Clinical applications of cell-free DNA (cfDNA) genotyping for cancer care

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Disclosures

• Consulting fees or honoraria from AstraZeneca, Ariad, Boehringer-Ingelheim, Clovis, Chugai, Genentech, Inivata, Sysmex

• Ongoing research collaborations with multiple pharma and biotech partners in this space
Case

- 49 yo M never-smoker p/w several weeks of cough, headache
  – Chest CT shows adenopathy, pulm nodules
Case

- 49 yo M never-smoker p/w several weeks of cough, headache
  - Chest CT shows adenopathy, pulm nodules
  - Brain MRI with 8mm cerebellar lesion, cannot rule out lepto
  - Supraclav biopsy shows NSCLC

- Presents to oncology 4 days post-biopsy
  - Path not yet finalized, genomics not started
Lung cancer genotyping

1984 - 2003

No known genotype

2004

No known genotype

2009

EGFR
KRAS
ALK PIK3CA BRAF HER2

2012

No known genotype

EGFR
KRAS
RET MET ROS1
ALK PIK3CA BRAF HER2
Case

• 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
  – Tumor genotyping pending

• Plasma genotyping of EGFR ordered
  – Seen on a Monday, blood drawn that day
  – Results reported on Wednesday
  – EGFR L858R detected at 34% AF
Case

- 49 yo M never-smoker p/w stage IV NSCLC metastatic to brain
- Erlotinib initiated, patient symptoms rapidly improve
Plasma ddPCR

- Droplet digital PCR is a highly sensitive, quantitative assay for detection of hotspot mutations (e.g. EGFR, KRAS, BRAF, PIK3CA)
  - 20,000 droplets generated each carrying mutant or wildtype DNA

Patient plasma → Extracted DNA → Emulsion of DNA + reagents, and amplification → Droplet flow cytometry → Quantification of allele prevalence
Plasma ddPCR

- In EGFR-mutant NSCLC, ddPCR can detect response and resistance

![Graphs showing concentration of mutation per 100 μL of plasma DNA over weeks on treatment for Patient 1 and Patient 2]

**Patient 1**

**Patient 2**

- EGFR exon 19 del
- EGFR T790M

Oxnard & Paweletz et al, CCR, 2014
Plasma ddPCR

- In EGFR-mutant NSCLC, ddPCR can detect response and resistance.

Patient 1

Patient 2

EGFR exon 19 del

EGFR T790M

Oxnard & Paweletz et al, CCR, 2014
Plasma ddPCR

- In EGFR-mutant NSCLC, ddPCR can detect response and resistance

**Patient 1**

**Patient 2**

---

**EGFR exon 19 del**

**EGFR T790M**

Oxnard & Paweletz et al, CCR, 2014
Plasma genotyping

• Several clinical applications to consider:
  1. Cancer genotyping at initial therapy
  2. Cancer genotyping at resistance
  3. Assessment of response / PD effect
  4. Cancer screening / diagnosis
Lung cancer genotyping

- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - Overall sensitivity of 64-82% for detection of known tumor genotype
  - Rate of detection increases with increased tumor burden
  - 3-day TAT

Sacher et al, JAMA Onc, 2016
Lung cancer genotyping

- We recently completed a prospective validation of plasma ddPCR in 180 patients with NSCLC
  - 100% specificity (0% FPR) for driver mutations
  - 63% specificity for T790M resistance mutation

*Sacher et al, JAMA Onc, 2016*
Lung cancer genotyping

• Now using our validated plasma ddPCR assay as a CLIA test at BWH
• DFCI has launched a clinical trial of plasma EGFR genotyping for rapid initiation of erlotinib (NCT02770014)

Advanced NSCLC in need of EGFR genotyping (TKI naïve) → Rapid plasma genotyping (2-3 day turnaround time) → Genotype detected in plasma → Phase II trial of treatment based on plasma genotype aiming to demonstrate very high RR → Proceed to tissue genotyping / biopsy and standard therapy off trial → Genotype not detected in plasma
Lung cancer genotyping

• Now using our validated plasma ddPCR assay as a CLIA test at BWH
• DFCI has launched a clinical trial of plasma EGFR genotyping for rapid initiation of erlotinib (NCT02770014)
• This is the setting in which the FDA approved the Cobas plasma assay as a screening test, with reflex to tumor analysis if negative
NGS of plasma cfDNA

Strengths of ddPCR genotyping

- Rapid
- Quantitative
- Inexpensive

Limitations of ddPCR genotyping

- Only tests for known genotypes
- Difficult to multiplex
- Cannot detect rearrangements

Paweletz et al, CCR, 2015
Using NGS of plasma, one may detect all targetable alterations in lung cancer rapidly and noninvasively.

