Liquid Biopsy in Clinical Practice: Implementation of the OncoBEAM™ RAS Platform in the Management of mCRC Patients

Sysmex Inostics
World CDx Boston
October 20th, 2016

Vishal Sikri
VP of Commercial Operations
Sysmex Corporation

Kobe
Sysmex Corporation

GLOBAL INTEGRATED NETWORK of Marketing, Sales, Production and R&D

- 190+ COUNTRIES SUPPLIED
- >10% R&D EXPENDITURE
- SUBSIDIARIES & AFFILIATES: 53 locations in 29 countries
- NUMBER OF EMPLOYEES: >7,500
- $2.3B FY2015 NET TOTAL SALES

TOP 10 LEADING IVD COMPANIES WORLDWIDE
What Does Sysmex Inostics Do?

Clinical Products and Services

- Assist in Therapy Selection
- Assessment of Drug Response
- Resistance and Recurrence Monitoring

Pharma/CRO Services

- Biomarker Assay Development
- CDx Kit Development
- Regulatory Registration & Approval
- cGMP Manufacturing
- Commercialization
Advantages of Plasma DNA Testing

» High compliance
  Minimally invasive, few risks

» Fresh DNA
  Archival tissue can be degraded & have different mutation profile

» Accessible
  Tissue not always accessible; e.g. NSCLC

» No selection bias
  Assess primary tumor and metastases w/ one sample
  Tumor intra/inter-heterogeneity not a problem

» Monitoring possible
  Allows monitoring for drug response and resistance
Detection of Cell-Free Tumor DNA

Localized vs Metastatic Disease

Bettegowda et al, Sci Tran Med Feb 2014
BEAMing (Beads, Emulsions, Amplification, Magnetics) has shown efficacy in several therapeutic clinical trials as well as in oncology patient testing applications.
Flow Cytometry Analysis Separates Wild Type from Mutant DNA

Flow cytometry analysis

- Wild-type DNA
- Mutant DNA
- Mutant & Wild-type DNA
CRO Partner to Pharmaceutical Companies

> 100 clinical trials; > 40,000 samples total

- Lung, 31%
- Skin, 22%
- Colon, 6%
- Liver, 2%
- Gastric (GIST), 6%
- Endomedrium, 3%
- Prostate, 2%
- Pancreas, 3%
- Ovarian, 1%
- Various, 9%
- Thyroid, 1%
- Breast, 15%
- Various, 9%

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> 100 clinical trials; > 40,000 samples total
Publications

- Van Cutsem et al. 2015, Journal of Clinical Oncology
- Tabernero et al. 2015, Lancet Oncology
- Dawson et al. 2013, New England Journal of Medicine
- Misale et al. 2012, Nature
- Morelli et al. 2015, Annals of Oncology
- Cardiello et al. 2014, Journal of Clinical Oncology
- Thress et al. 2015, Lung Cancer
- Karlovich et al. 2016, Clinical Cancer Research
- Oxnard et al. 2016, JCO
- Sorich et al. 2015, Annals of Oncology
- Diaz et al. 2012, Nature
- Diaz et al. 2012, Nature
- Misale et al. 2012, Nature
- Dawson et al. 2013, New England Journal of Medicine
- Cardiello et al. 2014, Journal of Clinical Oncology
Sysmex Inostics OncoBEAM™ Assays for Therapy Selection and Monitoring
## Concordance Between Tissue and Cell-Free DNA on Clinically Actionable Mutations

