Development and Clinical Validation of Liquid ddPCR Tests for Actionable Somatic Mutations in NSCLC

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Disclosure Information

Gary A. Pestano, PhD

I have the following financial relationships to disclose:
  Stockholder in Biodesix, Inc.
  Employee of Biodesix, Inc.
  Co-inventor on a patent application filed by Biodesix, Inc.

- and -

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

• Introduction to Biodesix

• The GeneStrat® Test
  – Targeted approach for rapid variant testing in patients with NSCLC
  – Practice studies demonstrating utility of GeneStrat in early treatment decisions

• Representative Blood-based Test Development Case Studies
  – EGFR T790M Detection¹
  – PD-L1 Expression²

• What’s on the horizon for Biodesix and our partners?

¹JMD May 2017, Mellert et al.; ²Poster presentation q10; Mellert, Jackson, Pestano
A fully-integrated multi-omic diagnostics company
## Why Blood?

**Tissue is the “Gold Standard” for Diagnosis of Cancer (and Variant Detection)**

<table>
<thead>
<tr>
<th></th>
<th>Blood (Plasma)</th>
<th>Tissue Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Turn-around Time</strong></td>
<td>Rapid (72 hours or less)</td>
<td>Ranges (median 12 days for newly diagnosed patients)</td>
</tr>
<tr>
<td><em>(draw to result)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Initial Diagnosis</strong></td>
<td>Minimally invasive; simple blood draw</td>
<td>Invasive; Up to 25% of NSCLC patients do not have tissue available at diagnosis</td>
</tr>
<tr>
<td><em>(sample availability)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serial Testing</strong></td>
<td>Enabled by blood draw</td>
<td>Difficult to justify re-biopsies</td>
</tr>
<tr>
<td><em>(monitoring)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quantitation</strong></td>
<td>ddPCR particularly suited for development of quantitative assays</td>
<td>The most prevalent tissue tests, IHC, are semi-quantitative at best</td>
</tr>
<tr>
<td><strong>Heterogeneity</strong></td>
<td>Detection of variants in circulation</td>
<td>Results are biased heavily by sampling; difficult to assess intra-tumor and inter-site heterogeneity</td>
</tr>
<tr>
<td><strong>Regulatory Approvals</strong></td>
<td>Only one plasma test is currently FDA approved (Roche COBAS EGFR v2)</td>
<td>Multiple tissue tests for molecular testing (IHC and FISH mostly) have FDA approval</td>
</tr>
<tr>
<td><strong>Clinical Practice</strong></td>
<td>Emerging</td>
<td>Established</td>
</tr>
</tbody>
</table>

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Limitations of Tissue Testing for NSCLC

- Nearly 80% of patients will not have mutation results available at their initial oncology consult\(^1\)

- Approximately 1 in 4 patients will begin treatment in advance of receiving their mutation results\(^2\)
  
  - Results may take too long
  - Tissue is often unavailable or insufficient for ordering biomarkers
  - Initial “actionable” biomarker order depletes tissue block; unable to test for PDL-1 status initially or NGS at progression if needed

\(^1\) Lim, C., et.al. 2015. Biomarker Testing and Time to Treatment Decision in Patients with Advanced Non-Small Cell Lung Cancer. Ann Oncol first published online April 28, 2015

Why ddPCR?

What this means for the Laboratory and for Precision Medicine

Detecting *EGFR* Drug Resistance Mutations in ctDNA

<table>
<thead>
<tr>
<th>Cost 5</th>
<th>qPCR</th>
<th>NGS</th>
<th>ddPCR™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnaround Time</td>
<td>qPCR</td>
<td>NGS</td>
<td>ddPCR™</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>qPCR</td>
<td>NGS</td>
<td>ddPCR™</td>
</tr>
</tbody>
</table>

(Bordi et al. 2015, Wiita and Schrijver 2011, Pender et al. 2015)

Decisive moments in precision medicine are revolutionizing care (https://info.bio-rad.com/DecisiveMoments.html?WT.mc_id=170504018473)
Currently Available Genetic Biomarker Testing for NSCLC

