Lessons Learned from ctDNA NGS in 25,000 Advanced Cancer Patients in Clinical Practice

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Guardant Health, Inc.

Disclosure: Employee
Guardant Health, Inc.
Outline/Key Points

1. Predictive Diagnostic – what are the critical performance characteristics?
   a) Why comprehensive genomic plasma testing “CGP testing” is critical?
   b) What sensitivity (limit of detection) is required for advance cancer patients?

2. Path to validate analytical claims when orthogonal reference methods for plasma do not exist

3. Clinical outcomes for different type of alterations and low MAF variants
Case 1: Multiple resistance mechanisms in \textit{EGFR} mutant NSCLC

- 49 year old female with NSCLC diagnosed at age 46
- \textit{EGFR} exon 19 deletion identified at diagnosis via tissue testing
- Progressing on afatinib after \(~18\) months
- Guardant360 performed to look for resistance mechanism without another biopsy
EGFR T790M Drives Resistance in 50% of EGFR mutated NSCLC

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the relative changes of observed cfDNA at different sample submission time points. The "Somatic Alteration Burden" value below refers to the maximum % cfDNA detected at each time point. Amplifications are not plotted.

Summary of Alterations & Associated Treatment Options

The percentage, or allele frequency, of altered cell-free DNA (% cfDNA) circulating in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment.

<table>
<thead>
<tr>
<th>Alteration</th>
<th>% cfDNA</th>
<th>cfDNA Amplification</th>
<th>FDA Approved in Indication</th>
<th>Available for Use in Other Indications</th>
<th>Clinical Drug Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>E746_A750 Del</td>
<td>0.2</td>
<td></td>
<td>Afatinib, Erlotinib, Gefitinib</td>
<td>Neckitumab, Panitumumab</td>
<td>Trials Available</td>
</tr>
<tr>
<td>EGFR</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T790M</td>
<td>0.2</td>
<td></td>
<td>Osimertinib, Erlotinib, Gefitinib</td>
<td>Afatinib, Neckitumab, Panitumumab</td>
<td>Trials Available</td>
</tr>
</tbody>
</table>
Beyond EGFR T790M – Genomic Mechanisms of Acquired Resistance

- EGFR T790M: 50%
- ERBB2 (HER2) amp: 12.0%
- MET amp: 4.0%
- EMT to SCLC RB1 loss or inactivating mutation: 6.0%
- PTEN loss or inactivating mutation: 4.0%
- BRAF mutation: 1.0%
- NF1 loss or inactivating mutation: 1.0%
- ERBB3 (HER3) amp or mutation: 2.0%
- EGFR exon 20 insertion: 4.0%
- EGFR L747S, D761Y, T854A: 0.1%
- Other: 3.3%
- PIK3CA mutation: 5.0%
- KRAS mutation: 1.0%
- MEK1 (MAP2K1) mutation: 5.0%
- EML-ALK and other fusions: 1.0%
- Other: 3.3%
Osimertinib Worked for 6 Months – Monitoring Just for EGFR T790M Would Have Missed the Full Picture

Ex19del+T790M+ clones responding to osimertinib
Ex19del+/T790M-/PIK3CA+ clones not responding to osimertinib
Emergence of sub-clonal RET fusion
### NCCN Guideline Somatic Genomic Targets

Eleven somatic genomic targets in seven cancer types across all four major types of genomic alterations

