Comprehensive Detection of Genetic Alterations in Circulation of Cancer Patients

Theresa Zhang, PhD
Vice President
Translational Research and Diagnostics
Empowering the fight against cancer

**BIOMARKER DISCOVERY**
Custom NGS Research & Development

- Identify biomarker targets for drug discovery pipeline

Larger, multi-analyte panels in tissue and plasma including whole exome sequencing and neoantigen prediction (RUO)

**HYPOTHESIS TESTING**
Clinical Biomarker & Clinical Trial Services

- Prospectively stratify patients for clinical trials

Robust prototype cost and risk profile at early stage (CLIA/IUO)

**GLOBAL PATIENT ACCESS**
IVD Product Development & Commercialization

- Inform patient treatment selection

Decentralized oncology testing system, sequencer agnostic using pre-existing installed based (PROGENEUS)

End to end solution & decentralized NGS diagnostics
...to reach CROs and molecular labs GLOBALLY

End-to-End Components

Co-developed assays
- Platform will run multiple assays
- Tissue or plasma
- Hybrid capture or amplicon PCR based

Web-based portal
Training and other support tools accessible from a PGDx web-based portal tailored to client laboratory set-up

Reagent kits
All the key reagents and protocols required to prepare genomic libraries for sequencing

On-site training
Hands-on training conducted on-site or at PGDx laboratory to build proficiency with methods

On-site server
PGDx supplied server houses bioinformatics pipeline to ensure ownership and security of valuable sample data

Validation support
Samples provided for well characterized mutations in both FFPE and ctDNA formats

Flexible data formats
Sequencing data and analysis integration for co-developed reporting format

Technical Support
Around the clock expert support for adopting CROs and molecular labs
Circulating cell-free tumor DNA (ctDNA)

- 180-200bp DNA fragments
- Half Life: 2 hours – Real Time
- Specific to Tumor
- Multi-clones & Multi-lesions

**ctDNA Challenge**
Very low amounts of ctDNA in the sea of wild-type DNA

Diaz et al., J Clin Oncol. 2014 Feb

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Sample Preparation

Cell-Free DNA Derived from Plasma

- End Repair
- A-tail

Adaptor Ligation (Molecular Barcoding)

Sequencing

Target Enrichment (Optimized Probe Design)

Analysis

Error Suppression

Single Base Substitutions

Genomic Rearrangements

Sequence Mutations

Insertions & Deletions

Amplifications
### Genes evaluated in PlasmaSELECT™ 64

*Full coding and specific exon analysis in 58 well characterized cancer genes, as well as amplification analysis for 19 genes*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
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</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>CDK4†</td>
<td>FLT3</td>
<td>MYCN†</td>
<td>PTCH1</td>
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<tr>
<td>ALK†</td>
<td>CDK6†</td>
<td>GNAS</td>
<td>NPM1</td>
<td>PTEN</td>
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<tr>
<td>AR‡</td>
<td>CDKN2A</td>
<td>HRAS</td>
<td>NRAS</td>
<td>RB1</td>
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<tr>
<td>ATM</td>
<td>CTNNB1</td>
<td>IDH1</td>
<td>NTRK1</td>
<td>RET†</td>
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<tr>
<td>BRAF*</td>
<td>DNMT3A</td>
<td>IDH2</td>
<td>NTRK2</td>
<td>RNF43</td>
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<tr>
<td>BRCA1</td>
<td>EGFR†</td>
<td>JAK2</td>
<td>NTRK3</td>
<td>ROS1†</td>
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</tr>
<tr>
<td>BRCA2</td>
<td>ERBB2‡</td>
<td>KIT‡</td>
<td>PALB2</td>
<td>TERT</td>
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</tr>
<tr>
<td>BRCA2*</td>
<td>ESR1</td>
<td>KRAS*</td>
<td>PIK3CA†</td>
<td>TP53*</td>
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</tr>
<tr>
<td>CCND1†</td>
<td>EZH2</td>
<td>MAP2K1</td>
<td>PIK3CB</td>
<td>TSC1*</td>
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</tr>
<tr>
<td>CCND2†</td>
<td>FGFR1†</td>
<td>MET†</td>
<td>PIK3R1</td>
<td>TSC2</td>
<td></td>
</tr>
<tr>
<td>CCND3†</td>
<td>FGFR2†</td>
<td>MTKR</td>
<td>POLD1</td>
<td>VHL</td>
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</tr>
<tr>
<td>CD274‡</td>
<td>FGFR3†</td>
<td>MYC†</td>
<td>POLE</td>
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<td></td>
</tr>
</tbody>
</table>

