Urinary ctDNA Platform for Diagnosis and Cancer Treatment Monitoring

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CHI Next Generation
Summit August 19, 2015
Circulating Tumor DNA (ctDNA)

Main Advantages of ctDNA

• Captures intra- and inter-tumor heterogeneity
• Systemic overview of cancer
• Frequent sampling options for monitoring applications
Technology Overview
Trovan gene Precision Cancer Monitoring (PCMSM) Platform

Extraction and Isolation of ctDNA

- 59%
- 92%
- ~0.7µg ctDNA/100 mL of urine

Mutant Allele Enrichment

- >100-fold enrichment

Platform Agnostic detection

- Integration with Commercially Available Platforms

Detection and Quantification

- Proprietary error rate detection and normalization.
- Single Molecule Detection

Ultra-Sensitive Detection and Quantitative Monitoring

ctDNA 2.0 ng/ul gDNA 1.4 ng/ul

ctDNA 4.9 ng/ul gDNA 0.04 ng/ul
Urine ctDNA Extraction

DNA extraction method enriches for short, fragmented DNA

- **Isolation without enrichment**
  - Smaller proportion of low MW DNA
  - Larger proportion of high MW DNA

- **Isolation with enrichment**
  - Higher proportion of small DNA
  - Less high MW DNA

- Automated magnetic bead-based urinary ctDNA isolation method being implemented in CLIA
  - Magnetic bead-based urinary ctDNA isolation kit in development
Urine Contains Approximately 10 Times the Amount of cfDNA as Compared to Plasma

<table>
<thead>
<tr>
<th></th>
<th>Total ng DNA per sample*</th>
<th>Total number of mutant KRAS copies per sample**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>urine</td>
<td>plasma</td>
</tr>
<tr>
<td>Median</td>
<td>989.5</td>
<td>61.6</td>
</tr>
<tr>
<td>10th Percentile</td>
<td>177.2</td>
<td>25.0</td>
</tr>
<tr>
<td>90th Percentile</td>
<td>4411.0</td>
<td>752.4</td>
</tr>
<tr>
<td>Number of samples</td>
<td>58</td>
<td>43</td>
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</table>

* Urine and plasma samples from Stage IV colorectal cancer patients at any time point on treatment
** Samples with detectable KRAS
Proprietary Mutant Allele Enrichment Method

- Platform independent
- Works with ddPCR or sequencing
- Sample type agnostic
- Works on tissue, blood and urine
- Non-allele specific technology, specific to hotspot of interest
NGS Provides Generic Assay Design for Detection and Quantitation

- Kinetically-driven PCR followed by NGS (MiSeq).
- The enrichment PCR assay utilizes a 31-46 bp footprint and selectively amplifies mutant DNA fragments.
- Following sequencing on MiSeq, a proprietary analysis algorithm allows accurate quantitation of input level of mutant DNA. Results are standardized by reporting number of copies detected per $10^5$ genome equivalents (GE).
- Enables unsupervised query for any mutation, eliminating the need for the creation of individual assays for specific mutations.

**Round 1**
- CS1 - TS1
- Gene target
- TS2 - CS2

**Round 2**
- PE1 – CS1
- 31bp product
- CS2 - BC - PE2

**Sequencing**
- TS = target sequence
- PE = paired end
- CS = common sequence
- BC = barcode
Examples of Standard Curves for Quantitation
EGFR and KRAS Assays

Unlike other NGS tests, PCM platforms counts absolute number of mutant DNA molecules in plasma or urine and not a ratio of mutant/wild-type DNA.

• Examples of master standard curve for EGFR Ex19 del and KRAS G12D
• 144 independent enrichments reactions/curve; spiked DNA input = 0-500 copies
• Accurate quantitation of absolute number of mutant DNA molecules in plasma or urine sample

ACCURATE DISEASE MONITORING WITH ANY TYPE OF THERAPIES
Establishing Clinical Utility
### Clinical Utility of ctDNA technology

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**Current Cancer Standard of Care**

- Monitoring for Progression and Emergence of Resistance
- Molecular Detection of Clinically Actionable Mutations
- Week 1: Assess Tumor Cell Kill by Therapy
- Beyond Week 1: Monitor Tumor Mutation Burden
- Monitoring for Minimal Residual Disease
- Tumor Recurrence
- Earlier Detection of Metastatic Disease
- Alternative to Tissue Biopsy
- Right Patients Treated with Right Therapy
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- Immediate Assessment of Drug Effect on Tumor
- Predict Best Response Weeks in Advance of Imaging
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- Anticipates and Enables Next Therapy to Target Tumor Resistance