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Targetable Genomic Alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSCLC</strong></td>
<td><strong>EGFR mt</strong> <strong>BRAF mt</strong> <strong>ERBB2 mt</strong> <strong>ALK fusion</strong> <strong>ROS1 fusion</strong> <strong>RET fusion</strong> <strong>MET amp and exon 14 skipping mt</strong></td>
</tr>
<tr>
<td><strong>Colorectal</strong></td>
<td><strong>KRAS mt</strong> <strong>NRAS mt</strong> <strong>BRAF mt</strong> <strong>MSI (or MMR IHC) if ≤ 70 or older if relative with CRC ≤ 50 or 2 relatives with CRC</strong></td>
</tr>
</tbody>
</table>
| **Breast**        | **ERBB2 (HER2) amp** **BRCA1/2 germline if:**
|                   | - early onset ≤ 45
|                   | - triple negative breast cancer ≤ 60
|                   | - male breast cancer at any age
|                   | - dx at any age & family hx breast, ovarian, pancreas or prostate |
| **Gastric & Gastro-esophageal** | **ERBB2 (HER2) amp** |
| **Melanoma**      | **BRAF mt** **KIT mt** |
| **GIST**          | **KIT mt** **PDGFRA mt** **BRAF mt** |
Guardant360 – No A Priori Knowledge Requirement

Critical exons completely sequenced and all four major classes of alterations

### POINT MUTATIONS - Complete* or Critical Exon Coverage in 70 Genes

<table>
<thead>
<tr>
<th>ATC1</th>
<th>ALK</th>
<th>APC</th>
<th>AR</th>
<th>ARAF</th>
<th>ARID1A</th>
<th>ATM</th>
<th>BRAF</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1</td>
<td>CCND2</td>
<td>CCNE1</td>
<td>CDH1</td>
<td>CDK4</td>
<td>CDK6</td>
<td>CDKN2A</td>
<td>CDKN2B</td>
<td>CTNNB1</td>
<td>EGFR</td>
</tr>
<tr>
<td>ERBB2</td>
<td>ESR1</td>
<td>EZH2</td>
<td>FBXW7</td>
<td>FGFR1</td>
<td>FGFR2</td>
<td>FGFR3</td>
<td>GATA3</td>
<td>GNA11</td>
<td>GNAQ</td>
</tr>
<tr>
<td>GNAS</td>
<td>HNF1A</td>
<td>HRAS</td>
<td>IDH1</td>
<td>IDH2</td>
<td>JAK2</td>
<td>JAK3</td>
<td>KIT</td>
<td>KRAS</td>
<td>MAP2K1</td>
</tr>
<tr>
<td>MAP2K2</td>
<td>MET</td>
<td>MLH1</td>
<td>MPL</td>
<td>MYC</td>
<td>NF1</td>
<td>NFE2L2</td>
<td>NOTCH1</td>
<td>NPM1</td>
<td>NRAS</td>
</tr>
<tr>
<td>NTRK1</td>
<td>PDGFRA</td>
<td>PIK3CA</td>
<td>PTEN</td>
<td>PTPN11</td>
<td>RAF1</td>
<td>RB1</td>
<td>RET</td>
<td>RHEB</td>
<td>RHOA</td>
</tr>
<tr>
<td>RIT1</td>
<td>ROS1</td>
<td>SMAD4</td>
<td>SMO</td>
<td>SRC</td>
<td>STK11</td>
<td>TERT</td>
<td>TP53</td>
<td>TSC1</td>
<td>VHL</td>
</tr>
</tbody>
</table>

### AMPLIFICATIONS

<table>
<thead>
<tr>
<th>AR</th>
<th>BRAF</th>
<th>CCND1</th>
<th>CCND2</th>
<th>CCNE1</th>
<th>CDK4</th>
<th>CDK6</th>
<th>EGFR</th>
<th>ERBB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>FGFR2</td>
<td>KIT</td>
<td>KRAS</td>
<td>MET</td>
<td>MYC</td>
<td>PDGFRA</td>
<td>PIK3CA</td>
<td>RAF1</td>
</tr>
</tbody>
</table>

### FUSIONS

<table>
<thead>
<tr>
<th>ALK</th>
<th>FGFR2</th>
<th>FGFR3</th>
<th>RET</th>
<th>ROS1</th>
<th>NTRK1</th>
</tr>
</thead>
</table>

### INDELS

| EGFR exons 19/20 | ERBB2 exons 19/20 | MET exon 14 skipping |
Guardant Health’s experience in running G360 as an LDT