### Rearrangement analysis for selected regions of 17 well-characterized cancer genes.

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</thead>
<tbody>
<tr>
<td>ALK</td>
<td>ETV6</td>
<td>MYC</td>
<td>PDGFRB</td>
<td>ROS1</td>
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<td>FGFR1</td>
<td>NTRK1</td>
<td>RAF1</td>
<td></td>
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</tr>
<tr>
<td>EGFR</td>
<td>FGFR3</td>
<td>PDGFRB</td>
<td>RET</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Micro-Satellite Instability (MSI) in plasma coming soon

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A breast cancer tumor was diluted with matched wild-type DNA to 0.10%, 0.20%, 0.50%, 1.0%, 10%, 25%, 50% and 100% tumor-derived DNA (0.10%, 0.20%, and 0.50% were evaluated in triplicate). For each sequence mutation, the observed allele fraction for the mutations identified in each case are plotted against the expected mutation allele fraction for that case.
The chronic myeloid leukemia cell line 562 was titrated with wild-type DNA to 0.10%, 0.20%, 0.50%, 1.0%, 10%, 25%, 50% and 100% tumor-derived DNA (0.10%, 0.20%, and 0.50% were evaluated in triplicate). Similar results were obtained for H2228 containing the EML4-ALK translocation and HCC78 containing the SLC34A2-ROS1 translocation.
<table>
<thead>
<tr>
<th>Performance Specification</th>
<th>Mutant Allele Fraction</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence Mutations (SBS/Indel)</td>
<td>≥0.50%</td>
<td>99.4%</td>
<td>&gt;99.999%*</td>
</tr>
<tr>
<td>Rearrangements</td>
<td>≥0.50%</td>
<td>94.4%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Amplifications (≥4-fold)</td>
<td>≥20%</td>
<td>97.2%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Amplifications (≥4-fold)</td>
<td>&lt;20%</td>
<td>varies depending on level of amplification and tumor content</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

*Per-base specificity provided for sequence mutation analyses

Can we improve the performance of amplification detection?
Ultrasensitive identification of MET amplification in plasma

**Approach #1:**
Detect the novel genomic junctions created by amplifications and deletions

**Approach #2:**
Compute fold change of read count between MET and reference genomic regions

Confidential Property of PGDx. Do not copy or distribute without written permission.
Somatic rearrangements associated with MET amplification detected in cfDNA

Analyses of Cell-Free DNA from Patients with *MET* Amplification

Analyses of Cell-Free DNA from Patients without *MET* Amplification
Detection of MET amplification at varying levels of tumor contribution.
Evaluation of MET amplification in dilution series (fold change of read count)

R² = 0.9892

Fold Change in #Reads

Tumor DNA Fraction (%)
<table>
<thead>
<tr>
<th>Structural Alterations</th>
<th>Mutant Allele Fraction</th>
<th>Assay Sensitivity</th>
<th>Assay Specificity</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplifications</td>
<td>≥0.50%</td>
<td>&gt;95%</td>
<td>&gt;99%</td>
<td>&gt;15 copies</td>
</tr>
<tr>
<td>Translocations</td>
<td>≥0.50%</td>
<td>&gt;95%</td>
<td>&gt;99%</td>
<td></td>
</tr>
</tbody>
</table>
Challenges for validating tests to detect rare structural alterations in plasma

• Lack of positive controls
  – Whole genome sequencing required to identify breakpoints

• Lack of orthogonal test for structural alterations in plasma

• Undefined gold standard tests even for tumor
Thank You

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