Proffered Papers session 3

Stephen Finn MB PhD FRCPath
Cancer Molecular Diagnostics and Pathology.
University of Dublin, Trinity College and St. James’s Hospital
DISCLOSURE SLIDE

Commercial Research Support: Janssen and Astellas

Honoraria: Merck, Pfizer, Astra Zeneca, Roche, Boehringer Ingelheim, BMS
Abstracts to discuss

• 58O_PR - Clinical and demographic features that influence EGFR mutation detection in plasma from patients with aNSCLC: The ASSESS experience. Dr. Normanno et al

• 134O_PR - Plasma ctDNA analysis for detection of EGFR T790M mutation in patients with EGFR mutation-positive advanced non-small cell lung cancer (aNSCLC) Dr. Jenkins et al Dr. Yang presenting

• 135O_PR - Plasma genotyping for predicting benefit from osimertinib in patients with advanced NSCLC Dr. Oxnard et al
The liquid Biopsy: Easy access to the primary and resistance phenotype(s)? Or not?
Abstract 580: ASSESS

- Large (n= 1162) multicentre, non-interventional, non comparative diagnostic study evaluating the utility of ctDNA for EGFR mutation testing in patients with NSCLC in Europe and Japan
- Mandatory plasma and tumor at diagnosis (Stage IIIA/B or recurrent after surgery, chemo naïve)

- Objectives:
  - Primary: Concordance between tissue/cytology and blood (plasma) Testing
  - Secondary: EGFR Mutation practices

- Presentation of impact of clinical (disease)/patient characterization the ability to detect mutations in plasma
Primary Results:

cDNA assessment is feasible

Accuracy of Plasma v Tumour

- Concordance 89%
  - Sensitivity: 46% (Real World Setting)
    - Sensitivity in plasma increases with disease burden
  - Specificity: 97%

- Presumably a wide array of technologies used for local practice tissue assessment?
  - Technology for cDNA? Digital v Non-Digital
• Increased sensitivity associated with burden of disease (Intuitive and demonstrated previously)
• Also in never Smokers
• Intriguing findings in relation to clinical characteristics and/or patient demographics on the ability to detect mutations in plasma
  ❖ EGFR Mutation detection in plasma higher in patients aged <65 v >65!!!
  ❖ Overall higher frequency of EGFR mutations in patients >65 but nevertheless detection in plasma higher in younger patients
  ❖ A window on disease biology as a function of age
• Similar sensitivity and PPV comparing Exon 19 Del v L858R
• Reassurance of similar trends in IGNITE
## AURA Studies: Technologies

<table>
<thead>
<tr>
<th></th>
<th>PHASE 1</th>
<th>PHASE 2</th>
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<tbody>
<tr>
<td>Plasma Assay</td>
<td>BEAMing</td>
<td>Cobas</td>
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<tr>
<td>Comparator Assay</td>
<td>ddPCR or Cobas</td>
<td>miSEQ NGS</td>
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<tr>
<td>Abstract</td>
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Abstract 1340: AURA Phase II Studies Osimertinib

- Plasma ctDNA of T790M
- EGFR Roche Cobas® v2
- T790M: Concordance of Cobas® v2 with NGS Methodology (miSeq)
- Sensitivity, Specificity and Concordance
  - Tissue
    - Sensitivity: 88.3%
    - Specificity: 97.3%
    - Concordance: 91%
  - Plasma
    - Sensitivity: 91.5%
    - Specificity: 91.1%
    - Concordance: 91.3%
Cobas® Plasma v Cobas® Tissue

Cobas® plasma performance with Tissue as Reference or “Gold Standard”

<table>
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<tr>
<th></th>
<th>L858R</th>
<th>Del Exon 19</th>
<th>T790M</th>
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<tbody>
<tr>
<td>Sensitivity (PPA)</td>
<td>75.6%</td>
<td>85.1%</td>
<td>61.4%</td>
</tr>
<tr>
<td>Specificity (NPA)</td>
<td>98.1%</td>
<td>98%</td>
<td>78%</td>
</tr>
<tr>
<td>Concordance (OPA)</td>
<td>90.9%</td>
<td>90%</td>
<td>65.4%</td>
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- Objective Response Rates (ORR) similar between tissue and plasma
- Similar figures to those seen in Karlovich et al Clin Cancer Research 2016*
- T790M Plasma v Tissue: Reflects Tumour Biology and Molecular Heterogeneity in the setting of resistance
- Issue of Negative T790M in plasma arises

