Capturing tumor heterogeneity and clonal evolution using ctDNA analysis

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NCI Workshop on Circulating Tumor DNA assays in Clinical Cancer Research
September 29, 2016
Blood plasma has cell-free circulating DNA that carries somatic mutations:

- Point mutations
- Rearrangements
- Amplifications
- Deletions
- Epigenetic changes
**Tumor-guided analysis**

- Panel Exome WGS

- Targeted Analysis

**Applications**
- Monitoring tumor burden
- Treatment Response
- Residual disease
- Recurrence

**Challenges**
- Tumor tissue sequencing required
- Patient-specific assays needed
- Turnaround time

**Tumor-independent analysis**

- Hotspot

- Panel Exome WGS

**Applications**
- Noninvasive tumor genotyping
- Alternative to re-biopsy
- Clonal evolution
- Acquired therapeutic resistance

**Challenges**
- Variable mutation signal
- Sequencing noise
- Multiple testing
- Limited input material
How well does ctDNA capture tumor heterogeneity?

- Treatment-sensitive genotype
- Treatment-resistance genotype
How concordant is plasma with tumor analysis?

- Technical factors affecting concordance
- Comparison of plasma with multi-regional sampling
- Clinical implications/opportunities and unanswered questions
Technical factors affecting concordance: limited analyte

• In healthy controls, median of 1500-1900 GEs/mL plasma (5-7 ng/mL)
Technical factors affecting concordance: sampling inefficiency

- Ligation-based library preps: at best, 30% efficient at incorporating <10ng DNA input
- PCR based preps: efficiency is a function of amplicon size
Technical factors affecting plasma-tumor concordance

• Pre-analytical processing
  – Delayed processing of blood leads to peripheral cell lysis, lowering measurable mutation fraction

• Background noise in the assay
  – Need to distinguish signal from PCR and library prep noise
ctDNA and tumor biopsy concordance in ovarian cancer

n=38 patients

Summary of results

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Plasma samples analyzed</td>
<td>69</td>
</tr>
<tr>
<td>Mutations detected by digital PCR &gt;2%</td>
<td>47</td>
</tr>
<tr>
<td>Mutations detected by TAm-Seq &gt;2%</td>
<td>46</td>
</tr>
<tr>
<td>Missed by TAm-Seq due to sampling error</td>
<td>1</td>
</tr>
<tr>
<td>Sensitivity of TAm-Seq for mutations &gt;2%</td>
<td>97.5%</td>
</tr>
<tr>
<td>Positive predictive value for mutations &gt;2%</td>
<td>97.5%</td>
</tr>
</tbody>
</table>

Genes

- TP53
- PTEN
- EGFR
- PIK3CA
- KRAS
- BRAF

Tumor-independent: ~1% AF
Tumor-guided: ~0.1% AF

Forshev, Murtaza and Rosenfeld et al. Science Translational Medicine 2012
Intra-tumor heterogeneity in ovarian cancer
Exome-wide comparison between ctDNA and tumor biopsies

- n=6 patients (advanced breast, ovarian and lung cancers)
- 2-5 plasma sample each

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature 2013
Comparison of tumor and plasma exomes

- 151 single nucleotide variants
  - 62% in both sets
  - 21% in plasma only
  - 17% in mets only

- 64 SNVs shared with primary
  - 100% detectable in plasma
  - 89% detectable in both plasma and mets

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature 2013
Multi-regional and longitudinal sampling from a patient with breast cancer

*Autopsy samples

Days of follow-up

<table>
<thead>
<tr>
<th>Days</th>
<th>Tumor samples</th>
<th>Plasma</th>
<th>Tamoxifen</th>
<th>Trastuzumab</th>
<th>Lapatinib Capecitabine</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>67</td>
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<tr>
<td>577</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>9</td>
<td>1077</td>
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<tr>
<td>1193</td>
<td>3</td>
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</table>

- Breast DCIS
- Breast* (Breast metastasis)
- Chest wall*
- Lymph Node
- Liver*
- Ovary*
- Vertebral metastasis

*Autopsy samples

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
Exome and deep amplicon sequencing of tumor and plasma samples

**Exome Analysis**
- Germline: 26x
- Tumor: 17x-74x
- Plasma: 77x-140x
- 362 non-synonymous SNVs

**Amplicon Sequencing**
- 350 amplicons in multiplexed droplet PCR
- 310 successfully amplified
- Germline: 4304x
- Tumor: 965x-2777x
- Plasma: 288x-8248x

Murtaza, Dawson, Rosenfeld, Caldas et al. Nature Communications 2015 and Nature 2013
Multi-regional tumor heterogeneity

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
Is ctDNA concordant with tumor biopsies?