Challenges of advanced genomics in plasma:
- Small quantities
- Mostly germline
- Fragmented

Paweletz et al, CCR, 2015
**NGS of plasma cfDNA**

- Ultra-high-efficiency cloning of cfDNA with tagged adaptors

- Methods that yield on-target rates >90% and generate “primer-indexed” reads

- Proprietary nucleic acids chemistry that neutralizes GC bias

- Synergies between chemistry and molecular biology reduce sequencing demands and turnaround times

Paweletz et al, CCR, 2015
NGS of plasma cfDNA

**Actionable genes**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Gene</th>
<th>Mutant allele</th>
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</thead>
<tbody>
<tr>
<td>A549</td>
<td>KRAS</td>
<td>G12S</td>
</tr>
<tr>
<td>H1666</td>
<td>BRAF</td>
<td>G466V</td>
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<td>ALK</td>
<td>EML4</td>
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**Cell line validation**

**Admix measurement**

<table>
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<tr>
<th>Sensitivity &amp; Specificity</th>
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<tr>
<td>100%/100% at ≥ 1.0%</td>
</tr>
<tr>
<td>90%/100% at ≥ 0.1%</td>
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</table>

**Paweletz et al, CCR, 2015**
### NGS of plasma cfDNA

#### Sample Genotype Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tissue Genotype</th>
<th>ddPCR</th>
<th>NGS</th>
<th>GE in NGS Library</th>
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<tr>
<td>105</td>
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![Graph showing allele frequency comparison between ddPCR and NGS](Image)

**Pearson Correlation 0.89, p < 0.00001**
NGS of plasma cfDNA

Paweletz et al, CCR, 2015
Resistance genotyping

- Osimertinib is approved in multiple countries for EGFR-mutant NSCLC with T790M+ resistance

Oxnard et al, JCO, 2016
Resistance genotyping

- Plasma from phase I trial sent for BEAMing
  - Similarly found that sensitivity was 70%-86%
  - Similarly found a high specificity (>95%) for driver EGFR mutations but only 69% specificity for T790M
Resistance genotyping

- Plasma from phase I trial sent for BEAMing
  - Despite the false positives, plasma T790M+ cases do well, like tumor T790M+
  - But plasma T790M- cases do better than expected

Oxnard et al, JCO, 2016

<table>
<thead>
<tr>
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<th>RR (95% CI)</th>
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<tr>
<td>T790M+ in plasma</td>
<td>63% (55, 70)</td>
</tr>
<tr>
<td>T790M- in plasma</td>
<td>46% (36, 56)</td>
</tr>
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Resistance genotyping

- Which is better, tumor vs plasma?
  - Tumor genotyping can clarify which plasma T790M-patients do better or worse on osimertinib

![Graph showing survival rates for different T790M statuses.](image)

Oxnard et al, JCO, 2016
Resistance genotyping

- Which is better, tumor vs plasma?
  - Tumor genotyping can clarify which plasma T790M- patients do better or worse on osimertinib
  - Tumor genotyping also clarifies which plasma T790M+ patients do better or worse on osimertinib

Oxnard et al, JCO, 2016
Resistance genotyping

- Plasma T790M is a compelling resistance biomarker but heterogeneity is a challenge
- Would be clinically valuable as a screening assay:

