Liquid Biopsy in Clinical Practice

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DISCLOSURES

I do not have anything to disclose.
The issues with tissue biopsies

- Risk
- Spatial heterogeneity
- Temporal heterogeneity
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The issues with tissue biopsies

- Risk
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  - Serial assessment of ctDNA 220 pts with mRCC using commercial 70-gene platform
  - ctDNA profile varied across line of therapy

The issues with tissue biopsies

- Risk
- Spatial heterogeneity
- Temporal heterogeneity

- Serial analysis of ctDNA from 24 pts with mCRC who responded to anti-EGFR therapy but subsequently relapsed, 23 out of 24 patients developed one or more mutations in genes involved in the MAPK signaling pathway

What is “Liquid biopsy”

- Non-invasive (or minimally invasive) technique for assessing tumors
- Blood, ascite fluid, urine, sputum, amniotic fluid, saliva…
  - Circulating tumor cells
  - Circulating cell-free DNA
  - Exosomes
- Potential applications:
- Potential pitfalls
Where does cfDNA come from?

- Patients with cancer have higher levels of cfDNA than patients without cancer.
- Accumulation of cfDNA in plasma occurs as macrophages are unable to keep with clearance from associated apoptosis and necrosis, leading to release of cfDNA into circulation.
- Most cfDNA fragments are 180-200bp, suggesting majority arises from tumor cell apoptosis.
Nucleosomes (N) and Transcription Factors (TF) Protect Genes from cfDNA Degradation$^{1,2}$

DNA released from apoptotic cells is degraded by DNAses unless protected by N or TF

$^1$ Shendure et al., Cell, 2016.
$^2$ Ulz et al., Nature Genetics, 2016
ctDNA Platforms

and more...
AF = Allele frequency / Allele fraction

- ctDNA is usually quantified as the allele frequency (as known as fraction of mutant alleles)
  \[
  \text{Mutated alleles} \div \text{Wild-type alleles + mutated alleles}
  \]
- Ratio:
- Advantage: using the wild-type copies as an intra-patient control for the exclusion of poor sample quality or poor sample handling during ctDNA extraction.
- Disadvantage: Other factors may significantly affect the number of wild-type copies, which in turn may bias the AF variable:
  - Infection, inflammation, trauma, even the treatment itself..
  - Problem for monitoring / comparison across timepoints…
ctDNA is an attractive biomarker since:

Mainly because…

• Mutations are highly specific to tumor cells
• Mutations are involved in pathogenesis
• Some mutations are actionable
• Half-life: ~ 30min
CHALLENGE:

Move this from the “Wow!” phase to clinical application.
ctDNA – clinical applications

- Screening
- Localized treatment
- Minimal residual disease
- Surveillance
- Recurrence
- Treatment response
- Systemic treatment for metastatic disease
- Non-invasive genotyping
- Resistance variants
MINIMAL RESIDUAL DISEASE
STAGE II CRC
Minimal Residual Disease

The Potential of Circulating Tumor DNA (ctDNA) to Reshape The Design of Clinical Trials Testing Adjuvant Therapy in Patients with Early Stage Cancers

Jeanne Tie*, Yuxuan Wang, Cristian Tomasetti, Lu Li, Simeon Springer, Isaac Kinde, Rachel Wong, Suzanne Kosmider, Ben Tran, Jayesh Desai, Theresa Hayes, Andrew Haydon, Desmond Yip, Michael Christie, Robert L. Strausberg, Luis Diaz Jr, Nickolas Papadopoulos, Kenneth Kinzler, Bert Vogelstein and Peter Gibbs

*Walter and Eliza Hall Institute of Medical Research, Australia
Minimal Residual Disease

250 Subjects with Stage II Colon Cancer
July 2011 – Sept 2014

Excluded (n = 19): ineligible (n = 8), withdrew consent (n = 4), insufficient blood (n = 7)

Blood Collection
- 4 – 10 weeks post-op (n = 231)
- 3-monthly follow-up blood collection (n = 167)

Standard Follow-Up
- +/- Adjuvant Chemotherapy*
- Surveillance
  - 3-monthly review + CEA
  - 6-monthly CT for 2 years

*Tie et al. ASCO 2016

*Adjuvant chemotherapy at clinician discretion [Chemo = 52 (23%); No chemo = 178 (77%)]

MD Anderson | ctDNA in Oncology
Minimal Residual Disease

Recurrence-Free Survival
(Patients *not* treated with chemotherapy)

HR: 18 (95% CI: 7.9–40), *p* < 0.001
Minimal Residual Disease

Recurrence-Free Survival

**Clinical Low-Risk**
(dMMR or pMMR + no poor prognostic features)

- Post-op ctDNA Negative (N = 122)
- HR: 28 (95% CI: 8.3–93)
- $p < 0.001$

- Post-op ctDNA Positive (N = 7)

**Clinical High-Risk**
(pMMR + at least one poor prognostic feature)

- Post-op ctDNA Negative (N = 42)
- HR: 7.5 (95% CI: 2.6–22)
- $p < 0.001$

- Post-op ctDNA Positive (N = 7)