### OncoBEAM™ Clinical Snapshot: Published Performance

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Marker</th>
<th>Stage</th>
<th>Patient No.</th>
<th>Tissue Analysis</th>
<th>Concordance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>EGFR</td>
<td>IV</td>
<td>78</td>
<td>SOC</td>
<td>99(^a)</td>
</tr>
<tr>
<td></td>
<td>KRAS</td>
<td>IV</td>
<td>78</td>
<td>SOC</td>
<td>92(^b)</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td></td>
<td>38</td>
<td>SOC</td>
<td>95(^c)</td>
</tr>
<tr>
<td>mCRC</td>
<td>Extended RAS</td>
<td>IV</td>
<td>238</td>
<td>SOC</td>
<td>93.3(^d)</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>PIK3CA</td>
<td>IV</td>
<td>34</td>
<td>BEAMing</td>
<td>100(^e)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF V600E</td>
<td>IV</td>
<td>42</td>
<td>Sanger</td>
<td>93(^f)</td>
</tr>
</tbody>
</table>

\(^a\) Reference: [1].
\(^b\) Reference: [2].
\(^c\) Reference: [3].
\(^d\) Reference: [4].
\(^e\) Reference: [5].
\(^f\) Reference: [6].
High Sensitivity Matters

Distribution of RAS MAFs in first line mCRC patients

- 39% of patients with MAFs <0.1%
- 29% of patients with MAFs 0.1-1%
- 21% of patients with MAFs >1%
- 11% of patients with MAFs >5%

Distribution of EGFR MAFs in EGFR-mutant NSCLC patients with T790M+ resistance

- 36% of patients with MAFs <0.1%
- 15% of patients with MAFs 0.1-1%
- 27% of patients with MAFs >1%
- 22% of patients with MAFs >5%

Distribution of PIK3CA MAFs in HR+/HER- recurrent breast cancer patients

- 32% of patients with MAFs <0.1%
- 22% of patients with MAFs 0.1-1%
- 23% of patients with MAFs >1%
- 23% of patients with MAFs >5%

50% of patients with MAFs <1%

42% of patients with MAFs <1%

45% of patients with MAFs <1%

Sysmex Inostics OncoBEAM™ RAS Kit (Joint Collaboration: Sysmex | Merck)

Bringing Service based Assays to Hospitals Globally
LDT to IVD
RAS Tissue Testing
From Physician Order to Test Results

ESMO guidelines recommend RAS testing to be completed within 7 days

OncoBEAM™ Platform & Workflow

Blood Collection → Plasma Preparation → DNA Extraction → PCR Amplification

Emulsion PCR → Breaking & Hybridization → Flow Cytometry → Analysis Flow Data & Report

48h Turnaround time

Qiavac 24 Plus System
QIAvac Connecting System
Vacuum Pump (230 V, 50 Hz)

Veriti Dx 96-well
Thermal Cycler

E12-200XLS+
(electronic pipettor)

Alpaqua 96S Super Magnet Plate

OncoBEAM/Cube6i
Flow Cytome
Dedicated Training Center
Applications in Metastatic Colorectal Cancer

<table>
<thead>
<tr>
<th>KRAS</th>
<th>NRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Codon 12</td>
<td>• Codon 12</td>
</tr>
<tr>
<td>• Codon 13</td>
<td>• Codon 13</td>
</tr>
<tr>
<td>• Codon 59</td>
<td>• Codon 59</td>
</tr>
<tr>
<td>• Codon 61</td>
<td>• Codon 61</td>
</tr>
<tr>
<td>• Codon 117</td>
<td>• Codon 117</td>
</tr>
<tr>
<td>• Codon 146</td>
<td>• Codon 146</td>
</tr>
</tbody>
</table>

34 different mutations multiplexed to run with 2-3 mL of plasma
Blood-based RAS Testing mCRC Therapy Selection and Detection of Resistance

**Therapy Selection**

- **Tissue**
  - RAS mutation status
  - WT
  - MUT

- **Blood**
  - Concordance of RAS status in blood vs tissue

**RAS Resistance Detection**

- **No tissue**
  - Monitoring of RAS mutation status
  - T0, T1, T2
  - Anti-EGFR Re-challenge?