- Launched as an LDT in June 2014
- Leader in liquid biopsy market: processed >25,000 samples from >2,500 ordering physicians to date.
Half of reported variants occur below 0.4% Variant Allele Fraction (N=20,000)
Somatic variant detection rate

Liver: 92%
CUP: 90%
Bladder: 90%
Ovarian: 89%
Cholangio: 87%
Lung: 87%
Prostate: 86%
Colorectal: 85%
Pancreatic: 84%
Carcin: 82%
Esophageal: 82%
Breast: 81%
Head & Neck: 79%
Melanoma: 72%
Glioblastoma: 57%

Avg: 85%
Path for Validation of Analytical Claims

- Unlike sheared genomic DNA or synthetic material, cell-line derived cfDNA are well suited to be used as contrived samples.

- We believe that the genomic profile of cell line-derived cell-free DNA (cfDNA) is highly similar to matched cell line-derived gDNA (specimen commutability).

- Using titrated cell line-derived cfDNA, analytical performance of the assay can be studied.

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Reportable Range</th>
<th>Unique Variants Tested</th>
<th>Sensitivity [95% CI]</th>
<th>PPV [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNVs</td>
<td>≥0.2%</td>
<td>90</td>
<td>98.9% [93.1-99.9]</td>
<td>100% [94.8-100%]</td>
</tr>
<tr>
<td>Indels</td>
<td>≥2 molecules</td>
<td>37</td>
<td>95.7% [84.0-98.7%]</td>
<td>100% [90.0-100%]</td>
</tr>
<tr>
<td>CNAs</td>
<td>≥2.20 copies</td>
<td>70</td>
<td>94.3% [85.3-98.2%]</td>
<td>93.0% [83.6-98.3%]</td>
</tr>
<tr>
<td>SVs</td>
<td>≥2 molecules</td>
<td>19</td>
<td>100% [79.1-100%]</td>
<td>100% [79.1-100%]</td>
</tr>
</tbody>
</table>
Analytic Specificity > 99.9999%
54-Gene Panel vs. Whole Exome Sequencing

A New Gold Standard for Specificity:
~1,560,000 base pairs sequenced -
(20 Samples x 78,000 BPs per sample)

<table>
<thead>
<tr>
<th>Reference</th>
<th>G360</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>365</td>
<td>1</td>
<td></td>
<td>366</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1,559,634</td>
<td></td>
<td>1,559,634</td>
</tr>
<tr>
<td>Total</td>
<td>365</td>
<td>1,559,635</td>
<td></td>
<td>1,560,000</td>
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</table>

Performance (95% CI)

<table>
<thead>
<tr>
<th></th>
<th>(95% CI)</th>
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<tbody>
<tr>
<td>Specificity</td>
<td>&gt;99.9999%</td>
</tr>
<tr>
<td></td>
<td>(&gt;99.9999%-100.0%)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>(98.7%-100.0%)</td>
</tr>
<tr>
<td>Concordance</td>
<td>99.9999%</td>
</tr>
<tr>
<td></td>
<td>(99.9999%-100.0%)</td>
</tr>
</tbody>
</table>

The single putative false positive sample was sent to another outside reference lab (Stanford Protein and Nucleic Acid Facility) where Sanger Sequencing found it to be a true positive.
High Analytic Specificity

68-Gene Panel vs. Whole Exome Sequencing

2nd Study Re-Confirms Near-Perfect Specificity:
~1,515,000 base pairs sequenced – 68-gene panel
(15 Samples x 101,000 BPs per sample)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G360 Positive</td>
<td>215</td>
<td>3</td>
<td>218</td>
</tr>
<tr>
<td>G360 Negative</td>
<td>0</td>
<td>1,514,785</td>
<td>1,514,785</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
<td>1,514,788</td>
<td>1,515,003</td>
</tr>
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</table>

Performance (95% CI)

<table>
<thead>
<tr>
<th>Performance</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>&gt;99.9998%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>Concordance</td>
<td>99.9999%</td>
</tr>
</tbody>
</table>

