“Liquid Biopsies” in gastrointestinal malignancies: Are we ready?
Disclosures

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Applications of Liquid Biopsies

- Resistance
- Treatment Response
- MRD
What is a “Liquid Biopsy”?

Liquid-Biopsy Sources

- Cerebrospinal fluid
  - Tumors of the central nervous system
- Saliva
  - Head and neck tumors
- Pleural fluid
  - Thoracic cancers
  - Metastatic cancers
- Peripheral blood
  - Circulating tumor cells
  - Exosomes
  - cfDNA
- Ascites
  - Metastatic cancers
- Stool
  - Gastrointestinal tract cancers
- Urine
  - Urinary tract cancers
  - cfDNA filtered from blood

Corcoran and Chabner, NEJM 2018
Different forms of liquid biopsy

Corcoran and Chabner, NEJM 2018
• Cell-free DNA (cfDNA) is shed into the bloodstream by cells (normal or cancer) throughout the body

• Thus, only a fraction of cfDNA may be ctDNA (tumor-derived)
Methods for ctDNA analysis

Sensitivity

~1%
Whole exome/whole genome sequencing

~0.1%
Targeted sequencing

~0.01%
Mutation-specific analysis (ddPCR, BEAMing)

Breadth of analysis
Resistance
Secondary (acquired) Resistance

Pre-treatment

+ Vemurafenib 15 weeks

A

B

Wagle et al, JCO 2011
Overcoming acquired resistance to therapy

- Initial targeted therapy
  - Tumor Biopsy
  - Molecular profiling
  - Identify targetable alteration

- Target Alteration

- Target Alteration + Resistance Alteration

- Therapy to overcome resistance
  - Re-Biopsy
  - Molecular profiling
  - Identify resistance mechanism
Tumor heterogeneity and acquired resistance

- Interlesional Heterogeneity between distinct metastatic lesions
- Intralesional Heterogeneity within a single metastatic lesion

- A single needle biopsy may vastly underrepresent molecular heterogeneity
- Liquid biopsy may detect alterations in ctDNA shed by tumor cells throughout the body

Adapted from Bardelli, ASCO 2013; Misale, Cancer Discovery 2014
Are single tumor biopsies sufficient to guide therapy?

(Ryan Corcoran in collaboration with Larry Blaszkowsky, Alberto Bardelli)
Russo et al, Cancer Discovery 2016
### Tumor heterogeneity complicates acquired resistance

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<th>EGFR ECD</th>
<th>Amplification</th>
<th>KRAS</th>
<th>NRAS</th>
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*Strickler et al, Cancer Discovery 2018*
Systematic liquid biopsy collection during targeted therapy

0 1 2 4 6

Disease Progression

Months from treatment start

Blood for ctDNA (ddPCR) X X X X X X
Blood for ctDNA (NGS) X X
Tumor biopsy (NGS) X X

• Integrated, disease center-wide protocol for all targeted therapy patients

• One page consent “brochure” given to patients at first clinic visit

• Fully staffed by CRCs/technicians

• Activated by single page/email from MD to CRC at time of treatment start
Liquid biopsy to identify heterogeneous resistance mechanisms

In 23 patients with matched tumor biopsies, ctDNA identified additional resistance mechanisms in 78% of cases.
Comparison of multiple tumor biopsies versus liquid biopsies in a BRAF-mutant colorectal cancer patient
Serial liquid biopsy and autopsy in a patient with a FGFR-2 fusion positive gastric cancer
Real-time adaptation of therapy guided by ctDNA

Treatment 1

Treatment 2

Treatment 3

Variant Allele Fraction

Target Alteration

Resistance Alteration 1

Resistance Alteration 2
Real-time adaptation of therapy guided by ctDNA

“Re-challenge” with anti-EGFR therapy in CRC

• 54M w metastatic CRC. 1st line FOLFOX bevacizumab for 15 months.
• Molecular testing revealed RAS wild type
• Received irinotecan + cetuximab for 16 months

Somatic Alteration Burden 49.6%

TP53 R282W 49.6%
KRAS amp 2+
HER2 amp 1+
• Received local ablation, followed by 3 months of Trifluridine/tipiracil
Case: “Re-challenge” with anti-EGFR therapy in CRC

- Received irinotecan + panitumumab. Response lasting ~6 months.
Conclusions

• Molecular profiling through ctDNA can be used to guide treatment decisions, particularly when inadequate tissue is available

• Diverse mechanisms can promote resistance in different patients **AND** in the **same** patient

• Single-lesion biopsies may not capture heterogeneity of resistance, missing alterations that might drive treatment failure

• Integrating liquid biopsy into clinical-decision making may be key to developing strategies to overcome heterogeneity of resistance

• Liquid biopsy may offer the ability to monitor emergence of resistance mechanisms in real-time and adjust therapy accordingly
Treatment Response
Serial ctDNA to predict treatment response

Systemic Therapy Treatment

Baseline 2 Weeks 4 Weeks 8 Weeks 16 Weeks Progression

Every 8 weeks until progression

Treatment Start
Change in ctDNA and Tumor Markers at 4 weeks
Progression-Free Survival by % Change in ctDNA

