Liquid Biopsies and Molecular Monitoring of Genitourinary Malignancies

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Disclosures

• Employee of Foundation Medicine and Equity owner in Roche Holdings
• Consultant and Equity owner of Tango Therapeutics
• Consultant and equity owner of Celsius Therapeutics
Blood Provides a Rich Source of Tumor-derived Material

Circulating tumor cells (CTCs) are shed into circulation from primary tumor and metastases\textsuperscript{3,4}

Healthy and tumor tissue release cell-free DNA and RNA into circulation through apoptosis, necrosis and lysis of circulating cells\textsuperscript{3,5,6}

Membrane-encapsulated extracellular vesicles (EVs) are released from healthy and tumor cells\textsuperscript{3}

Tumor-educated platelets (TEPs) may contain tumor-derived RNA and alternatively spliced transcripts\textsuperscript{3}

Tissue and Liquid Biopsies

Solid vs liquid biopsy

**Solid biopsy**
- Considered the ‘gold standard’ for cancer diagnosis and allows both morphological and molecular assessment
- Involves a relatively invasive procedure
- May not be feasible for some tumors, especially when not amenable or when highly necrotic
- May not provide sufficient sample for all necessary pathological workup
- Requires more surgical infrastructure and has longer turn-around time than liquid biopsy
- Is not suitable for longitudinal monitoring
- Single site biopsy may not represent tumor heterogeneity

**Liquid biopsy**
- Not yet comparable to solid biopsy with respect to evidence for clinical utility and applicability in initial cancer diagnosis and management
- Less invasive than solid biopsy
- May be used when tissue biopsies cannot be performed due to inaccessibility
- Provides an option when tissue samples are limited or exhausted
- Requires less surgical infrastructure and has shorter turn-around time than tissue biopsy
- Is suitable for repeat sampling during longitudinal monitoring
- Can capture the genomic heterogeneity of all cancerous lesions

Overview of the Liquid Biopsy (1)

- Circulating Tumor Cell assays
  - Launched in 2003
  - Relatively slow adoption
  - Utilized in diseases with high tumor cell shedding rates: prostate cancer
  - Difficult to perform molecular tests on captured cells

- ctDNA (circulating tumor DNA extracted from blood)
  - Popularly known as “The Liquid Biopsy”
    - NGS-based for mutation detection and characterization
    - Non-NGS based for “molecular monitoring”
    - Also in widespread development for early cancer detection

- Other “liquid” samples used for Molecular Studies
  - Bone marrow for hematologic malignancies
  - Urine for bladder, renal and prostate cancers
    - Monitoring response to intra-vesicle BCG and other local treatments
  - CSF for brain and spinal cord tumors
The Liquid Biopsy (2)

- Detection of ctDNA uses NGS techniques
- Two main types of assays:
  - Hybrid-capture based
  - Non-hybrid capture based
- Three major commercial Liquid Biopsy assays in the USA
  - Guardant 360 (1 companion diagnostic FDA-approved indications)
  - Foundation One Liquid CDx (24 companion diagnostic FDA-approved indications)
  - Tempus
- Can serve as a surrogate indicator of overall “disease burden”
  - Too expensive to be used as a frequently ordered monitoring test
  - “Tumor Fraction” emerging as a prognosis guide
- Primary role is to identify genomic alterations that can indicate potential benefit of targeted therapies
- Can provide information on biomarkers associated with immune checkpoint inhibitor response
  - bTMB
  - MSI
  - Others
• Different primary tumor types have different frequencies of yielding informative liquid biopsy results
  • Some tumors shed DNA from intact and apoptotic cells more easily than others
    • Breast, prostate and lung cancers more readily shed ctDNA into peripheral blood
    • Pancreas, ovary less readily shed ct DNA into peripheral blood
    • CNS tumors (GBM) rarely if ever yield informative ctDNA in peripheral blood
  • In many settings, tumor stage is more predictive of successful liquid biopsies than tumor type

• The use of other biomarkers to “predict” obtaining an informative liquid biopsy is emerging:
  • PSA of 5 ng/dL as a “requirement” to order a liquid biopsy on a prostate cancer patient
  • Other serum ELISA-based biomarkers in other tumor types guiding liquid biopsy selection: CEA, CA 19-9, others

• Clonal hematopoiesis genes (“CHiP”) must be differentiated in liquid biopsies

• A non-informative liquid biopsy is costly and loses time for the patient

• When deciding between a liquid biopsy and a metastatic site tissue biopsy for sequencing:

  “It is not tissue first. It is not liquid first. It is the patient first”
Tumor Heterogeneity: ctDNA can Capture Multiple Mechanisms of Acquired Resistance

Multiple solid tumor biopsies show diverging resistance mechanisms in different metastases in a patient with advanced BRAF V600E CRC.