The samples with the three putative false positives were sent to another outside reference lab (Stanford) where Sanger Sequencing found all three to be true positives.
ctDNA NGS to ctDNA ddPCR

Using highly-validated clinical ddPCR assays as reference, G360 demonstrated perfect qualitative and excellent quantitative accuracy even at low allelic fractions

**Quantitative Calibration Important for Relative Variant Allele Fractions**
Cell-free DNA sensitivity may be limited when tumor DNA is not shed into circulation. Tissue DNA sensitivity may be limited because samples fail to capture tumor heterogeneity.
Temporal Heterogeneity Confounds Plasma to Tissue NGS Concordance: Blinded External Validation Study

Prospective study of 102 Consecutive NSCLC patients

Concordance (%)

- ≤2 Weeks: 100%
- ≤2 Months: 92%
- ≤6 Months: 94%
- >6 Months: 60%

p = 0.038*
ctDNA PPV vs tissue biopsy (NSCLC, CRC, Breast, Others)

- Truncal driver mutations
  - PPV remained high (94.5%, n=71) for low MAF truncal mutations (<0.5%)
  - * The single ctDNA-positive, tissue-negative ALK fusion responded to crizotinib

- Subclonal mutations indicate likely resistance
  - Discordant resistance cases likely reflect evolution on therapy after initial tissue biopsy
Mutations in First 20,000 Guardant360 Patients Reflect Prevalence in TCGA Tumor Tissue Compendia

The secondary resistance mechanism EGFR T790M frequently found in ctDNA cohort, but rare in TCGA (surgical/treatment naïve)
ctDNA Fusion Breakpoint Patterns Mirror TCGA

**ALK**

**EML4**

<table>
<thead>
<tr>
<th>EML4 intron</th>
<th>Breakpoints in ctDNA (n=41)</th>
<th>Breakpoints in COSMIC (n=375)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>46%</td>
<td>47%</td>
</tr>
<tr>
<td>6</td>
<td>37%</td>
<td>35%</td>
</tr>
<tr>
<td>20</td>
<td>5%</td>
<td>14%</td>
</tr>
</tbody>
</table>

Morán T, et al. 2013 *Transl Lung Cancer Res*
Clinical Outcomes for Different Variant Types in CtDNA NGS, and Low Variant Allele Fraction Calls In Particular.
EGFR Mutations Respond to TKI Whether Measured in Tissue (A) or Plasma (B)

A - EGFR L858R or exon 19 Del Measured in Tissue (N = 86)
Median PFS (95% CI):
- Erlotinib arm 10.4 mos (8.4 – 12.9)
- Chemotx arm 5.1 mos (4.5 – 5.8)

B - EGFR L858R or exon 19 Del Measured in Plasma (N = 49)
Median PFS 995% CI) by qPCR or TaqMan:
- Erlotinib arm 12.3 mos (8.4 – 14.7)
- Chemotx arm 5.5 mos (4.5 – 6.7)

Whether measured in tissue or blood, EGFR L858R and ex19 deletions responded to erlotinib. This is intuitive since the mutations in the blood come from the tissue.
Plasma *EGFR* T790M Responds to 3rd Generation TKI Even When Tissue Negative

<table>
<thead>
<tr>
<th></th>
<th>Plasma (BEAMing dPCR) vs. Tissue “Non-Reference Standard” (N=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>81%</td>
</tr>
<tr>
<td>Specificity</td>
<td>58%</td>
</tr>
</tbody>
</table>

**Response Rate (CR and PR)**
- Tissue: 61% (45%-75%) (25/41)
- Plasma: 59% (42%-73%) (24/41)

**Disease Control Rate (CR, PR, and SD)**
- Tissue: 98% (86%-100%) (40/41)
- Plasma: 90% (76%-97%) (37/41)
Case Two: Low-MAF ALK Fusion NSCLC but Tissue QNS for Genotyping

Clinical Case:

— 58-year-old female non-smoker presents with metastatic NSCLC

— Progression on empirical chemotherapy but tissue is QNS for genotyping despite three biopsy attempts

— Guardant360 demonstrated TWO MOLECULES of EML4-ALK fusion at 0.1% VAF.