<table>
<thead>
<tr>
<th>Source</th>
<th>Mutations/Fusions</th>
<th>Platform</th>
<th>Performance</th>
<th>Turnaround Time</th>
<th>Medicare Reimbursed</th>
<th>Source Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company 1</td>
<td>*EGFR, *T790M, *ALK, ROS1, RET, *KRAS, *BRAF</td>
<td>ddPCR</td>
<td>Sensitivity: 91%&lt;sup&gt;1&lt;/sup&gt;</td>
<td>72 hours</td>
<td>Yes</td>
<td>(cDNA)</td>
</tr>
<tr>
<td>Company 2</td>
<td>ALK, RET1, MET, PD-L1, PTEN, ROS1, TOP2A, TP, TRKpan, TUBB3</td>
<td>NGS, IHC</td>
<td>Specificity: 100%&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Company 3</td>
<td>*EGFR, *T790M, *ALK</td>
<td>nPCR w/ NGS</td>
<td>EGFR: 83%, T790M: 64%, ALK: 88%&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5 days</td>
<td>Yes</td>
<td>Tissue</td>
</tr>
<tr>
<td>Company 4</td>
<td>*EGFR, T790M, ALK, ROS1, RET, KRAS, BRAF, MET, HER2</td>
<td>nPCR</td>
<td>EGFR: NA, ALK: 100%&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Company 5</td>
<td>EGFR, EGFR T790M, ALK1, ROS11, KRAS1, PD-L1</td>
<td>ddPCR</td>
<td>Specificity: 100%&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5-7 days</td>
<td>Yes</td>
<td>Blood (CTCs)</td>
</tr>
<tr>
<td>Company 6</td>
<td>*EGFR, *T790M, *KRAS, *BRAF</td>
<td>ddPCR w/ NGS</td>
<td>URINE: EGFR Exon 19: 67%, EGFR L858R: 75%, T790M: 72%&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2 weeks</td>
<td>Yes</td>
<td>Blood or urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BLOOD: EGFR Exon 19: 90%, EGFR L858R: 97%, T790M: 93%&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ddPCR, droplet digital PCR; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation (high-throughput) DNA sequencing; nPCR, reverse transcriptase PCR.

2. No published data could be located.

Table 1. Currently available genetic biomarker testing for NSCLC.
Blood-based Nucleic Acids for Cancer Mutation Testing

Circulating DNA and RNA

- Tumor cells can release/shed nucleic acids into the blood
- Plasma is spun down (high speeds) and the DNA purified
- Plasma is spun at slower speed to recover circulating RNA that is either free or attached to carriers such as exosomes and/or platelets
- More representative of tumor burden and heterogeneity than tissue biopsy
Nucleic Acid Testing from Blood

Measurement of DNA and RNA gene fusion transcripts

# What is GeneStrat?
A series of tests for the sensitive, accurate and rapid resulting of blood-based genetic mutations

## DNA Tests
- **cfDNA BCT**
  - **Gene**: EGFR
  - **Variant**: L858R, Del19 (ΔE746-A750), T790M
  - **Gene**: KRAS
  - **Variant**: G12C, G12D, G12V
  - **Gene**: BRAF
  - **Variant**: V600E

## RNA Tests
- **cfRNA BCT**
  - **Gene**: ALK
  - **5’ Fusion Partner (# of transcripts)**: EML4 (3)
  - **Gene**: ROS1
  - **5’ Fusion Partner (# of transcripts)**: CD74 (2), SDC4 (2), SLC34A2 (2), EZR (1), TPM3 (1)
  - **Gene**: RET
  - **5’ Fusion Partner (# of transcripts)**: KIF5B (6), CCDC6 (1), TRIM33 (1)
How is GeneStrat being used?

Perform Blood-based Testing First

Result back to physician in 72 hours

Physician may recommend targeted therapy options and treat sooner

Result back to physician in 2-4 weeks

Negative samples reflex to analysis of tissue biopsy results

Extended time to result

Tissue-based testing confirms mutation negative tumor status

Physician may recommend targeted therapy options
Blood-based genomic tests are improving treatment decision and start times
By Krish Bhadra MD, Hestia Mellert PhD and Gary Pestano PhD

CLP June 2017
Impact of Liquid Biopsy on Time to Treatment

Shortening Time from Diagnosis to Treatment in NSCLC: Are Blood-Based Biopsies the Answer?