Tie et al. ASCO 2016
### Minimal Residual Disease

**RFS: Univariate and Multivariate Analyses**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th></th>
<th>Multivariate*</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P</td>
<td>HR</td>
</tr>
<tr>
<td>T stage, T3 vs T4</td>
<td>4.0</td>
<td>0.002</td>
<td>8.1</td>
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<tr>
<td>Lymph node yield, ≥12 vs &lt;12</td>
<td>3.1</td>
<td>0.009</td>
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<tr>
<td>Lymphovascular invasion, no vs yes</td>
<td>2.4</td>
<td>0.03</td>
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<tr>
<td>MMR status, deficient vs proficient</td>
<td>3.6</td>
<td>0.08</td>
<td>--</td>
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<tr>
<td>Clinical Risk Group, low vs high</td>
<td>3.2</td>
<td>0.002</td>
<td>--</td>
</tr>
<tr>
<td>Post-op ctDNA status, negative vs positive</td>
<td>18</td>
<td>&lt;0.001</td>
<td>28</td>
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</table>
MONITORING TREATMENT
PREDICTING RESISTANCE
Monitoring Treatment

Dynamic changes in ctDNA levels by treatment

Monitoring Treatment

Retrospective cohort of patients receiving Regorafenib or TAS102

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A

<table>
<thead>
<tr>
<th>KRAS-mut Allele Frequency</th>
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<tbody>
<tr>
<td>0 1 2 3 4 5 6 7</td>
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B

<table>
<thead>
<tr>
<th>Treatment start</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/16 5/21/16 6/10/16 6/30/16</td>
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C

<table>
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<tr>
<th>Restaging</th>
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<td>5/16 5/21/16 6/10/16 6/30/16</td>
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PI: Allan Pereira
RECURRENTE AFTER METASTASECTOMY
Recurrence after liver resection

58 CRC patients undergoing curative intent hepatic metastectomies

Curatively Resected Cohort

N = 53

Detected Preoperative Mutations

N = 39

Detected Postoperative Mutations

N = 17

No detected Postoperative Mutations

N = 22

No Detected Preoperative Mutations

N = 14*

Detected Postoperative Mutations

N = 2

No detected Postoperative Mutations

N = 12

R2 resection or residual disease remaining (preop and postop mutations detected in 4)

N = 5

*one preoperative sample failed processing
Recurrence after liver resection

• Median time from preoperative blood collection to surgery was 4.5 days
• Median time from surgery to postoperative blood collection was 14 days.

Recurrence Free Survival

Overall Survival

• Median RFS was 7.6 months for ctDNA detected and 15.8 months for non-detected ctDNA, p=0.004
• 2-year RFS was 9% for ctDNA detected and 43% for non-detected ctDNA

P-value 0.004
HR 2.6 (95%CI 1.2-5.7)

P-value 0.06
HR 3.5 (95%CI 0.8-15.7)

Overman et al. 2017 ASCO annual meeting
Guiding decisions in referring patients with mCRC to matched biomarker-related clinical trials.
Matched biomarker-related clinical trials

A – All patients (N=128)

- Mutation/amplification present 78%
- NO alteration present 22%

B – Any genomic alteration present (N=100)

- Potentially actionable mutation 38%
- Potentially actionable 50%
- Both mutations and amplifications actionable 6%
- Amplifications actionable 6%

C – Potentially actionable genomic alteration (N=50)

- Clinical Trial Identified 60%
- No Trial Identified 28%
- Ongoing search 12%
Potential pitfalls
Predictors of Negative ctDNA

Multivariate Logistic Regression

- **OR > 1**
  - Favors positive cfDNA

- **OR < 1**
  - Favors negative cfDNA

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% C.I. for EXP(B)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age (continuous)</td>
<td>0.94</td>
<td>0.91 - 0.98</td>
<td>0.004</td>
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<tr>
<td>Liver Metastasis</td>
<td></td>
<td></td>
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<tr>
<td>- Absent</td>
<td>0.19</td>
<td>0.08 - 0.45</td>
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<tr>
<td>- Present</td>
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<td>(Reference)</td>
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<tr>
<td>Lymph nodes</td>
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<tr>
<td>- Absent</td>
<td>0.28</td>
<td>0.12 - 0.70</td>
<td>0.006</td>
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<tr>
<td>- Present</td>
<td></td>
<td>(Reference)</td>
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<tr>
<td>Moment Plasma Ordered</td>
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<tr>
<td>- During treatment</td>
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<td>(Reference)</td>
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<tr>
<td>- After Progression</td>
<td>10.95</td>
<td>4.23 - 28.33</td>
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<td>TP53 (tissue)</td>
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<tr>
<td>- WT</td>
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<tr>
<td>- Mutated</td>
<td>3.19</td>
<td>1.32 - 7.72</td>
<td>0.01</td>
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</table>
Thank You!
Contact

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allan.pereira.onco@gmail.com