— Major durable response to crizotinib

— From no options to response with a simple blood test
**MET Exon 14 Skipping Indel at 0.1% MAF in Undergenotyped Patient**

72-year-old male never-smoker with metastatic lung adenocarcinoma. Had 3 prior lines: carbo/taxol/avastin, then nivolumab, then carbo/pem. Could no longer walk on the beach.

Guardant360 reveals *MET* mutation: *MET* inhibitor produced PR (30%) with major clinical response, ECOG performance now 0.
### Guardant360 Publications (1-6)

<table>
<thead>
<tr>
<th>Author/Journal (Year)</th>
<th>Key Findings</th>
<th>AV, CV, CU</th>
</tr>
</thead>
</table>
| **Zill et al. 2015** Cancer Discovery (pancreatic cancer and cholangiocarcinoma) | • 35% of tissue biopsies insufficient in 100% “rescued” with Guardant360, i.e. ctDNA positive for driver mutation  
• 92% sensitivity for 5 major genes | CV |
| **Ko et al. 2015** Clinical Cancer Research (pancreatic cancer) | • 100% concordance for KRAS | CV |
| **Kim et al. 2015** Oncotarget (colorectal ca/melanoma) | • 90%+ sensitivity for KRAS and BRAF  
• Mean turnaround 10 days (N=75 patients) | CV |
| **Lanman et al. 2015** PLOS One (multiple stage III/IV solid tumor cancers) | • Analytic sensitivity to 1-2 molecules (0.1% variant allele fraction), analytic specificity 99.9999%  
• Clinical sensitivity 85%, clinical specificity 99.6%, accuracy 99.3% | AV, CV |
| **Lokhandwala et al. 2016** Clin Lung Cancer (lung cancer) | • Lung biopsy average cost ($14,600) driven by complication rate (19.3%) in Medicare population | Health Econ |
| **Raghav et al. 2016** Oncotarget (advanced colorectal carcinoma) | • 22% of anti-EGFR inhibitor-resistant metastatic colorectal cancer patients MET amplified, but not treatment-naive or RAS mutant | CV |

Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)
# Guardant360 Publications (7-11)

<table>
<thead>
<tr>
<th>Author/Journal (Year)</th>
<th>Key Findings</th>
<th>AV, CV, CU</th>
</tr>
</thead>
</table>
| Liang et al. 2015 Breast Cancer Res Treat (metastatic breast cancer) | • 100% specificity for ERBB2 (HER2) amplification  
• 86% clinical response rate | CV, CU |
| Schwaederlé et al. 2015 Oncotarget (advanced pan-cancer) | • 54-gene/3-gene amp test version, 69% were actionable w/ FDA-approved drug  
• Mean turnaround 13 days (95%CI 12-13), N=171 | CV, CU |
| Piotrowska et al. 2016 J Thor Oncol (non-small cell lung cancer (NSCLC)) | • Case report of EGFR T790M Guardant360 “rescue” and response when biopsy tissue was insufficient for genotyping | CV, CU |
| Schwaederlé et al. 2016 Clin Canc Res (advanced pan-cancer) | • CtDNA-targeted patients with response or stable disease in 5 of 12 evaluable patients (42%) superior to 2 of 28 unmatched patients (7.1%), p=0.02 | CV, CU |
| Villaflor et al. 2016 Oncotarget (advanced NSCLC) | • 1st clinic-based NSCLC cohort, 83% had ctDNA detected  
• Tissue not genotyped in 54%  
• Six (11%) of all ctDNA positive patients had EGFR mutations (two were T790M), one initial EGFR driver was found in plasma but not tissue  
• 8 responded (75%) & 2 stable – for 100% disease control rate  
• Median PFS of 11.5 months in the expected range based on published studies using tissue genotyping | CV, CU |

Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)
<table>
<thead>
<tr>
<th>Author/Journal (Year)</th>
<th>Key Findings</th>
<th>AV, CV, CU</th>
</tr>
</thead>
</table>
| Thompson et al. 2016 Clinical Cancer Research (advanced NSCLC) | • Prospective clinic-based NSCLC cohort (N=102), 53 1<sup>st</sup> line, 47 at progression (> 2<sup>nd</sup> line)  
• Tissue not genotyped in 51% – 7 not biopsy-able and 45 QNS  
• CtDNA positive in 84.3%, including 8 with EGFR T790M where tissue failed  
• 31% had on-label Rx, 55% off-label and 70% were trial eligible | CV, CU |
| Hong et al. 2016 Cancer Discovery In Press (advanced colorectal cancer (mCRC)) | • Prospective combination therapy study of 17 mCRC patients with BRAF V600E: 35% response rate (RECIST) and 88% disease control rate - median PFS 7.7 months vs. 2.5 months  
• Guardant BRAF V600E correlated to plasma ddPCR: R<sup>2</sup> = 0.99  
• BRAF V600E cfDNA correlated with radiographic response | CV, CU |
| Rozenblum et al. 2016 Journal of Thoracic Oncology In Press (advanced NSCLC) | • 19 tissue insufficient for tissue NGS, treatment decision was changed in 32% (6 of 19) who had NCCN genomic targets EGFR (2), RET (2), MET (1), ERBB2 (HER2) (1)  
• Matched therapy resulted in three PR and two SD out of five treated (60% objective response, 100% disease control rate by RECIST)  
• All but one of the six with genomic targets (84%) were missed with local EGFR qPCR and ALK FISH only testing | CV, CU |

Note: Most Clinical Utility Studies are actual clinical practice (outside of an investigational setting)
“Blood to Blood” Validation of ctDNA NGS to ddPCR and Response for \( \text{BRAF}^{V600E} \) in Colorectal Cancer

1. Six of 17 evaluable patients (35%) achieved a radiographic response by RECIST 1.1 criteria with vemurafenib, cetuximab & Irinotecan. Median progression-free survival was 7.7 months.

2. \( \text{BRAF}^{V600E} \) cfDNA trends correlated with radiographic changes (response and progression)

3. Acquired mutations from cfDNA in genes critical to MAPK signaling were observed at progression before their first restaging scan.
Prospective cfDNA-based Matching Trial, an Interim Analysis: NEXT-2 NSCLC

87% Response Rate and 100% Disease Control Rate

Targeted Alteration % cfDNA
- EGFR T790M: 6.3%
- EGFR Exon 19 deletion: 0.5%
- EGFR L858R: 0.2%
- ALK fusion: 3.2%
- other: 4.8%
- other: 0.6%
- other: 0.5%
- other: 0.8%
- other: 3.8%
- other: 0.1%
- other: 12.7%
- other: 0.1%
- other: 0.2%
- other: 40.6%
- other: 1.5%
- other: 0.1%

ECOG PS
- 1: 1
- 1: 1
- 1: 1
- 1: 1
- 1: 1
- 2: 1
- 1: 2
- 1: 1
- 1: 1
- 1: 2
- 1: 1
- 1: 1
- 1: 2
- 1: 1

Line of Therapy
- 1: 1
- 1: 1
- 2: 1
- 2: 2
- 2: 2
- 2: 1
- 3: 1
- 1: 3
- 1: 9
- 1: 1
- 1: 3
- 2: 2
- 2: 2

Best Response (RECIST 1.1)
- 9%: -7%
- 1: -35%
- 1: -35%
- 1: -39%
- 1: -40%
- 1: -41%
- 2: -41%
- 1: -41%
- 1: -42%
- 1: -54%
- 1: -54%
- 1: -56%
- 1: -63%
- 2: -65%

