Development of highly automated Next Generation Sequencing IVD tests for Solid Tumours

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Introduction

With the increased use of NGS (Next Generation Sequencing) in clinical settings, Vela Diagnostics has developed four automated NGS based In Vitro diagnostics oncology disease panels (Sentosa® SQ Melanoma Panel, Sentosa® SQ CRC Panel, Sentosa® SQ NSCLC Panel and Sentosa® SQ Thyroid Cancer Panel to aid medical decision-making.

The panels are based on target amplification of disease related genes harbouring actionable/ prognostic related somatic hotspot mutations.

Design: Target gene list of the panels

<table>
<thead>
<tr>
<th>Targets for Sentosa® SQ Melanoma Panel</th>
<th>Targets for Sentosa® SQ CRC Panel</th>
<th>Targets for Sentosa® SQ NSCLC Panel</th>
<th>Targets for Sentosa® SQ Thyroid Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF/MKR</td>
<td>MKR</td>
<td>MKR</td>
<td>MKR</td>
</tr>
<tr>
<td>AKT/CTNNB1</td>
<td>CTNNB1</td>
<td>CTNNB1</td>
<td>CTNNB1</td>
</tr>
<tr>
<td>CDKN2A/PIK3CA</td>
<td>PIK3CA</td>
<td>PIK3CA</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>MAP2K1/FGFR3</td>
<td>FGFR3</td>
<td>FGFR3</td>
<td>FGFR3</td>
</tr>
<tr>
<td>FGFR3/RET</td>
<td>RET</td>
<td>RET</td>
<td>RET</td>
</tr>
<tr>
<td>GNA11/PTEN</td>
<td>PTEN</td>
<td>PTEN</td>
<td>PTEN</td>
</tr>
<tr>
<td>GNA11</td>
<td>RET</td>
<td>RET</td>
<td>RET</td>
</tr>
<tr>
<td>Total Number of Targeted Mutations</td>
<td>127</td>
<td>113</td>
<td>105</td>
</tr>
</tbody>
</table>

Methods and Workflow

**Sample ID**

- Extraction and Library Prep
- Template Prep
- Sequencing
- Data Analysis
- Result Upload to LIS

**Conclusion**

- **Sentosa® SQ Melanoma Panel**: clinical sensitivity and specificity has been determined for the most frequent and clinically important target mutations in Melanoma samples – BRAF V600E.
- The reference method is Sanger Sequencing.
- 4 runs were tested using FFPE material carrying 39 mutations (BRAF V600E, 22 with vs 17 without BRAF V600E).
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- All targets were successfully recovered.

**Sentosa® SQ CRC Panel**: clinical sensitivity and specificity has been determined for the most frequent and clinically important target mutations in CRC samples – KRAS G12A/C/D/R/S/V/G13D.

- The reference method is KRAS PCR Test.
- 3 lots of Sentosa® SQ CRC Panel Kit used.
- 62 samples tested: 16 positive, 46 negative.
- Clinical sensitivity: 100% (95%CI: 96.34-100%)
- Clinical specificity: 100% (95%CI: 96.34-100%)
- Negative 0 80
- Positive 74 0

**Sentosa® SQ NSCLC Panel**: clinical sensitivity and specificity has been determined for the most frequent and clinically important target mutations in NSCLC samples – EGFR and ALK.

- The reference method is Sanger Sequencing.
- 3 lots of Sentosa® SQ NSCLC Panel Kit used.
- 62 samples tested: 16 positive, 46 negative.
- Clinical sensitivity: 100% (95%CI: 96.34-100%)
- Clinical specificity: 100% (95%CI: 96.34-100%)
- Negative 0 80
- Positive 73 0

**Sentosa® SQ Thyroid Cancer Panel**: clinical sensitivity and specificity has been determined for the most frequent and clinically important target mutations in Thyroid Cancer samples – BRAF V600E.

- The reference method is Sanger Sequencing.
- 3 lots of Sentosa® Thyroid Cancer Panel Kit used.
- 62 samples tested: 16 positive, 46 negative.
- Clinical sensitivity: 100% (95%CI: 96.34-100%)
- Clinical specificity: 100% (95%CI: 96.34-100%)
- Negative 0 80
- Positive 72 0

**Conclusion**

- All the panels (viz. Sentosa® SQ Melanoma Panel, Sentosa® SQ CRC Panel, Sentosa® SQ NSCLC Panel and Sentosa® SQ Thyroid Cancer Panel) have been verified and validated with ≥95% analytical and clinical sensitivity-specificity.
- In conclusion: robust, minimal hands-on, workflow-automated oncology NGS IVD panels holds great promise to contribute significantly to personalized cancer diagnosis and treatment.

Table: Comparison of Clinical Validation

<table>
<thead>
<tr>
<th>Panel Type</th>
<th>Mutation</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive Calls</th>
<th>Negative Calls</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E</td>
<td></td>
<td>100 (95%CI: 96.34-100%)</td>
<td>100 (95%CI: 96.34-100%)</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>KRAS G12A/C/D/R/S/V/G13D</td>
<td></td>
<td>100 (95%CI: 96.34-100%)</td>
<td>100 (95%CI: 96.34-100%)</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
<td>100 (95%CI: 96.34-100%)</td>
<td>100 (95%CI: 96.34-100%)</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
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</tr>
</tbody>
</table>

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