  A
  
  Acquired resistance to EGFR TKI
  
  All pts undergo biopsy, FDA-approved FFPE assay for T790M
  
  T790M+ → Third gen. EGFR TKI
  
  T790M− → Chemotherapy

  B
  
  Acquired resistance to EGFR TKI
  
  FDA-approved plasma assay for T790M and sensitizing mutations
  
  T790M+ → Skip biopsy, start third gen. EGFR TKI
  
  T790M− → Biopsy, FDA approved FFPE assay for T790M
  
  T790M+ → Third gen. EGFR TKI
  
  T790M− → Chemotherapy

Oxnard et al, JCO, 2016
Plasma response

- Levels of circulating mutations appear to track with disease status

- Various patterns of plasma response kinetics seen using ddPCR

Sacher et al, JAMA Onc, 2016
Plasma response

• Mok et al, CCR, 2015
  – Studied advanced EGFR-mutant NSCLC
  – Drop in plasma EGFR levels on therapy
Plasma response

- Mok et al, CCR, 2015
  - Studied advanced EGFR-mutant NSCLC
  - Drop in plasma EGFR levels on therapy
  - Worse PFS in those without plasma “CR”

Mok et al, CCR, 2015
Plasma response

- Have studied plasma ddPCR to complement dose finding in phase I trials:
  - Combination of CDK4/6 and MEK inhibition in KRAS-mutant cancers
  - Compared plasma and tumor response
Plasma response

• Available data supports the idea that levels of tumor DNA in plasma track with disease status
• Unclear if it will be practical / clinically valuable / cost effective to routinely monitor cfDNA in patients with advanced cancer
• There could be a role for monitoring assays which are very rapid and very inexpensive
Case

• 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions
  – Stage II adenocarcinoma resected 3 years prior, followed by adjuvant chemo
  – Surveillance CT shows new sclerotic lesions in bilateral ribs
  – Bone scan confirms abnormal uptake in ribs, spine, pelvis suspicious for a metastatic process
Case

- 74 yo M never-smoker with a prior history of resected NSCLC p/w bone lesions
Case

- 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence
- Plasma genotyping for EGFR & KRAS
  – Positive for EGFR L858R, 3.5% AF
- Does this confirm recurrence of his NSCLC?
Case

• 74 yo M never-smoker with h/o resected NSCLC p/w suspected recurrence

• Plasma genotyping for EGFR & KRAS
  – Positive for EGFR L858R, 3.5% AF

• Does this confirm recurrence of his NSCLC?
  – We confirmed the diagnosis with a bone biopsy before starting erlotinib
Cancer screening

• Bianchi et al, JAMA, 2015
  – Through a collaboration with Illumina, investigators queried NIPT results from 125,426 asymptomatic pregnant women
  – Aneuploidy identified in 3757 (3%)
  – In 8 cases, clinician voluntarily informed the lab that cancer was subsequently diagnosed, and the patient was consented for further study
  – All had abnormal NIPT; 7 had fetal karyotype performed and all were normal
Cancer screening

• Bianchi et al, JAMA, 2015
Cancer screening

• Bianchi et al, JAMA, 2015
  – Cancers detected in cfDNA were largely advanced or hematologic malignancies:
    • 4 cases of lymphoma
    • 1 case of leukemia
    • Stage IIIC colorectal cancer
    • Stage IIIB anal cancer
    • Stage IV neuro-endocrine carcinoma
Cancer screening

• Bianchi et al, JAMA, 2015
  – Cancers detected in cfDNA were largely advanced or hematologic malignancies:
  – Can plasma NGS be used to identify early-stage, curable cancers pre-diagnosis?
  – What will the false positive rate of such a screening approach be?
  – What if plasma NGS is abnormal but extensive imaging does not identify a cancer?
Conclusions

• cfDNA genotyping is a powerful tool for noninvasive genotyping
  – Can be rapid and convenient
  – Offers insight into the heterogeneity of resistance
  – Can allow noninvasive monitoring
  – However, not all tumor shed tumor DNA

• PCR and NGS assays likely have complementary roles going forward for clinical application and research
Acknowledgements

• Lowe Center for Thoracic Oncology
  – Pasi Jänne, Ryan Alden, Adrian Sacher, Emmy Hu
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  – Cloud Paweletz, Yanan Kuang, Nora Feeney
• Department of pathology, BWH
  – Lynette Sholl, Neal Lindeman
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  – AstraZeneca, Resolution Bioscience, Guardant, Illumina, Astellas
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  – Damon Runyon Cancer Research Foundation, DOD, Anna Fuller Fund, Stading-Younger Cancer Research Fund