Blood Testing for EML4-ALK Fusion Transcripts in Non-Small Cell Lung Cancer Patients by ddPCR: A CLIA Lab Experience

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Disclosure Information

AACR Annual Meeting 2016
Hestia Mellert, PhD

I have the following financial relationships to disclose:
Stockholder in: Biodesix, Inc.
Employee of: Biodesix, Inc

- and -

I will not discuss off label use and/or investigational use in my presentation.
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Outline

• Introduction to Biodesix

• GeneStrat
  – Overview of blood-based targeted mutation profiling test for cancer
  – Droplet Digital PCR Technology

• EML4-ALK
  – an RNA-based blood test
  – Clinical Practice

• Future Directions
Biodesix®

Blood-based molecular diagnostics for cancer

Discovery

Development

Clinical Validation

CLIA/CLEP certified

Commercialization

PROSE: Prospective Randomized Phase III Trial
• Product Development
  – Sensitive and rapid mutation testing from blood
  – Arrangement with Bio-Rad for early access to new and improved content
  – Multiple collaborations with diagnostic companies, pharmaceutical companies and major academic centers

• GeneStrat Assays
  – EML4-ALK
    • Variant 1 (E13:A20)
    • Variant 2 (E20:A20)
    • Variant 3 (E6:A20)
  – EGFR
    • *sensitizing mutations*: Exon 19 deletion E746-A750 and L858R
    • *resistance mutation*: T790M
  – KRAS
    • G12C, G12D, G12V
  – BRAF
    • V600E
GeneStrat Test Workflow

Day 1
- Whole Blood Collection Kit used by Requesting Physician

Day 2
- Secure LIMS Accessioning at Biodesix
- Plasma cfDNA Isolation
- Plasma Circulating RNA Isolation
- Reverse Transcription of Circulating RNA to cDNA

EGFR, KRAS and BRAF
- ddPCR and Data Analysis using QuantaSoft

Day 3
- Test Results Report sent to Requesting Physician

EML4-ALK
Droplet Digital PCR

A Powerful Solution for High-Resolution Cancer Research

1. Make Droplets
   - Droplet Generator

2. Cycle Droplets
   - Bulk PCR Thermal Cycler

3. Read Droplets
   - Droplet Reader

Booth # 1730

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Example of a 2D plot Showing Droplet Distribution
EML4-ALK

Background and Clinical Utility

• Rearrangement of the EML4 and ALK loci leads to expression of fusion transcripts

• Gene Expression

• Circulating RNA test for detection of transcripts for EML4-ALK and a control gene (Glucuronidase; GUSB)

• The prevalence of EML4-ALK variants 1, 2, and 3 represent ~78% of the total ALK mutations in Non-Small Cell Lung Cancer¹

• XALKORI® (crizotinib), ZYKADIA® (ceritinib) and ALECENSA™ (alectinib)

EML4-ALK

Summary of Development Studies

• Verification
  - Design, synthesis and testing of an *in-vitro* RNA positive control
  - Multiplexed detection of EML4-ALK and control gene by RT-ddPCR
  - RNA isolation method optimization
  - Reverse transcription method optimization
  - Pre-analytic processing of cDNA optimization

• Validation
  - Analytic Lower Limit of Detection
  - Precision: Intra-day, Inter-day and Inter-operator
  - Robustness: 21 consecutive days of testing
  - Normal healthy donor specificity evaluation
  - Clinical sensitivity and specificity with cancer donors
  - Final acceptance testing
**EML4-ALK**

*Representative results using analytic RNA*
EML4-ALK

*Precision testing with analytic RNA*

- **Intra-Day**
- **Inter-Day**
- **Inter-Operator**

Number of EML4-ALK Copies Detected

Concentration of Input RNA

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EML4-ALK

Robustness – 21 Consecutive Days of Testing

Number of Copies

Time in days

Control Gene

ALK

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EML4-ALK

Clinical Validation

• Description of Samples
  • 24 samples with positive and negative reference results established using FISH or PCR
  • All samples were assessed using Biodesix’ GeneStrat ALK test
  • Calculated sensitivity and concordance is based on the prevalence of the variants covered by the test\(^1\)

• Sample Workflow using CLIA-lab SOPs
  • Circulating RNA extracted from 1-3 mL frozen plasma
  • Sample amplification conducted in duplicate wells using Bio-Rad’s QX200 ddPCR system

EML4-ALK

Representative Clinical Validation Samples

Variation Not Detected

Variation Detected
## EML4-ALK

**Concordant Positive Results and Copy Number**

<table>
<thead>
<tr>
<th>Reference Result (FISH or PCR methods)</th>
<th>GeneStrat Mutant EML4-ALK copies per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>3920</td>
</tr>
<tr>
<td>Detected</td>
<td>3360</td>
</tr>
<tr>
<td>Detected</td>
<td>1072</td>
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<tr>
<td>Detected</td>
<td>956</td>
</tr>
<tr>
<td>Detected</td>
<td>762</td>
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<td>532</td>
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<td>20</td>
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<tr>
<td>Detected</td>
<td>17</td>
</tr>
<tr>
<td>Detected</td>
<td>8</td>
</tr>
</tbody>
</table>
# EML4-ALK

## Test Performance Summary

<table>
<thead>
<tr>
<th>Analytic Validation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Ship Stability</td>
<td>7 days</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>0.2%</td>
</tr>
<tr>
<td>Precision (Inter/Intra-run and Inter-Operator)</td>
<td>Passed</td>
</tr>
<tr>
<td>Robustness (21-days)</td>
<td>Passed</td>
</tr>
<tr>
<td>Normal Healthy Donor Specificity (n=10)</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Validation with Cancer Donors (n=24)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>~85%</td>
</tr>
<tr>
<td>Concordance</td>
<td>~92%</td>
</tr>
</tbody>
</table>

Clinical Sensitivity and Concordance based on EML4-ALK variants 1, 2, and 3 representing ~78% of the total ALK mutations in NSCLC
The CLIA Experience

*Commercial Test Metrics for the EML4-ALK Test*

- Samples were selected over a contiguous 3 month period
- 272 samples were included in this study
- 95% of test results were generated within 72 hours of sample receipt
- 2% of the EML4-ALK variant tests were detected
Future Directions

• Bio-Rad Digital Biology Center
  • Advancing the Clinical Utility of the QX200™ System
    • Launched CE-IVD marked system
    • Pursuing FDA 510(k) clearance

• GeneStrat Extensions
  • ROS1 and RET fusion assays
  • Additional DNA and RNA multiplexed assays
  • Clinical utility studies
    • Detection associated with stage of metastatic disease
    • Therapeutic monitoring and progression
    • Treatment decision making
Thank you to all of the patients who support our studies

Questions?

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For follow up visit us at Booth # 2438