- **Chemotherapy** (e.g. FOLFIRI)

- **Monitoring of RAS mutation status**
  - MUT
### Concordance Study Results

#### Tissue SOC RAS result

<table>
<thead>
<tr>
<th>OncoBEAM™ RAS CRC Plasma RAS Result</th>
<th>Mutation detected</th>
<th>No mutation detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation detected</td>
<td>112</td>
<td>7</td>
<td>119</td>
</tr>
<tr>
<td>No mutation detected</td>
<td>9</td>
<td>110</td>
<td>119</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>117</td>
<td>238</td>
</tr>
</tbody>
</table>

**Overall Agreement = 93.3%**

- **Positive Percent Agreement = 92.6%**
- **Negative Percent Agreement = 94.0%**

### Cutoff Setting

- RAS Tissue Mutation Rate: 50.8%
- RAS OncoBEAM™ Blood Mutation Rate: 50.0%
Value of Sensitivity of OncoBEAM™ RAS CRC Kit

Offers the sensitivity *NEEDED* to detect mutations at low levels in recurrent patients

40% of mCRC cases diagnosed are **recurrent cases**
% Mutant Fraction: **1.49%**

60% of mCRC cases diagnosed are **primary diagnosed cases**
% Mutant Fraction: **9.63%**

**HIGH Sensitivity Matters**
High Sensitivity is Required for Reliable cfDNA Profiling

In First-line metastatic Colorectal Cancer, 50% of patients show ctDNA at <1% fraction

Concordance study, VHIO, n=150, retrospective, patients anti-EGFR naïve, testing plasma BEAMing + tissue SOC & BEAMing (RAS panel). WGIC 2016
Clinical Performance: Blood versus Tissue

Clinical Relevance: PFS and OS in RAS wild-type RAS population

Next Steps: Monitoring

1. Is the therapy working?
2. Is the cancer progressing?
Emerging Application: Patient Selection for Re-challenge with Anti-EGFR Therapy

Can Liquid Biopsy identify...

- Patients with RAS-WT mCRC...
- Who progress and have detectable RAS mutations during therapy...
- Which decrease during a pause in anti-EGFR therapy...
- Who may respond to anti-EGFR re-challenge?
RAS Mutant Clones Dynamically Evolve in Response to Pulsatile anti EGFR- Therapy

Applications in Lung Cancer

Lung Cancer Gene Mutation Assays
Advancing Precision Medicine with a Tube of Blood

**EGFR**

- Deletion 19
- L858R
- T790M
- C797S

11 different mutations multiplexed to run with 2 mL of plasma

**ALK**

- C797S
Emerging Mutation Detection Directs Therapy in NSCLC

Tissue

Blood

EGFR mutation status

Chemotherapy

TKI therapy

WT

MUT

Blood

Monitoring for emerging T790M resistance mutation

T0  T1  T2
### Mutations Detected of Patients with Distant Metastatic (M1b) vs. Local (M1a/M0) Disease

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Disease classification</th>
<th>Total patients with mutation detected</th>
<th>Subset with mutation detected in plasma by BEAMing</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activating mutations</td>
<td>M1a/M0</td>
<td>18</td>
<td>7</td>
<td>39%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>M1b</td>
<td>55</td>
<td>52</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>T790M</td>
<td>M1a/M0</td>
<td>15</td>
<td>4</td>
<td>27%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>M1b</td>
<td>49</td>
<td>47</td>
<td>96%</td>
<td></td>
</tr>
</tbody>
</table>

» Activating EGFR mutations were detected in plasma of 95% of M1b vs. 39% of M1a/M0 patients

» T790M mutations were detected in plasma of 96% of M1b vs. 27% of M1a/M0 patients

Karlovich et al. (2016) Clin Cancer Res. 15;22(10):2386-95
Paradigm Shift is Occurring

Blood based testing first...