- Five oncology centers, 179 patients
- Average TAT for Biodesix results was 33 hours (1.3 business days) vs. 12 days for tissue-based results
- For Biodesix Lung Reflex:
  - 20% of patients had actionable mutations (EGFR, ALK, BRAF, KRAS)
  - 21% of patients had a VeriStrat Poor status
- **All patients** that received Biodesix Lung Reflex testing at ECU initiated treatment **within 7 days**
- With Biodesix Lung Reflex at diagnosis:
  - Pulmonologists can utilize results to personalize procedural decisions
  - Patients are referred on to the medical oncologist with results in-hand for rapid treatment decisions
  - Can help patients receive appropriate treatment faster, and may positively impact patient outcomes

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1 Bowling M; Mattingley J; Bhadra K; Pritchett M; Skibo S; Walker P. Contributing Institutions: ECU Pulmonology, North Carolina; Gunderson Lutheran, Wisconsin; CHI Memorial Hospital, TN; Pinehurst Medical Clinic, NC; 21st Century Oncology, NC; Leo Jenkins Cancer Center, NC. Abstract number 143. Program PS01.16 IASLC Chicago 2016.
Turn Around Time for EGFR Mutations (US)

96% Reported in 72 hours or less

Hours from receipt to result:
- 0-24: 80%
- 24-48: 14%
- 48-72: 2%
- >72: 4%
How Does ddPCR Testing Work in Commercial Laboratory?

Overview of the GeneStrat Test

• Specimen Collection Kit at physician’s office
• Draw and ship FedEx Priority Overnight to our laboratory
• Plasma separation and isolation of circulating nucleic acids
• Droplet Digital™ PCR is conducted on duplicate samples arranged in batches by variant with controls
• Results are analyzed and a Test Result Report is generated for each variant and specimen received
• Results are encrypted and electronically communicated to the ordering physician
GeneStrat Commercial Test Workflow

Day 1

Ambient shipped whole blood from Physician to Biodesix

Day 2

Secure LIMS accessioning

Plasma cfDNA Isolation

Plasma cRNA isolation + reverse transcription

EGFR
KRAS
BRAF

EML4-ALK
ROS1
RET

PD-L1 Under Development

Day 3

Droplet Digital PCR

Data Analysis

Variant

Control

Test results to physician

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Case Studies: EGFR and PD-L1 Test Development using Droplet Digital PCR
Development of Liquid Biopsy Tests

• Test Development Process
  – Feasibility, Verification, Validation, Transfer and On-market performance monitoring

• Representative development examples for liquid biopsy ddPCR tests
  – EGFR T790M in Monitoring
  – PD-L1 over-expression in Blood

¹Mellert et al. JMD 2017
EGFR T790M
Clinical Validation

Description of Testing:

• All samples were assessed using the CAP/CLIA-lab SOPs for GeneStrat EGFR

• T790M was tested along with the sensitizing mutations L858R and del19 (E746-A750)

• T790M potential negatives were pre-selected based on KRAS positive status

• 55 samples were included in the validation set
**EGFR T790M**

Representative Clinical Validation Samples

![Graph showing Variant Positive and Variant Negative samples for Wild-Type EGFR with T790M Mutation data.](image-url)
# EGFR T790M

## Test Performance Summary

<table>
<thead>
<tr>
<th>Test System Metric</th>
<th>EGFR T790M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from sample ship to results (TAT)</td>
<td>24 hours in transit; 48 hours to test result</td>
</tr>
<tr>
<td>Sample Collection Kit (Ship Stability)</td>
<td>Up to 7 days</td>
</tr>
<tr>
<td>Analytic Sensitivity (LOD)</td>
<td>0.02%</td>
</tr>
<tr>
<td>Clinical Validation Set</td>
<td>n=55</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>86.7%</td>
</tr>
<tr>
<td>Concordance</td>
<td>96.4%</td>
</tr>
<tr>
<td>Precision</td>
<td>Passed</td>
</tr>
</tbody>
</table>
Example of EGFR Copy Number Monitoring in Plasma from NSCLC

Biodesix tests for EGFR L858R, del19 mplex and T790M

Table 2 and Fig 3 from ASCO Poster presented by AstraZeneca*

Table 2. Median PFS based on clearance or detection of EGFR mutations in plasma samples taken 3 and 6 weeks post initiation of osimertinib

