Reinventing Liquid Biopsy Research for Rare Variant Detection using NGS

China Ion World Tour 2016

Proprietary & Confidential

The world leader in serving science
Tissue Biopsy versus Liquid Biopsy

**Tissue Biopsy**

- Challenges
  - Tissue biopsy is invasive
  - Tissue biopsy is limited
  - Solid tumor may not be accessible
  - Tissue Biopsy doesn’t capture tumor heterogeneity

**Liquid Biopsy**

- Advantages
  - Non invasive – samples can be taken at multiple time points
  - Less expensive and faster turnaround time
  - Good indicator of tumor heterogeneity
  - Potential to monitor both treatment and recurrence

For Research Use only. Not for use in diagnostic procedures.
Oncomine cfDNA Assays—3-day Clinical Research Workflow

- Validated limit of detection down to 0.1% (with 20ng input) for SNV hotspots and indels
- Validated using a single tube of blood down to 0.1% LOD
- Demonstrated cfDNA input amounts down to 1ng
- cfDNA variant caller for optimized analysis
  - Variant caller displays metrics for all libraries sequenced on a single run along with concentration of detected variants at hotspots in targeted regions

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Oncomine™ Lung cfDNA Assay Content

**Oncomine Lung cfDNA Assay Gene List:**

- ALK
- BRAF
- EGFR
- ERBB2
- KRAS
- MAP2K1
- MET
- NRAS
- PIK3CA
- ROS1
- TP53

Assays designed to identify:
- Primary tumor drivers
- Resistance mutations

Tag Sequencing Technology enables detection of rare variants present down to 0.1%
- Sensitivity: 90% or greater
- Specificity: 98%

35 amplicon panel for lung

168 hotspot mutations in 11 genes

SNVs & indels only

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New Proprietary Sequencing/Amplification Technology

New Proprietary Technology enables:
• Low limits of detection (LOD) down to 0.1% with a very high confidence Level.
  • 90% Sensitivity at 0.1%
  • 100% Sensitivity at 0.5%

• Improved Error Correction
  • Analysis tools are able to identify and remove randomly incorporated errors such as those from PCR or handling of material
  • 98% Specificity at 0.1%

New Proprietary Technology is not AmpliSeq!
Coverage, Limit of Detection, and cfDNA Input Requirements

- 1 ng cfDNA: 0.6% LOD
- 5 ng cfDNA: 0.25% LOD
- 10 ng input: 0.15% LOD
- 20 ng input: 0.1% LOD
- 30 ng input: 0.05% LOD

Minimum coverage vs. ng of input cfDNA
## Variants Called from Horizon cfDNA Multiplex Reference Set

<table>
<thead>
<tr>
<th>Sample</th>
<th>EGFR E746_A750 delELREA</th>
<th>EGFR L858R</th>
<th>EGFR T790M</th>
<th>EGFR V769_D770 insASV</th>
<th>KRAS G12D</th>
<th>NRAS A59T</th>
<th>NRAS Q61K</th>
<th>PIK3CA E545K</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% HDX</td>
<td>0.06</td>
<td>0.17</td>
<td>0.06</td>
<td>0.10</td>
<td>0.22</td>
<td>0.17</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>1% HDX</td>
<td>0.72</td>
<td>1.07</td>
<td>0.75</td>
<td>0.74</td>
<td>1.14</td>
<td>1.15</td>
<td>1.15</td>
<td>2.29</td>
</tr>
<tr>
<td>5% HDX</td>
<td>4.52</td>
<td>4.86</td>
<td>6.32</td>
<td>3.97</td>
<td>6.34</td>
<td>6.11</td>
<td>6.94</td>
<td>5.29</td>
</tr>
<tr>
<td>100% WT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All 8 somatic variants at 0.1% were detected.
No false positives detected.
# Mutation Correlation between FFPE and cfDNA

Late-stage lung cancer samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Variant</th>
<th>FFPE</th>
<th>cfDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EGFR-L858R</td>
<td>71.42%</td>
<td>2.62%</td>
</tr>
<tr>
<td>2</td>
<td>TP53-R158L</td>
<td>51.89%</td>
<td>4.32%</td>
</tr>
<tr>
<td>3</td>
<td>MET-T1010I, KRAS-G12C</td>
<td>43.87%, 34.62%</td>
<td>51.75%, 0.28%</td>
</tr>
<tr>
<td>4</td>
<td>N/A</td>
<td>No detection</td>
<td>No detection</td>
</tr>
<tr>
<td>5</td>
<td>EGFR-L858R, MET-T1010I, TP53-Y220C</td>
<td>58.44%, 41.93%, 35.54%</td>
<td>7.28%, 48.72%, 1.93%</td>
</tr>
<tr>
<td>6</td>
<td>TP53-R158L</td>
<td>10.19%</td>
<td>1.26%</td>
</tr>
</tbody>
</table>

**Bold**—somatic mutations  
Normal font face—germline mutations

As expected, see a higher allelic fraction from FFPE compared to cfDNA

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How the Oncomine™ Lung cfDNA Assay is used today

- 30-50% of the time, no solid biopsy can be taken in NSCLC and/or
- They will take a cfDNA sample in addition to a solid tumor sample because they are looking for EGFR mutations when deciding treatment

- Pathology lab in Barcelona, Spain that monitors the treatment with dPCR. They collect cfDNA monthly and if they see a change in the dPCR results, they immediately run the Oncomine Lung cfDNA Assay to monitor the evolution of the cancer.
NGS cfDNA Assays*—Reinventing How Researchers Targets Cancer

Data for NGS cfDNA Assays*

Application—Ion 530 Chip and Ion 540 Chip
Demonstrated on Proton and Ion PGM Systems

Tube of blood
20 ng input—0.1% LOD
Flexible down to 1 ng

Genes covered for Lung Assay:
ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, TP53

Lung Assay Critical Hotspots:
EGFR: T790M, L858R, Exon 19 del
KRAS: G12X, G13X, Q61X,
BRAF: V600E
ALK Exon 21-25,
PIK3CA: E545K, H1047R, E542K,
and more

*The content provided herein may relate to products that have not been officially released and is subject to change without notice.

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Oncomine™ Colon cfDNA Assay Content

**Oncomine™ Colon cfDNA Assay Gene List:**

- AKT1
- BRAF
- CTNNB1
- EGFR
- ERBB2
- FBXW7
- GNAS
- KRAS
- MAP2K1
- NRAS
- PIK3CAS
- SMAD4
- TP53
- APC

Assays designed to identify:
- Primary tumor drivers
- Resistance mutations

Tag Sequencing Technology enables detection of rare variants present down to 0.1%
- Sensitivity: 90% or greater
- Specificity: 98%

48 amplicon panel for Colon

245 hotspot mutations in 14 genes (SNVs & indels only) and coverage of APC exon 16

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Oncomine™ Breast cfDNA Assay Content

Assays designed to identify:
• Primary tumor drivers
• Resistance mutations

Tag Sequencing Technology enables detection of rare variants present down to 0.1% 
• Sensitivity: 90% or greater
• Specificity: 98%

26 amplicon panel for Breast

Covering 157 hotspot mutations in 10 genes – SNVs & indels only

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