Abbreviations: IGF, insulin-like growth factor; MAb, monoclonal antibody; cfDNA, cell-free DNA
Treatment Decision Changed in 32% (6 of 19) of NSCLC with Biopsy Insufficient for Tissue NGS

Local EGFR & ALK Testing Missed 83% (5 of 6) ctDNA-Detected Alterations

Rozenblum et al. 2016 Journal of Thoracic Oncology
Note: 6th patient with CCDC6-RET fusion not yet evaluated
* Clinically stable disease; ^copy number in plasma
Prospective cfDNA-based Matching Trial, an Interim Analysis: NEXT-2 Gastric Carcinoma

67% Response Rate and 100% Disease Control Rate

**Matched Therapy**

<table>
<thead>
<tr>
<th>ctDNA amp</th>
<th>lapatinib</th>
<th>taxol + AKT1i</th>
<th>FGFR2b MAb</th>
<th>capcitabine + oxaliplatin + lapatinib</th>
<th>taxol + AKT1i</th>
<th>capcitabine + oxaliplatin + lapatinib</th>
<th>IND</th>
<th>capcitabine + oxaliplatin + lapatinib</th>
<th>capcitabine + oxaliplatin + lapatinib</th>
<th>capcitabine + oxaliplatin + lapatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.81</td>
<td>N/A</td>
<td>3.92</td>
<td>5.08</td>
<td>N/A</td>
<td>10.43</td>
<td>10.28</td>
<td>2.55</td>
<td>12.15</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**ECOG PS**

- Line of Therapy: 3 2 6 1 2 1 1 1 1 1

**Abbreviations:** AKT1i, AKT1 inhibitor; MAb, monoclonal antibody; ctDNA, circulating tumor DNA; IND, investigational new drug
86% Response Rate to ctDNA-Detected *ERBB2* (HER2) Amplification in Metastatic Breast Cancer

<table>
<thead>
<tr>
<th>Patient <em>ERBB2</em> Amplified in plasma</th>
<th>Tissue HER2+ Confirmed by IHC and/or FISH</th>
<th>Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.6</td>
<td>✓</td>
<td>paclitaxel/trastuzumab/pertuzumab</td>
<td>✓</td>
</tr>
<tr>
<td>3.9</td>
<td>✓</td>
<td>paclitaxel/trastuzumab/pertuzumab</td>
<td>✓</td>
</tr>
<tr>
<td>7.1</td>
<td>✓</td>
<td>paclitaxel/trastuzumab/pertuzumab</td>
<td>✓</td>
</tr>
<tr>
<td>2.3</td>
<td>✓</td>
<td>trastuzumab/pertuzumab</td>
<td>✓</td>
</tr>
<tr>
<td>2.7</td>
<td>✓</td>
<td>trastuzumab/emtansine/lapatinib</td>
<td>✓</td>
</tr>
<tr>
<td>8.6</td>
<td>✓</td>
<td>trastuzumab/emtansine/lapatinib</td>
<td>✓</td>
</tr>
<tr>
<td>2.7</td>
<td>✓</td>
<td>trastuzumab emtansine monotherapy</td>
<td>✘</td>
</tr>
<tr>
<td>Total</td>
<td>100% Concordant</td>
<td></td>
<td>86% Response Rate</td>
</tr>
</tbody>
</table>

Liang et al. 2015 *Breast Cancer Research & Treatment*
Case Three: MET Gene Amplification in NSCLC

Summary of Alterations & Associated Treatment Options
The percentage, or allele frequency, of altered circulating cell-free DNA (% cfDNA) in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turnover, size, heterogeneity, vascularization, disease progression, or treatment.