Liquid biopsy captured all 4 resistance mechanisms:

- **Brain lesion**
  - BRAF V600E, AF 54.7%
  - EGFR amp
  - KRAS G12S not detected
  - NRAS Q61R not detected

- **Liver biopsy 1**
  - BRAF V600E, AF 36.4%
  - EGFR amp, not detected
  - KRAS G12S, AF 6.4%
  - NRAS Q61R, AF 3.1%

- **Liver biopsy 2**
  - BRAF V600E, AF 61.6%
  - EGFR amp, not detected
  - KRAS G12S, AF 22.4%
  - NRAS Q61R, not detected

- **Subcutaneous lesion**
  - BRAF V600E, AF 45.4%
  - EGFR amp, not detected
  - KRAS G12S, AF 0.2%
  - NRAS Q61R, not detected
ctDNA Is Detectable but Variable Across Tumor Types

Tumor fraction estimation based on aneuploidy and variant information

Percentage of liquid biopsies

Tissue of origin

CRC = colorectal cancer; NSCLC = non-small cell lung cancer.
## ctDNA Genomics Across Multiple Tumor Types

### All solid tumors: NTRK1/2/3, MSI-H/dMMR, TMB-H*

<table>
<thead>
<tr>
<th>Breast¹</th>
<th>NSCLC²</th>
<th>Prostate³</th>
<th>Ovarian⁴</th>
<th>Colorectal⁵,⁶</th>
<th>Melanoma⁷</th>
<th>Thyroid⁸</th>
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<tr>
<td>BRCA1/2</td>
<td>EGFRᵃ</td>
<td>BRCA1/2ᵃ</td>
<td>BRCA1/2ᵃ</td>
<td>KRAS</td>
<td>BRAF</td>
<td>BRAF</td>
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<tr>
<td>PIK3CAᵃ</td>
<td>ALKᵃ</td>
<td>ATMᵃ</td>
<td>RAD51B/C/D</td>
<td>NRAS</td>
<td>ROS1</td>
<td>RET</td>
</tr>
<tr>
<td>ERBB2</td>
<td>ERBB2ᵇ</td>
<td>BARD1</td>
<td>CHEK1/2</td>
<td>BRAF</td>
<td>KIT</td>
<td>KIT</td>
</tr>
<tr>
<td>(HER2)</td>
<td>ROS1</td>
<td>BRIP1</td>
<td>CDK12</td>
<td>ERBB2</td>
<td>NRAS</td>
<td>ALK</td>
</tr>
<tr>
<td></td>
<td>KRAS</td>
<td>PALB2</td>
<td>FANCL</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>METᵃ</td>
<td>FANCA</td>
<td>RAD54L</td>
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<table>
<thead>
<tr>
<th>Bladder⁹</th>
<th>Gastric¹⁰/GEJ¹¹</th>
<th>Endometrial¹²</th>
<th>GIST¹³</th>
<th>Pancreatic¹⁴</th>
<th>Bone¹⁵</th>
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</thead>
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<tr>
<td>FGFR2</td>
<td>FGFR3</td>
<td>ERBB2 (HER2)</td>
<td>KIT</td>
<td>BRCA1/2ᵃ</td>
<td>IDH1</td>
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<td></td>
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<td>ERBB2 (HER2)</td>
<td>SDH</td>
<td>BRAF</td>
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<td></td>
<td></td>
<td>BRAF</td>
<td>ERBB2</td>
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<td></td>
<td></td>
<td></td>
<td>NF1</td>
<td>POLD1</td>
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<td></td>
<td></td>
<td>FGFR</td>
<td>POLD1</td>
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<td></td>
<td></td>
<td></td>
<td>PDGFRA</td>
<td>POLD1</td>
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</table>

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**Notes:**
- dMMR = mismatch repair deficient; GEJ = gastroesophageal junction; GIST = gastrointestinal stromal tumor; MSI-H = microsatellite instability-high; NCCN = National Comprehensive Cancer Network; NSCLC = non-small cell lung cancer; TMB-H = tumor mutational burden high.
- Additional genes recommended for germline testing in various tumor types:
  1. APC, ATM, BAPI, BRCA1/2, CDH1, CDK4, CDKN2A, CHEK2, EGFR, MITF, MLH1, MSH2/6, MUTYH, NFI, PALB2, PMS2, POLD1, POLE, PTEN, RET, RNF43, SMAD4, STK11, and TP53.
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**References:**
*See the specific NCCN Guidelines® for detailed testing recommendations; testing is not recommended for some of these biomarkers in certain guidelines.*

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**Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for:**
Liquid Biopsies and Germline Testing

- The liquid biopsy is not a “formal” germline test
- The patient has not consented for a germline test
- Genetic counseling program has not been activated before the test was obtained
- However, the liquid biopsy is highly informative of the germline status as:
  - Both the ctDNA and the wbcDNA will harbor the germline mutation
  - The variant allele frequency should be at or near 50%
  - The Liquid Biopsy report will alert the ordering physician that formal germline testing on another blood sample is recommended
Elevated tumor fraction (TF) is prognostic for worse overall survival in the four tumor histologies studied.