<table>
<thead>
<tr>
<th>Group</th>
<th>Median PFS, (95% CI)</th>
<th>ORR</th>
<th>Patients alive at DCO n (%)</th>
<th>P-value (log rank test)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance of plasma EGFR mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 6 weeks</td>
<td>n=62</td>
<td>11.1 months (9.3, 12.6)</td>
<td>87%</td>
<td>36 (73.1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Detectable plasma EGFR mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 6 weeks</td>
<td>n=19</td>
<td>6.7 months (4.1, 7.7)</td>
<td>53%</td>
<td>5 (26.3%)</td>
<td></td>
</tr>
<tr>
<td>3 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance of plasma EGFR mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 3 weeks</td>
<td>n=44</td>
<td>11.1 months (8.2, 12.7)</td>
<td>80%</td>
<td>33 (75.0%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Detectable plasma EGFR mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 3 weeks</td>
<td>n=20</td>
<td>5.7 months (4.1, 10.1)</td>
<td>75%</td>
<td>4 (26.0%)</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; DCO, data cut-off; EGFR, epidermal growth factor receptor; ORR, objective response rate; PFS, progression-free survival

Figure 3. PFS based on clearance or detection of EGFR mutations in plasma samples taken A) 3 weeks and B) 6 weeks post initiation of osimertinib

New Product Feasibility

PD-L1 in Blood

• Therapeutic options for patients with non-small cell lung cancer (NSCLC) continue to expand with the approval of immunotherapies.

• PD-L1 testing can be clinically challenging due to lack of tissue and complexities associated with immunohistochemistry (IHC) including multiple antibodies, various scoring methods and heterogeneous expression.

• Moreover, various thresholds have been established for diagnostic tests being used in the context of different checkpoint inhibitors in order to direct clinical practice.

• We hypothesized that a test that delivered PD-L1 results from plasma read out as continuous variables may be of increased utility in the selection of therapeutic options
Blood-based PD-L1 Test Feasibility¹

Requirements

• A blood-based assay that measures PD-L1 mRNA transcripts

• Utility for NSCLC patients when tissue is not available

¹Please see Poster q10 at the break – Mellert et. al.
Objectives

Key Take-Away Messages

• Showed how Droplet Digital™ PCR technology is being used for liquid biopsy testing
  – Blood based testing offers supplementary information to tissue
  – Clinical studies have used results to examine monitoring for therapeutic effectiveness
  – Oncology practices embraced the rapid turn-around times of this type test as it may allow for faster treatment decisions

• Reviewed performance data from an on-market ddPCR test (GeneStrat)
  – Highly sensitive, specific and robust tests have been developed
  – DNA and RNA analytes can be reproducibly assessed from blood
  – Rapid turn-around times have become the expectation for liquid biopsy testing
What’s next from Biodesix and Our Partners?

• Biodesix
  – Extending the clinical utility of ddPCR for biomarkers in blood
    • Highly multiplexed assays (gene fusions) and over-expression (eg. PD-L1 and other immune response markers for immunotherapy)
  – Expanding test access to OUS markets: current examples are in Israel and India
  – Collaborations on IVD test development (US and oUS)
    • The GeneStrat EGFR Specimen Collection kit is CE-IVD registered

• Bio-Rad DBC
  – High-throughput/multiplexed ddPCR platforms and assays
  – Progress toward Universal Dx for ddPCR in US and EU
The Future is Bright!
Cleveland Clinic Top 10 Innovations 2017

Top 10 for 2017

1. The Microbiome to Prevent, Diagnose, and Treat Disease
   When it comes to life-saving potential and market opportunities, it turns out the gut is a gold mine.

2. Diabetes Drugs that Reduce Cardiovascular Disease and Death
   Nearly a decade ago, a wave of new diabetes drugs hit the market with promises of lowering blood sugar.

3. Cellular Immunotherapy to Treat Leukemia & Lymphomas
   In 2016, nearly 16,000 children and adolescents will be diagnosed with cancer. About 4,000 of these cases will be diagnosed with leukemia.

4. Liquid Biopsies to Find Circulating Tumor DNA
   Imagine going to the doctor for a checkup, and routine bloodwork. The bloodwork comes back with a diagnosis of cancer.

5. Automated Car Safety Features and Driverless Capabilities
   It’s the scene of everyone’s dreams. Parents especially spend countless nights, lying in bed, hoping the phone not to ring. Yet, too often, it does.
Discussion