<table>
<thead>
<tr>
<th>Alteration</th>
<th>% cfDNA</th>
<th>cfDNA Amplification</th>
<th>FDA Approved in Indication</th>
<th>Available for Use in Other Indications</th>
<th>Clinical Drug Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>AMP</td>
<td>+++</td>
<td>None</td>
<td>Cabozantinib, Crizotinib</td>
<td>Trials Available</td>
</tr>
</tbody>
</table>

A

B

PRE 02-09-2015
POST 09-11-2015

Crizotinib

Courtesy Nir Peled MD, Sheba Medical Center, Tel Aviv University
MET Amplification: a Function of anti-EGFR Therapy in Metastatic Colorectal Cancer

![Bar chart showing MET amplification rates in different groups](chart.png)
MET Amplification: An Actionable Resistance Target in Metastatic Colorectal Cancer (ctDNA)
“Comprehensive” Means Fusions & Amps Too!

“plasma NGS is now moving into the clinical space and can make accurate and precise calls”

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PCR Assays</th>
<th>NGS Assays</th>
<th>Capture-Based Targeted NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele-Specific PCR</td>
<td>Emulsion PCR</td>
<td>Amplicon-Based Targeted NGS</td>
</tr>
<tr>
<td>Variants potentially detected</td>
<td>Known recurring mutations</td>
<td>Known recurring mutations</td>
<td>Any exonic mutations, copy number gains</td>
</tr>
<tr>
<td>Quantitation</td>
<td>Semiquantitative (against standard curve)</td>
<td>Absolute or relative quantitation, wide dynamic range</td>
<td>Quantitation of relative AF, but vulnerable to PCR amplification bias</td>
</tr>
<tr>
<td>Speed and complexity</td>
<td>Rapid, relatively easy to interpret</td>
<td>Rapid, relatively easy to interpret</td>
<td>Potentially rapid, less complex bioinformatics</td>
</tr>
<tr>
<td>Examples</td>
<td>Cobas (Roche); therascreen (Qiagen)</td>
<td>Droplet digital PCR (Biorad); BEAMing (Sysmex Inostics)</td>
<td>Tam-seq (Inivata)</td>
</tr>
</tbody>
</table>
Should the Hurdle for Clinical Utility for a Diagnostic be Higher Than for a Matched Therapy Drug?

“High ORR (e.g. statistically exceeding an ORR of 30%) is an appropriate end point for single-arm trials aiming to demonstrate breakthrough activity of a single-agent anticancer therapy”

<table>
<thead>
<tr>
<th>Percentage (95% CI)</th>
<th>ORR</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single-Agent Therapies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>70 (49-87)</td>
<td>86 (74-94)</td>
<td>68 (46-85)</td>
<td>88 (76-95)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>67 (45-84)</td>
<td>91 (81-97)</td>
<td>76 (53-92)</td>
<td>87 (75-94)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>50 (29-71)</td>
<td>91 (81-97)</td>
<td>71 (44-90)</td>
<td>81 (70-90)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>42 (22-63)</td>
<td>95 (85-99)</td>
<td>77 (46-95)</td>
<td>79 (68-88)</td>
<td></td>
</tr>
<tr>
<td><strong>30</strong></td>
<td>33 (16-55)</td>
<td>98 (91-100)</td>
<td>89 (52-100)</td>
<td>78 (66-87)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>33 (16-55)</td>
<td>98 (91-100)</td>
<td>89 (52-100)</td>
<td>78 (66-87)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>29 (13-51)</td>
<td>98 (91-100)</td>
<td>88 (47-100)</td>
<td>77 (65-86)</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>25 (10-47)</td>
<td>100 (94-100)</td>
<td>100 (54-100)</td>
<td>76 (65-85)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>13 (3-32)</td>
<td>100 (94-100)</td>
<td>100 (29-100)</td>
<td>73 (62-82)</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>13 (3-32)</td>
<td>100 (94-100)</td>
<td>100 (29-100)</td>
<td>73 (62-82)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>13 (3-32)</td>
<td>100 (94-100)</td>
<td>100 (29-100)</td>
<td>73 (62-82)</td>
<td></td>
</tr>
</tbody>
</table>
31% of Lung Adenocarcinoma is Targetable

NCCN Genomic Targets: EGFR, BRAF, MET, ERBB2 (HER2), ALK, ROS1, RET