Troagene PCM adds information that imaging does not currently provide at much earlier time points.
Prospective Blinded Study of BRAF V600E Mutation Detection in Cell-Free DNA of Patients with Systemic Histiocytic Disorders

### Background
- Erdheim Chester disease (ECD) and Langerhans Cell Histiocytosis (LCH) are histiocytic diseases
- Histiocytic diseases harbor somatic mutations (i.e., BRAF V600E)
- Patients with BRAF V600E mutation respond to BRAF inhibitors
- Histiocytic tissue technically challenging for tissue biopsy

### Objectives
- **Concordance:**
  - Tissue biopsy (gold standard) vs. urine BRAF ctDNA
  - Urine vs. plasma BRAF cfDNA
- **Longitudinal:**
  - Urine ctDNA BRAF status during treatment regimen

### Study Design
- Urine and Plasma from patients
- Blinded study: tissue biopsies (CLIA) correlated after ctDNA results
- Pre-specified cutpoint

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1Hyman et al., Cancer Discov. 2015 Jan;5(1):64-71
Histiocytic Disorders: ECD/LCH Standard of Care

**ECD/LCH ALL STAGES**

- Molecular diagnosis of BRAF mutation – patients are placed on targeted therapy
- Re-staging of cancer

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**TROVAGENE**

**STUDIES**

- Detection
  - BRAF V600E
- Monitoring Therapeutic Response
  - BRAF V600E

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**MSKCC, MDACC, NIH**

(30 patients)
Urinary ctDNA Outperforms Tissue Biopsies\(^1\)

- BRAF mutational status obtained for 100% of patients by urine and only 70% of patients by tissue biopsy
- 100% concordance between tissue, urine and plasma in treatment naive patients

\(^1\)Hyman et al., Cancer Discovery 2015 Jan;5(1):64-71
Effect of Therapy on BRAF V600E Allele Burden in ctDNA of Patients with Systemic Histiocytosis

Comparison of BRAFV600E allele burden in treatment-naïve urine samples and urinary samples acquired anytime during therapy

Effect of RAF inhibitors on ctDNA BRAFV600E mutant allele burden in 7 consecutive patients monitored weekly during treatment with RAF inhibitors

1Hyman et al., Cancer Discov. 2015 Jan;5(1):64-71
Correlation between Longitudinal ctDNA and Radiographic Response

*BRAFV600E burden in urine correlates with radiographic response.*

*BRAFV600E allele burden in urine changes dynamically with therapy.*

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1Hyman et al., Cancer Discov. 2015 Jan;5(1):64-71
Dynamic Monitoring Captures Early Progression and Response

Successful monitoring tumor dynamics independent of drug class used

Patient’s tumor progressed within 1 wk of Anakinra withdrawal
- Demonstration of need for higher frequency urine-based testing to monitor

Patient responded to Vemurafenib, BRAF inhibitor
- Demonstrates that continually monitoring patient enables optimal therapy over time

\(^1\)Hyman et al., Cancer Discov. 2015 Jan;5(1):64-71
# Clinical Utility Enabled by Trovagene Technology

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Trovagene PCM adds information that imaging does not currently provide at much earlier time points.
Replacing Tissue Biopsy in Metastatic NSCLC with ctDNA
Detecting EGFR and Monitoring ctDNA EGFR for Treatment Response and Early Acquisition of EGFR T790M Resistance Mutation

Metastatic NSCLC Patients at Diagnosis, EGFR Exons19 and 21 Status in Tissue

Matched Urine and Plasma Samples

Evaluate EGFR Concordance between Urine and Tissue

Evaluate EGFR Concordance between Plasma and Tissue

Urine Samples Serial Collections on Erlotinib

cDNA Monitoring for Response and Early Acquisition of EGFR T790M

Detection of T790M 3 months prior to detection of progression by imaging*

Metastatic NSCLC Patients, 1st Line anti-EGFR Therapy

Urine Samples Serial Collections on Second Line Therapy

EGFR T790M Concordance between Urine and Tissue

Detection of EGFR T790M Mutation at any time point
14/14 Patients 100%

*Preliminary Results
Urine Identifies More Patients with EGFR T790M Resistance Mutation than Tissue and Earlier than Tissue¹

