Rigorous Validation of Clinical Circulating Tumor DNA For Cancer Molecular Profiling

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Background

- Profiling cell-free circulating tumor DNA (cfDNA) for the genomic alterations which drive oncogenesis promises to provide information important for understanding cancer biology, informing therapy selection when conventional biopsies are unavailable and monitoring of therapy response to therapy.
- To assess the accuracy of profiling cancer genomics in cfDNA, we characterized the performance of a new targeted NGS cfDNA assay in detecting substitutions, indels, gene fusions, and copy number amplifications in cell-line mixtures containing alterations down to ~0.5% allele frequency across 62 high relevance genes.
- To investigate clinical utility, we compared the results of comprehensive genomic profiling with clinical outcomes across matched pairs of FFPE (FoundationOne™) and cell-free DNA extracted from blood (FoundationOneCT™) samples from 32 patients with lung cancer at different disease stages.

Materials and Methods

- High conversion efficiency was obtained through optimized cfDNA isolation and NGS library construction to maintain sample complexity for >50x coverage.
- Mixtures of model and synthetic samples were used to assess accuracy for all classes of genomic alterations: 1500X base substitutions at 0.1%-78%, 1500X indels 1.39 bp at 0-51%, 48 rearrangements 1-50X and 35 copy number amplifications in T/B reference cell lines.
- Base substitutions, indels, and Chks were orthogonal validated using ddPCR for VAF < 5% and FoundationOne for VAF > 5%. Breakpoint PCR was used to validate gene fusions.
- Paired FFPE samples underwent analysis by the FoundationOne assay (Hampton et al. 2013).

6 Cancer Cell-line mixtures were diluted into normal sample to generate events with allele frequency <1%.

Analytic validation

For samples which meet or exceed 5,000X unique median exon coverage the FoundationOneCT assay achieves high sensitivity and PPV for detection of base substitutions, insertions/deletions, genomic rearrangements, and copy number amplifications.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>VAF</th>
<th>Sensitivity</th>
<th>Positive Predictive Value (PPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Substitutions</td>
<td>0.5%</td>
<td>98.0% (99.6-100%)</td>
<td>99.0% (98.6-100%)</td>
</tr>
<tr>
<td>Insertions/Deletions [1-40 bp]</td>
<td>0.1%</td>
<td>67.7% (60.7-74.5%)</td>
<td>93.6% (89.2-96.2%)</td>
</tr>
<tr>
<td>Rearrangements</td>
<td>10%</td>
<td>99.8% (99.5-100%)</td>
<td>98.8% (95.3-99.8%)</td>
</tr>
<tr>
<td>Copy Number Amplifications</td>
<td>1%</td>
<td>96.6% (71.1-100%)</td>
<td>&lt;0.05% (97.0-100%)</td>
</tr>
</tbody>
</table>

Reproducibility (average concordance between replicates): 50.0% intra-batch precision, 100% inter-batch precision.

Conclusions

- FoundationOneCT is rigorously validated and run in a CAP-accredited GCL lab to provide sensitive and specific results for substitutions, indels, rearrangements and copy number amplifications to patients with cancer:
  - While both specificity and PPV were assessed, PPV is the most clinically meaningful measure of specificity for an NGS assay as per base specificity will always be ~99.99% due to the large number of bases interrogated.
  - Cell-free DNA samples are less amenable to DNA detection due to typically lower tumor fraction when compared to tissue biopsies.
  - In late-stage lung cancer patients FoundationOneCT finds high concordance (94%) to tissue biopsy results with increased concordance for alterations known to be associated with cancer in the COSMIC database. The increased concordance of known alterations is likely a result of their being clonal in nature.
  - Standard-of-care biopsies remain the gold standard for CDD, but FoundationOneCT provides a viable option for patients whose tissue biopsy is not an option.