly to run routinely. Consequently, several targeted NGS assays have been designed to measure TMB. In this study, we compared TMB measurements from four targeted NGS assays using a common source of specimens. Concordance and accuracy of TMB values, cutoffs, and clinical interpretations were assessed.

Methods

TMB testing was completed or first attempted by Foundation Medicine (FoundationOne®), followed by on-site analysis by OmniSeq (Immune Report Card®), Illumina (TruSight Oncology 500™), and Theragene (Oncomine™ Tumor Mutation Load) from a subsequent central exome sequencing. Genomic DNA from 161 FFPE specimens representing 24 tumor types was extracted following a standard protocol in a centralized facility (Figure 2). Each laboratory followed its own protocol for reporting TMB values (Figure 3). Pairwise Pearson product-moment correlations (R) were performed to estimate concordance of TMB values between platforms (Figure 4). 150 gold standards were established (7 TMB-high, 143 TMB-low) for which at least three of four platforms were concordant when using a TMB-high cutoff of ≥10. Each platform was assessed for TMB interpretation accuracy at this threshold (Figure 5).

Assay Robustness

Table 1: Performance of TMB platforms across a common set of cases.

<table>
<thead>
<tr>
<th>Platform</th>
<th>Samples Attempted</th>
<th>TMB Resulted</th>
<th>Sample Fail</th>
<th>% Resulted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina (TruSight Oncology 500™)</td>
<td>161</td>
<td>153</td>
<td>8</td>
<td>95%</td>
</tr>
<tr>
<td>OmniSeq (Immune Report Card®)</td>
<td>213</td>
<td>202</td>
<td>11</td>
<td>95%</td>
</tr>
<tr>
<td>Theragene (Oncomine™ Tumor Mutation Load)</td>
<td>161</td>
<td>154</td>
<td>7</td>
<td>96%</td>
</tr>
<tr>
<td>Foundation Medicine (FMI)</td>
<td>NA</td>
<td>177</td>
<td>18</td>
<td>NA</td>
</tr>
</tbody>
</table>

Conclusions

- TMB performance is robust across platforms using a wide range of solid tumor specimens.
- There is general concordance between the platforms, but low number of TMB high samples limit statistical analysis.
- Pair-wise linear regression model fits did not significantly improve concordance between platforms (p>0.05).
- Each platform is highly accurate when using a TMB-high cutoff of ≥10, which improves when restricted to NSCLC.
- Majority of FP calls are boundary related to the TMB-high cutoff of ≥10.
- Further studies utilizing additional NGS platforms and gold standard samples are required.