Deep Sequencing of Circulating Tumor DNA for Cancer Detection and Monitoring

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Disclosure Information

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  – Founder/stock holder: CiberMed
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Potential Clinical Applications of ctDNA

- **Screening**
  - Non-invasive Genotyping

- **Tumor Burden**
  - Local tx response
  - Minimal Residual Disease
  - Surveillance
  - Systemic tx response
  - Resistance variants

- **Time**
  - Local tx (e.g. surgery, RT)
  - Systemic tx (e.g. chemo, TKI)
Clinical Utility of ctDNA Detection

- Clinical utility of ctDNA is largely still being established
- Utility has been documented for non-invasive genotyping
  - EGFR activating mutations in NSCLC (FASTACT-2 trial - Mok et al. *CCR* 2015)
- Other potential applications are in early stages of being explored
  - Monitoring treatment resistance mechanisms
  - Minimal residual disease
NGS-based ctDNA Detection
## Comparison of ctDNA Detection Limits in 10 mL Blood Draw

<table>
<thead>
<tr>
<th>ctDNA detection method</th>
<th>Detection limit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger sequencing</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>~10%</td>
</tr>
<tr>
<td><strong>Whole exome sequencing</strong></td>
<td>~5%</td>
</tr>
<tr>
<td>Whole genome sequencing</td>
<td>~1%</td>
</tr>
<tr>
<td><strong>WholeAmplicon NGS (e.g eTAm-Seq)</strong></td>
<td>~0.3%</td>
</tr>
<tr>
<td>Allele-specific PCR (e.g. Intplex)</td>
<td>~0.1-0.05%</td>
</tr>
<tr>
<td><strong>Barcoded Amplicon NGS (Safe-SeqS)</strong></td>
<td>~0.05-0.1%</td>
</tr>
<tr>
<td>Digital PCR (e.g. ddPCR, BEAMing, etc.)</td>
<td>~0.00025%</td>
</tr>
</tbody>
</table>

*50% efficiency, 90% probability of detection*
Tracking Multiple Mutations Increases Maximizes Sensitivity

10 cc blood → 5 cc plasma
~30 ng cfDNA
~5,000 hGEs*

*50% recovery rate assumed


M. Diehn / Stanford
Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq)

Population-level analysis

Patient-level analysis

Recurrent mutations

ACCTGTCG
ACCTGTCG
ACCTGACG
ACCTGACG

CAPP-Seq selector

Personalized biomarker for every patient
Sensitive and Specific Detection of Circulating Tumor DNA

- **Initial cohort:**
  - Pre-treatment plasma samples from patients with Stage I-IV NSCLC

- **% ctDNA:**
  - 0.019 – 3.2%

- **ctDNA concentrations:**
  - 1.9 – 226 pg/mL

Decreasing Sequencing Errors in Deep Sequencing-based cfDNA Analyses

Barcoding —
Polishing —

*cfDNA (n = 12)*

A>C  A>G  A>T  C>A  C>G  C>T  G>A  G>C  G>T  T>A  T>C  T>G

Error rate

0.03%
0%

Comparison of iDES-enhanced CAPP-Seq to Digital PCR

$R = 0.93$

$P < 0.0001$
Resistance Mechanisms in EGFR Mutant NSCLC
• Activating EGFR mutations occur in 15-50% of lung adenocarcinomas
• Sensitize tumors to EGFR tyrosine kinase inhibitors
  – First line: erlotinib, gefitinib, afatinib
• Resistance invariably develops
  – EGFR T790M is most frequent mechanism (~50-60%)
• “Third generation” EGFR TKIs target both activating and T790M mutations
  – Osimertinib, rociletinib, and others
Frequency of First-line EGFR TKI Resistance Mutations in Tumor Biopsies

~5-15% of patients with more than one mechanism
Heterogeneity of Resistance Mechanisms in Response to EGFR TKIs

• Hypotheses
  – First-line EGFR TKI treatment frequently leads to intra-patient heterogeneity in resistance mechanisms
  – Patients with multiple resistance mechanisms respond less well to third generation EGFR TKIs

