Introduction

PD-L1 expression and MSI status are useful predictive biomarkers for checkpoint inhibition (ICIs) in some tumor types. However, studies have demonstrated that the identification of all potential patients who may benefit from ICIs will require the analysis of both tumor cell and tumor microenvironment biomarkers. For example, recent data suggests that tumor mutation burden (TMB) and tumor mutational signatures as well as inflammation signatures and T cell clonality in the tumor microenvironment may serve as independent predictive biomarkers of CI responses. The need for evaluation of multiple biomarkers may be a concern for the clinical lab, as the cost of doing so can present a challenge in the clinical setting. Here we conduct a study to evaluate whether a panel of tumor biomarkers can be accurately detected using FFPE DNA and RNA isolated from plasma using Oncomine TagSeq TMB and other Oncomine assays. Multiple methods for assessing TMB and mutation signatures were compared. The impact of sample quality on biomarker analysis was also evaluated.

Materials and Methods

Samples and DNA/RNA extraction

A set of 10 CRC (Adenocarcinoma, Grade 2) and their matched plasma samples were used in this study. DNA and RNA were co-extracted from FFPE sections using RecoverAll™ kit. cDNA was extracted from plasma using the MaxiScript™ kit.

DNA QC

KAPA INGENIA Quantification and QC kit was used to evaluate FFPE DNA quality. A higher ratio of Q20 to Q1 was helpful in identifying samples with an increased tumor DNA fraction. To calculate overall tumor DNA content, DNA extracted from each sample was assessed using KAPA INGENIA Quantification and QC kit with the ratio of Q20 to Q1 of ≥ 5.

RNA QC

KAPA INGENIA Quantification and QC kit was also utilized to evaluate the RNA quality. The RNA integrity number (RIN) was utilized to assess the sample quality. A high RIN value indicates better sample quality, whereas a low RIN value indicates poor-quality RNA.

Conclusions

The Oncomine™ TML Assay generated TMB results that correlated with WES. There was a closer correlation between TMB results generated by Oncomine Assay and WES. The Oncomine™ TML Assay can provide comprehensive mutation spectrum analysis using FFPE DNA, even for FFPE samples with very low input amount.

References


OCAv3 pool2 failed. Total reads <100K.

Table 1. Comparison of TMB results from different assays

<table>
<thead>
<tr>
<th>Sample</th>
<th>TMB (mutations/Mb)</th>
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<th>TMB (mutations/Mb)</th>
<th>TMB (mutations/Mb)</th>
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<tbody>
<tr>
<td>Sample 1</td>
<td>0.123</td>
<td>0.124</td>
<td>0.125</td>
<td>0.126</td>
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<tr>
<td>Sample 2</td>
<td>0.127</td>
<td>0.128</td>
<td>0.129</td>
<td>0.130</td>
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</table>

Table 2. Concordance of mutations in matched cfTNA and FFPE tumor tissue

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mutation Type</th>
<th>Mutation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>KRAS G13D</td>
<td>Concordant</td>
</tr>
<tr>
<td>Sample 2</td>
<td>TP53 R282W</td>
<td>Discordant</td>
</tr>
</tbody>
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Table 3. Concordance of mutations in matched cfTNA and FFPE tumor tissue

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Figure 1. TMB measured by TML and WES in 10 high quality FFPE CRC samples

Figure 2. TMB concordance with MSI status (22 variable quality FFPE samples)

Figure 3. Concordant TP53 R231G mutation detected in plasma EXO-06336-K.

Figure 4. Discordant MET exon 14 splicing mutation detected in plasma EXO-0390163

Conclusions

• Both the Oncomine™ TML Assay and OCAv3 provide comprehensive mutation spectrum analysis using FFPE DNA, even for FFPE samples with very low input amount.

• The Oncomine Pan-Cancer Cell-Free Assay successfully detected MSI and MSI-H samples with 10% MSI-H high and smaller than 10% in some samples but the panel is not large enough to calculate TMB scores.

References