<table>
<thead>
<tr>
<th>PyClone Tumor Cluster</th>
<th>Stem Mutations</th>
<th>Metastatic clade mutations</th>
<th>Private, unclassified and plasma only mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Tumors</strong></td>
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<td></td>
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<tr>
<td><strong>Lymph node</strong></td>
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<tr>
<td><strong>Metastatic Tumors</strong></td>
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<tr>
<td><strong>Plasma Samples</strong></td>
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</tbody>
</table>

- **Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015**
Circulating levels of somatic mutations reflect clonal hierarchy

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
ctDNA tracks differential treatment response across metastatic deposits

Tumor-dependent clustering

Days of follow-up

Relative Plasma Abundance

P1.1  P3.1  M2.1
M3.1  M3.2  M3.3

Tamoxifen and Trastuzumab
Lapatinib and Capecitabine

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
ctDNA tracks differential treatment response across metastatic deposits

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
ctDNA can identify potential drivers of treatment resistance

Cell Cycle
Receptor tyrosine kinase ERBB4 mediates acquired resistance to ERBB2 inhibitors in breast cancer cells

Kaleigh Canfield, Jiaqi Li, Owen M. Wilkins, Meghan M. Morrison, Matthew Ung, Wendy Wells, Charlotte R. Williams, Karen T. Liby, Detlef Vullhorst, Andres Buonanno, Huizhong Hu, Rachel Schiff, Rebecca S. Cook & Manabu Kurokawa

Accepted author version posted online: 15 Jan 2015.

Murtaza, Dawson, Caldas and Rosenfeld et al. Nature Communications 2015
Heterogeneity for an actionable mutation

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**Graph:**
- **X-axis:** Days of follow-up (580 to 1077 days)
- **Y-axis:** Allele fraction of PIK3CA mutation (0 to 0.035)
- **Lines:**
  - Blue line: PIK3CA p.E542K allele fractions
  - Diamond markers: Not significantly detectable above background
  - Diamond markers: Significantly detectable above background

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**Table:**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutated Genes</th>
<th># Mut</th>
<th>#</th>
<th>Freq</th>
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</thead>
<tbody>
<tr>
<td>TP53</td>
<td>354</td>
<td>346</td>
<td>31.3%</td>
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</tr>
<tr>
<td>PIK3CA</td>
<td>378</td>
<td>340</td>
<td>30.8%</td>
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<tr>
<td>CDH1</td>
<td>135</td>
<td>133</td>
<td>12.0%</td>
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<tr>
<td>GATA3</td>
<td>127</td>
<td>119</td>
<td>10.8%</td>
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<tr>
<td>KMT2C</td>
<td>100</td>
<td>82</td>
<td>7.4%</td>
<td></td>
</tr>
</tbody>
</table>

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**Text:**
- Tamoxifen and Trastuzumab
- Lapatinib and Capecitabine

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**References:**
- Murtaza, Dawson, Rosenfeld, Caldas et al. Nature Communications 2015; TCGA Cell 2015
How do we interpret plasma-tumor concordance for any given mutation?

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
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<tr>
<td>+</td>
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<td>-</td>
<td>+</td>
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</table>
Summary and Unanswered Questions

• Quantitative levels of somatic mutations in ctDNA represent phylogeny and fraction of systemic tumor each mutation represents
  – Can we use relative levels of multiple mutations to prioritize actionable mutations or further stratify patients?

• Founder mutations are more readily detectable in plasma compared with sub-clonal mutations
  – Can we use multiple founder mutations to achieve greater sensitivity for detecting ctDNA in low disease burden states?

• Longitudinal ctDNA analysis captures clonal evolution in real-time, as it happens
  – Can we leverage this, beyond re-genotyping, to maneuver tumor evolution towards improved outcomes?
  – Can we use this to understand treatment scheduling (intermittent dosing or ordering when multiple options are available)?
Thanks and happy to take questions.

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Current and former lab members

Tania Contente-Cuomo  Nieves Perdigones  Pankti Shah  Havell Markus

Ahuva Odenheimer  Alex Nazareno  Jun Zhao  Sridhar Srivatsa

Collaborators

Cambridge:
Nitzan Rosenfeld  Carlos Caldas  Dana Tsui  Sarah-Jane Dawson  Tim Forshew  Davina Gale  Francesco Marass  Oscar Rueda  H Raza Ali  Suet-Feung Chin

Patients