A. Conventional paradigm

Acquired resistance to EGFR-TKI

All patients undergo biopsy, FDA approved FFPE assay for T790M

T790M positive

Third gen. EGFR-TKI

T790M negative

Chemotherapy

B. Proposed paradigm for use of plasma diagnostics

Acquired resistance to EGFR-TKI

FDA approved plasma assay for T790M and sensitising mutations

T790M positive

Skip biopsy, start 3rd gen. EGFR-TKI

Biopsy, FDA approved FFPE assay for T790M

T790M positive

3rd gen. EGFR-TKI

T790M negative

Chemotherapy
## Applications in Breast Cancer

- **ESR1**
  - E380Q
  - S463P
  - V534E
  - P535H
  - L536H
  - L536P
  - L536R
  - L536Q
  - Y537N
  - Y537S
  - Y537C
  - D538G

- **PIK3CA**
  - C420R
  - E542K
  - E545G
  - E545K
  - Q546K
  - M1043I
  - H104R
  - H1047L
  - H104Y

- **AKT1**
  - E17K

22 different mutations multiplexed to run with 2 mL of plasma
## Breast Cancer Panel (AKT1/PIK3CA/ESR1)

**Buparlisib Plus Fulvestrant Produced a Clinically Meaningful PFS Improvement in Patients With ctDNA PIK3CA Mutations**

<table>
<thead>
<tr>
<th>ctDNA PIK3CA Mutant</th>
<th>Buparlisib + Fulvestrant n=87</th>
<th>Placebo + Fulvestrant n=113</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, months</td>
<td>7.0 (5.0–10.0)</td>
<td>3.2 (2.0–5.1)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.56 (0.39–0.80)</td>
<td></td>
</tr>
<tr>
<td>One-sided nominal P value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ctDNA PIK3CA</th>
<th>Buparlisib + Fulvestrant n=199</th>
<th>Placebo + Fulvestrant n=188</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-mutant n=387</td>
<td>6.8 (4.7–8.5)</td>
<td>6.8 (4.7–8.6)</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.05 (0.82–1.34)</td>
<td></td>
</tr>
<tr>
<td>One-sided nominal P value</td>
<td>0.642</td>
<td></td>
</tr>
</tbody>
</table>

**Graphs:**
- **Left:** Probability of Progression-free Survival (% vs. Time (Months))
  - Buparlisib + fulvestrant (n/N=48/87)
  - Placebo + fulvestrant (n/N=90/113)

- **Right:** Probability of Progression-free Survival (% vs. Time (Months))
  - Buparlisib + fulvestrant (n/N=124/199)
  - Placebo + fulvestrant (n/N=126/188)

**Cl,** confidence interval; **ctDNA,** circulating tumor DNA; **HR,** hazard ratio; **PFS,** progression-free survival.

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Baselga et al. SABS 2015
OncoBEAM™ BCP – BELLE2 Clinical Trial

Critical Results:

» When PIK3CA mutation status was analyzed with archival tissue, small differences in overall response was seen

» When PIK3CA mutation status was analyzed using the OncoBEAM™ BCP assay from blood samples, PIK3CA mutant patients demonstrated a significant OR benefit (18.4 vs. 3.5 months) with the addition Buparlisib
Summary

1. High sensitivity needed to detect low mutant molecules
   » Value of low level mutant fractions shown in colon, lung and breast Cancers
2. Large multiplexing capabilities with 2 mL of plasma (1 tube of blood)
3. Assays validated to run on blood collected in Streck tubes or EDTA tubes
4. Most assays have analytical sensitivities of 0.02% - 0.04%
6. Global Regulatory experience: US, EU, Japan and China
   » Experienced with Class III submissions
7. Large Commercial Capabilities: selling in 190+ countries
   » Selling direct in most large markets.
   » Oncology focused support team
8. Versatile technology: LDT workflow modified for IVD use
   » Testing locally in hospital labs
9. Experienced in bringing Companion Diagnostics to market
   » Launched OncoBEAM™ RAS CRC Kit for anti-EGFR therapy determination in March 2016
Sensitivity Matters in Liquid Biopsy