Log-rank: p<0.0001
Hazard ratio: 3.29

ctDNA decreased by >30% mPFS = 175 days
ctDNA decreased by <30% mPFS = 59.5 days

Log-rank: p<0.0001
Hazard ratio: 5.48

ctDNA decreased by >30% mPFS = 226 days
ctDNA decreased by <30% mPFS = 62 days
Change in ctDNA and tumor markers over time

- % Change in ctDNA (2 weeks)
  - PR: p=0.99, p=0.83
  - SD: p=0.47
  - PD: p=0.32

- % Change in ctDNA (8 weeks)
  - PR: p=0.0090
  - SD: p=0.0000
  - PD: p<0.0001

Response categories: PR (Partial Response), SD (Stable Disease), PD (Progressive Disease), CB (Combined Response: PR+SD)
Longitudinal ctDNA changes during the first 100 days of therapy
MRD
Residual disease: The Problem

Stage III CRC:
All patients get adjuvant chemo
>50% cured by surgery alone

Stage II CRC:
SOC is NO adjuvant chemo
10-15% of patients recur

We have no way to determine who is cured and who will recur
Strategy for sensitive and specific detection of residual disease

- Personalized mutation-specific assay for detection of ctDNA
- Sequencing panel to identify one or more mutations
ctDNA detection requires high sensitivity and specificity

- **SENSITIVITY**: Trace amounts of ctDNA present in a background of normal cfDNA (~0.01%)

- **SPECIFICITY**: Need to have high confidence in detection methods to base treatment decisions on a positive result
Prediction of relapse in stage II CRC

Post-operative ctDNA

- Postoperative ctDNA-negative (n = 164)
- Postoperative ctDNA-positive (n = 14)

HR, 18 (95% CI, 7.9–40)

Clinical Risk Factors

- Clinical low risk (n = 129)
- Clinical high risk (n = 49)

HR, 3.3 (95% CI, 1.6–7.0)

Post-operative CEA

- CEA normal
- CEA elevated

HR = 2 (95% CI: 0.3 - 10)
P = 0.527

Tie et al, STM 2016
Prognostic but can it be a predictive biomarker???

Reinert et al
JAMA Onc 2019

9 months lead time to radiographic recurrence
Efficacy of adjuvant chemotherapy

• ctDNA detectable in 20 of 96 (21%) post surgery
  • inferior recurrence-free survival
  • 47% vs 76% despite adjuvant

• ctDNA detectable in 15 of 88 (17%) post chemo
  • 3-year RFI
  • 30% when ctDNA was detectable after chemotherapy and 77% when ctDNA was undetectable
Serial tracking improves sensitivity

47% detectable with 1 post op draw

82% detectable with serial draws

35% improvement
Integrated genomic and epigenomic plasma for recurrence prediction

Tumor-specific methylation patterns as a potential biomarker
Integrated assay has high PPV for CRC recurrence

Positive predictive value (PPV) = 100%,
Negative predictive value (NPV) = 76%,
Hazard ratio for recurrence = 9.22 (p < 0.0001)

ctDNA Detection Post-Completion of Standard of Care Therapy (N = 69 patients)

<table>
<thead>
<tr>
<th>Assay Performance by Analysis</th>
<th>Genomic (N)</th>
<th>Integrated Genomic and Epigenomic (N)</th>
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<tbody>
<tr>
<td>PPV (N of patients with ctDNA detected who recurred)</td>
<td>100% (11 / 11)</td>
<td>100% (14 / 14)</td>
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<tr>
<td>NPV (N of patients with ctDNA not detected who were recurrence free)</td>
<td>72% (42 / 58)</td>
<td>76% (42 / 55)</td>
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<td>Sensitivity for recurrence within one year of surgery</td>
<td>56% (9 / 16)</td>
<td>69% (11 / 16)</td>
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<tr>
<td>Specificity for recurrence within one year of surgery</td>
<td>96% (51 / 53)</td>
<td>94% (50 / 53)</td>
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Patients with persistent ctDNA were significantly more likely to experience disease recurrence in a shorter time period.

<table>
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<th>Recurred</th>
<th>Recurrence Free</th>
<th>Median Time to Recurrence (days)</th>
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<tr>
<td>Persistent ctDNA</td>
<td>11</td>
<td>0</td>
<td>182</td>
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<tr>
<td>Cleared ctDNA</td>
<td>3</td>
<td>3</td>
<td>333</td>
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<td>Negative ctDNA</td>
<td>7</td>
<td>19</td>
<td>NR* (median follow-up: 580 days)</td>
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Detecting residual disease with circulating tumor DNA

Observation only

Additional therapy
Potential to transform adjuvant therapy

Residual disease, tumor recurrence  Cured with surgery alone  Cured with adjuvant therapy
SU2C CRC Dream Team: Early treatment of occult metastatic disease following standard adjuvant therapy in stage III CRC

- Surgery
- SOC Adjuvant
- cfDNA assessment for residual disease
  - Positive
    - All other patients: FOLFIRI x 6 months
    - Exploratory cohorts
      - MSI patients: Nivolumab
      - BRAF V600 patients: Enco/Bini/Cmab x 6 months
  - Negative
    - SOC observation
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*First and foremost the patients and their families

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