<table>
<thead>
<tr>
<th></th>
<th>FFPE Tumor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T790M</td>
<td>Positive</td>
<td>Negative</td>
<td>Not yet tested</td>
<td>total</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Urine Positive</td>
<td>14</td>
<td>3</td>
<td>8</td>
<td></td>
<td>25</td>
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<tr>
<td><strong>Total</strong></td>
<td>14</td>
<td>3</td>
<td>18</td>
<td></td>
<td>35</td>
<td></td>
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**Urine % Sensitivity**

100% (14/14)²

¹Interim results

²Urine positive for T790M at any time point on treatment

¹Husain et al., ASCO 2015
Urinary Detection of EGFR T790M Resistance Mutation in Advance of Radiographic Progression

**Early T790M Detection (Days to Radiographic Progressive)**

<table>
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<tr>
<th>Patient</th>
<th>Days to Radiographic Progressive</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>111</td>
</tr>
<tr>
<td>Patient 3</td>
<td>52</td>
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<tr>
<td>Patient 10</td>
<td>56</td>
</tr>
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<td>Patient 20</td>
<td>29</td>
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1Husain et al., ELCC 2015
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<td>Imaging (every 6-8 weeks)</td>
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Current Cancer Standard of Care

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Monitoring Urinary ctDNA KRAS G12/13 Correlates with Treatment Response

30 Metastatic CRC (mCRC) patients undergoing treatment with chemotherapy or chemotherapy + surgery

Monitor Urine KRAS ctDNA for Response or Treatment Failure

Interim analysis in 4 patients:
Urine ctDNA KRAS dynamics correlates with treatment response

1Barzi et al, Cancer Markers and Liquid Biopsies 2015
Urinary ctDNA KRAS Detects Response in Advance of Imaging and CEA

Patient 1: Best Response – Partial Response

Patient 3: Best Response – Complete Response

Patient 6: Best Response – Partial Response

Patient 8: Best Response – Progressive Disease

1 Barzi et al, Cancer Markers and Liquid Biopsies 2015
Study Design

Retrospective prospective study of archived samples from 182 patients with unresectable, locally advanced or metastatic PC undergoing treatment with chemotherapy (Danish BIOPAC study)

640 Plasma Samples from 182 Patients

- ctDNA KRAS detection at baseline for association with OS
  - 176 of 182 patients with evaluable plasma at baseline
- ctDNA KRAS monitoring for response to chemotherapy and association with OS
  - 617 evaluable plasma samples
  - Timepoints: baseline, before cycle 2 of chemotherapy, every 2-3 months at time of CT scans

- Patient demographics: 84 females and 92 males, median age 68, range 45-89 years
- Locally advanced (n=50) or metastatic (n=132) pancreatic cancer
- Palliative treatment with gemcitabine or FOLFIRINOX
Significant Association Between Baseline KRAS Copies and Overall Survival

- Estimated Kaplan-Meier survival plots for males, age < 65, receiving gemcitabine, with baseline KRAS counts above and below 5.5 copies/100K GE.
- Similar results obtained for female and older patients.
Longitudinal Dynamics of KRAS ctDNA Burden after 2 Weeks of Chemotherapy Correlates with OS Better than the Baseline KRAS

- Plots of KRAS counts over time and hazard ratios relative to a patient with $\leq 5.5$ cps/100K GE KRAS at all time points. Estimated and actual patient survival is shown.
Acknowledgements

Filip Janku, MD

Omar Abdel-Wahab, MD
David Hyman, PhD MD
Eli Diamond, MD

UCSF Helen Diller Family Comprehensive Cancer Center
Eric Collisson, MD
Margaret Tempero, MD

UC San Diego Moores Cancer Center
Hatim Husain, MD
Razelle Kurzrock, MD

UNIVERSITY OF COPENHAGEN
Julia Johansen, MD
Inna Chen, MD

USC Norris Comprehensive Cancer Center
Afsaneh Barzi, MD
Heinz-Josef Lenz, MD

Genomac
Marik Minarik, PhD
Lucie Benešová PhD
Thank you!