• Approach
  – Perform CAPP-Seq on plasma from 43 patients who progressed on first-line EGFR TKIs and were subsequently treated with rociletinib
  – Analyze baseline and emergent resistance-associated somatic alterations
ctDNA Detection Summary

Chabon et al. *Nature Communications* 2016
Intra-patient heterogeneity of resistance mechanisms to first-line EGFR TKIs

- T790M Only: 54%
- T790M + SCNA: 34%
- T790M + SNV: 7%
- T790M + SCNA + SNV: 5%

46% with > 1 resistance mechanism after 1st line EGFR TKI
Rociletinib Resistance Mechanisms

- **Putative resistance mechanism definition**
  - Absent before treatment and emerged at progression
  - Increased in relative abundance from baseline to progression

- **Putative mechanism(s) identified in 65% of patients (72% of evaluable)**

- **Significant intra- and inter-patient heterogeneity**
  - 9 genes involved
  - 21% of patients develop multiple resistance mechanisms (*)

### Table

<table>
<thead>
<tr>
<th>SCNA</th>
<th>Pt ID</th>
<th>Percentage of Patients</th>
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<tbody>
<tr>
<td></td>
<td>CO4</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>CO6</td>
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<tr>
<td></td>
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<td>CO43</td>
<td>2%</td>
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<tr>
<td></td>
<td>CO46</td>
<td>2%</td>
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</tbody>
</table>

### Diagram

- Genes involved: MET, ERBB2, EGFR, PIK3CA, KRAS, CDKN2A, RB1, ALK, KIT, MET
- Symbols indicate percentage of patients:
  - *: 9%
  - **: 12%
  - ***: 26%
Emergence of EGFR C797S in a Single Patient

**Study** | **Compound** | **Evaluable Patients** | **C797S Present Study** | **C797S Prevalence**
---|---|---|---|---
Present Study | Rociletinib | 40 | 1 | 2% 
Piotrowska *et al.* Cancer Discovery 2015 | 9* | 0 | 2%
Novel EGFR L798I Resistance Mutation

Pre-Treatment: 10.3%
Progression: 20.4% 12.8%

T790M L798I

Day of Study
1 42 252

SLD (mm)

CO34

Mutant copies/mL

Day of Study
1 50 100 200 250 300

Ex19Del T790M L798I ND

ND
EGFR L798I Mutation Causes Rociletinib Resistance

Ba/F3 cells stably expressing EGFR constructs

Rociletinib dose [nM]

RLU mean ± SEM

- EGFR Ex19Del
- EGFR Ex19Del+T790M
- EGFR Ex19Del+T790M+L798I
**MET amplification mediates innate & acquired resistance**

### Innate Resistance

- **Day of Study**
  - -13
  - 40
  - 64

- **Normalized Copy Number**
  - CO7
  - MET
  - SLD
  - ND

- **Mutant copies/mL**
  - EGFR Ex19Del
  - EGFR T790M
  - PIK3CA E542K
  - PIK3CA E545K
  - ND

### Acquired Resistance

- **Day of Study**
  - -12
  - 77
  - 161

- **Normalized Copy Number**
  - CO10
  - MET
  - SLD
  - ND

- **Mutant copies/mL**
  - EGFR Ex19Del
  - EGFR T790M
  - EGFR S768I
  - TP53 Y205C
  - ND
Presence of Multiple Resistance Mechanisms predicts poor outcome

Expanded MET Cohort:
• 16 patients with T790M+/MET+
• 33 patients with T790M+/MET-
Summary

- ctDNA analysis has many potential clinical applications
- NGS-based methods such as CAPP-Seq can achieve similar sensitivity as ddPCR and facilitate broad molecular profiling and monitoring
- Simultaneous monitoring of multiple resistance mechanisms may allow personalized targeting of emerging resistance mutations
- Detection of MRD requires ultrasensitive assays and may allow personalized therapy
  - Randomized trial in Stage II colorectal cancer ongoing (J. Tie et al.)
- More prospective clinical studies required to validate preliminary findings and to establish clinical utility
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