Tumor fraction is measured by an emerging method of calculation based on an aneuploidy approach that determines the relative “quantity” of extracted DNA obtained from tumor cells divided by the quantity of all that of all the DNA extracted from the blood sample. A cut-off of 10% TF was used for a “low” vs “high” designation.

Case Study: Liquid Biopsy in Prostate Cancer

68 y/o M with advanced prostate cancer

- Presents to oncology clinic after 2 years of abiraterone treatment, PSA rising
- Prefers to avoid chemotherapy, interested in oral options
- Has a bone biopsy from 2 years prior

What do you do?

F1LCDx is sent and shows a BRCA2 mutation

Report indicates the possibility of a germline mutation

Starts olaparib and is referred for germline counselling and possible testing
68 y/o M with advanced prostate cancer

Liquid Biopsy Findings
Considerations: When to Draw for Liquid Biopsy?

**ctDNA levels highest in patients with mCRPC experiencing clinical progression**

Biochemical progression = serial PSA rise on 2 separate occasions after achieving a nadir PSA value

Clinical progression = first appearance of new radiological disease

**ctDNA Fraction (%)**

<table>
<thead>
<tr>
<th></th>
<th>Untreated mHSPC N=73</th>
<th>mHSPC on ADT N=33</th>
<th>Biochemical progressive mCRPC N=75</th>
<th>Clinically mCRPC N=69</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>0.6</td>
<td>1.8</td>
<td>3.0</td>
<td>100</td>
</tr>
<tr>
<td>P</td>
<td>&lt;.01</td>
<td>&lt;.05</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

ADT = androgen deprivation therapy; ctDNA = circulating tumor DNA; mCRPC = metastatic castration-resistant prostate cancer; mHSPC = metastatic hormone-sensitive prostate cancer; PSA = prostate-specific antigen.

“Our proposed threshold for clinical utility of liquid biopsy assessment is a PSA of >5 ng/ml, at which level 78% of patients would be expected to have a circulating TF of at least 1%, and 23% would have a TF of at least 30%. Conversely, at PSA concentrations of <5 ng/ml in the metastatic prostate cancer setting, a tumor biopsy would be expected to yield more robust CGP results than a liquid biopsy. Liquid and tissue CGP are fundamentally two complementary diagnostics and must be used in parallel to optimize diagnostic yields and to aid treatment decisions for cancer patients.”

Antonarakis ES et al. Prostate. 2022 May;82(7):867-875.
**BRCA2 Loss vs BRCA 2 Point Mutation in mCRPC**

Case Study: Liquid Biopsy in Urothelial Bladder Cancer: *CDH1* Mutation

- TURBT specimen showing diffuse infiltration by urothelial carcinoma in a 61-year-old man
- The entire trigone, anterior and left side of the bladder wall was involved with deep smooth muscle invasion
- The bladder appeared to be fixed to the pubic bone on initial imaging studies
- Additional MRI of the pelvis showed diffuse bladder wall thickening with infiltration into pelvic fat and invasion into the right seminal vesicle
- The pathology report was a high grade urothelial carcinoma plasmacytoid type

“Plasmacytoid Urothelial Carcinoma”
Case Study: Liquid Biopsy in Urothelial Bladder Cancer: CDH1 Mutation

- Comprehensive genomic profiling revealed a MS-stable tumor with low tumor mutational burden of 5 mutations/Mb. A w532* CDH1 mutation was identified. Additional alterations included both a short variant (Q35*) and short deletion (exon10-11) of RB1
- BRD4 and NOTCH3 amplifications were identified along with short variant mutations in TERT promoter (-124C>T) and TP53 (E285K)
- Liquid biopsies reveal similar CGP results as tissue samples when tumor fraction is >10% and ctDNA levels are high
- CDH1 mutated UBC (plasmacytoid and non-plasmacytoid types) appear to be associated with resistance to immunotherapy and sensitivity to chemotherapy
Clonal Hematopoiesis (CH)

CH adds a layer of complexity when interpreting liquid biopsy results

- Genomic findings from cell-free DNA (cfDNA) may originate from non-tumor somatic alterations, including CHIP\(^1\)

- CH is an age-related condition in which peripheral blood cells accumulate mutations in driver genes known to be associated with hematological malignancies\(^2\)