135O - Plasma genotyping for predicting benefit from Osimertinib in patients with advanced NSCLC AURA Trial

- Sensitivity of plasma genotyping 82-86\% for sensitizing mutations
  - But sensitivity is 70\% for T790M

- Reassurance of sensitivity for T790M association with sensitizing mutation
  - But T790M- sensitizing Mutation negative is uninformative

  When is a False Positive NOT a False Positive?

- “False Positive” Rate: 3-4\% for Sensitizing Mutations BUT 31\% for T790M
  - Spotlight shone on these false positives (n=18)
  - Orthogonal assessment
  - 14 confirmed, 4 remained discordant, all with very low allele frequency
    - Cut off issue
  - No false positives for T790M in 100 cases without any sensitizing mutation in EGFR

- High ORR in patients with tumour or plasma T790M
• Heterogeneity Issue: Provides strong support for concept of ctDNA representing a gestalt situation
  - Biopsy alone misses T790M

• False Negative T790M an issue
  - Reflex to tissue

• Concept of uninformative plasma test is very helpful:
  - T790M – Sensitizing +
  - T790M - and Sensitizing – (Status Unknown)

• Proposed Paradigm for use of plasma genotyping: See below
The Liquid Biopsy Centre Stage

Narrow Definition: A blood test that is associated with cytopathological assessment of CTCs

Broader Definition: ctDNA, ctRNA and Exosomes

Appeal of the “Liquid Biopsy”
Diagnosis, Prognosis, Theranostics, Prediction, Biology

Heterogeneity:
Liquid Biopsy as a Gestalt

Accessibility:
Blood, serum, plasma, urine, pleural fluid etc.

Temporal Heterogeneity of Disease:
Serial Access
# Tissue V Plasma

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<thead>
<tr>
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<th>Tissue Re-biopsy</th>
<th>Plasma ctDNA</th>
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<tbody>
<tr>
<td>Ease of Access/Patient Comfort</td>
<td>X</td>
<td>✔</td>
</tr>
<tr>
<td>Serial Access</td>
<td>X</td>
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<tr>
<td>Assessing potential Other Resistance Phenotypes</td>
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<td>X</td>
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<tr>
<td>Small Cell Transformation</td>
<td>✔</td>
<td>X</td>
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<tr>
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<td>Turn Around Time</td>
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Abstracts: Refine and add granularity to the ctDNA and T790M story

Summary:
• ctDNA feasible and practical at diagnosis and progression
• Especially useful when positive!!
• ctDNA is probably superior to biopsy alone as a screen for T790M if followed by a tissue test when T790M- and Sensitizing mutation-
• For T790M many False Positives are True Positives
• Use the sensitizing mutation as a control
• Beware the negative test
Testing Paradigm for acquired resistance to EGFR-TKI

1. **Plasma ctDNA with validated assay**
   - **T790M +**
     - Proceed to 3rd Generation Therapy
   - **T790M -**
     - Biopsy Validated test for T790M
       - **T790M Positive**
         - Third Generation Therapy
       - **T790M -**
         - Chemotherapy
## Tissue V Plasma

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**We need to use plasma and tissue together**

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The relative frequencies of the various mechanisms of acquired resistance.

- **MET amplification**: 3%
- **Small cell + MET**: 1%
- **Small cell**: 1%
- **Small cell + T790M**: 2%
- **MET + T790M**: 3%
- **HER2 + T790M**: 4%
- **Unknown**: 18%
- **T790M**: 60%
Unanswered Questions

• Technology
  - Non Digital: Cobas®, Therascreen™ ARMS
  - Digital: Droplet Digital PCR, BEAMing
  - NGS

• Focus:
  - T790M burden to monitor disease?
  - Allele Frequency
  - EGFR sensitizing and resistance mutations only?
  - How to integrate other resistance candidates: Small Cell, PIK3CA, Her2 Amp, IGF1R, Met Amp
  - Resistance to third Generation TKIs? Role of assessment for C797S
  - Assessing T790M C797S cis and trans alleles