- Genes with alterations that may be derived from CHIP include, but are not limited to: ASXL1, ATM, CBL, CHEK2, DNMT3A, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2, TP53, and U2AF1\(^2\)

Molecular Monitoring of Solid Tumors Using ctDNA Obtained from a Blood Sample

• Uninformed monitoring in late stage disease
• Informed monitoring in early and mid-stage disease
  • Earlier detection of relapse in patients believed to have been cured
  • Earlier detection of progression in a patient under-going anti-cancer systemic treatment
    • Target therapy and resistance mechanisms
    • Immunotherapy and resistance mechanisms
    • Chemotherapy and resistance mechanisms
• Informed monitoring requires prior tissue-based or blood based NGS
  • Monitoring assay based on the results of the NGS assay
  • Monitoring assay “converted” to a relatively expensive platform such as digital droplet PCR allowing for the test to be performed frequently and on a regular basis to “monitor” the patient’s tumor status
• Methylation of DNA Promoter genes is an emerging method of monitoring therapy response
  • Limited clinical data at this time
  • Best methods for detection and which methylated genes to search for are not fully established
Clinical Applications for ctDNA Monitoring

tctDNA monitoring applications are diverse and may vary across tumor types and stage

<table>
<thead>
<tr>
<th><strong>Molecular Residual Disease (MRD)</strong></th>
<th><strong>Surveillance</strong></th>
<th><strong>Treatment Response Monitoring (TRM)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single time point</td>
<td>Multiple time points</td>
<td>Multiple time points</td>
</tr>
<tr>
<td>Early-stage cancer</td>
<td>Early-stage cancer</td>
<td>Advanced/metastatic cancer</td>
</tr>
<tr>
<td>Following curative intervention (such as surgery) to detect ctDNA for adjuvant treatment decisions</td>
<td>Surveillance following surgery or adjuvant therapy to detect recurrence earlier</td>
<td>Detection of ctDNA over time to monitor treatment response or resistance to systemic therapy</td>
</tr>
<tr>
<td>Was complete tumor resection achieved during surgery?</td>
<td>Is the patient recurring after surgical or adjuvant treatment intervention?</td>
<td>Is the patient responding to therapy?</td>
</tr>
<tr>
<td>Should I give this patient adjuvant therapy based on ctDNA status?</td>
<td>Can I detect recurrence sooner than imaging?</td>
<td>Can I detect progression sooner than imaging?</td>
</tr>
<tr>
<td></td>
<td>Can I adjust adjuvant therapy?</td>
<td>Can I switch to an alternative therapy, if available?</td>
</tr>
</tbody>
</table>
ctDNA Monitoring Assays Can Be Tissue-Informed or Tissue-Naïve

Initial sequencing of tumor tissue to inform ctDNA can be followed by non-invasive, easily repeatable, real-time ctDNA monitoring.

<table>
<thead>
<tr>
<th></th>
<th><strong>Tissue-informed</strong></th>
<th><strong>Tissue-naïve</strong></th>
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<tbody>
<tr>
<td>Gene coverage</td>
<td>Small personalized and tumor-informed gene panel</td>
<td>Large panel of commonly altered genes</td>
</tr>
<tr>
<td>Tissue sequencing</td>
<td>Required</td>
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<tr>
<td>Key applications</td>
<td>• Detection of MRD</td>
<td>• Detection of MRD</td>
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<tr>
<td></td>
<td>• Assessment of treatment response</td>
<td>• Assessment of heterogeneity</td>
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<tr>
<td></td>
<td>• Serial recurrence monitoring</td>
<td>• Detection of actionable alterations</td>
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<tr>
<td></td>
<td></td>
<td>• Identification of resistance drivers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Serial monitoring</td>
</tr>
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<td>Germline and/or CH</td>
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</tr>
<tr>
<td>alteration screening</td>
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</table>
Molecular Monitoring can be used to select bladder cancer patients who might benefit from post-cystectomy adjuvant treatments.

Retrospective analysis of IMvigor010 (Phase III, randomized clinical trial of atezolizumab vs observation in high risk adjuvant MIBC) demonstrated Signatera.
Molecular Monitoring Can Detect Recurrence in Bladder Cancer an Average Of 152 Days Before Radiologic Imaging Can Find Recurrent or Metastatic Disease

Conclusions

• Blood based genomic testing is emerging rapidly as a clinically useful approach both to determining underlying driver mutations that can be targeted and biomarkers of immunotherapy response.

• When deciding whether to order a blood based or tissue based molecular test, which test to order first is best based on the status of the patient.

• Blood based molecular monitoring for solid tumor relapse and progression shows substantial promise to allow earlier treatment adjustments with potential to improve clinical outcomes for these patients.

• When used appropriately at the right time, liquid biopsies have potential to add significant additional precision in the modern care of the cancer patient.