Advantages and Disadvantages of ctDNA vs CTC Assays: how to move the needle forward towards clinical application

Julie E. Lang, MD, FACS
Associate Professor of Surgery
Director, University of Southern California Breast Cancer Program
Disclosures

- Speaker bureau – Genomic Health
- Research funds – ANGLE Parsortix
Circulating tumor (ctDNA) assays

- Nucleic acids shed into the bloodstream in patients with cancer are linked to apoptosis and necrosis of cancer cells in the tumor microenvironment. Vast amount wt DNA.
- Cell free DNA (cfDNA) has been identified in the peripheral blood plasma fraction of healthy individuals but patients with cancerous tumors have higher quantities of ctDNA and detection is associated with poorer prognosis.
- Nucleic acids have a half-life in the circulation ranging from 15 minutes to several hours.
Is ctDNA the ideal circulating biomarker?

- Technically easier to isolate than CTCs
- DNA is more stable than cells or RNA
- Proportion of ctDNA related to tumor burden and overall survival
- High sensitivity and dynamic range
- Pool of cancer cells/disease sites – represents heterogeneity
  - repertoire of genetic alterations
  - level of genetic instability
  - number and properties of subclones
Potential disadvantages of ctDNA

- Requires *a priori* knowledge of the target of interest in most cases
- Somatic mutations in only three genes (TP53, PIK3CA and GATA3) occurred at >10% incidence across all primary breast cancers*
- Not all DNA mutations are expressed
- Limitation of available material (NGS detection of mutations < 1% AF challenging)
- Blood cell death under therapy could spike ctDNA fraction (not reflecting cancer cell death)
- Source not clear – lytic, apoptotic tumor cells or are they derived from CTCs?
- Large background of ‘normal’ cfDNA (detected in healthy volunteers)

*TCGA, Nature, 2012*
Potential confounders for ctDNA

- The majority of specific DNA markers is in the cell bound fraction
- Cytotoxic chemotherapy leads to leukocyte and erythrocyte apoptosis, which may release cell bound DNA into plasma. An increase in these markers could be from the death of these cells rather than tumor cells.
- Unclear if ctDNA is released from cancer cells because they are dying from therapy or if because they are resistant to therapy.
Certain medical conditions may cause elevation of circulating DNA – need to focus on tumor specific genes/mutations

Zhong et al, Clinical Chemistry 2007
Elevated Levels of Circulating DNA in Cardiovascular Disease Patients

Figure 1. Levels of circDNA in the plasma of CVD patients and healthy control samples (in Log10 scale).


http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0105221
Examples of ctDNA assays

- Digital droplet PCR – requires knowledge of gene mutation of interest (i.e., PIK3CA). Up to 5 mutations may be assayed (typically).
- CAPP-Seq – hundreds of known recurrent mutations (139 genes, SNVs in 521 exons and 13 introns for NSCLC)
- Guardant 360 - 70 genes including SNVs, CNVs, indels, and rearrangements across more than 150,000 base pairs
ctDNA is more sensitive than CA15-3

A  CA 15-3 vs. ctDNA

<table>
<thead>
<tr>
<th></th>
<th>CA 15-3</th>
<th>ctDNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detected</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (&gt;32.4 U/ml)</td>
<td>21</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Not elevated (≤32.4 U/ml)</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>ctDNA sensitivity, 26/27 (96%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 15-3 sensitivity, 21/27 (78%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (&gt;32.4 U/ml)</td>
<td>67</td>
<td>4</td>
<td>71</td>
</tr>
<tr>
<td>Not elevated (≤32.4 U/ml)</td>
<td>27</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>20</td>
<td>114</td>
</tr>
<tr>
<td>ctDNA sensitivity, 94/114 (82%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 15-3 sensitivity, 71/114 (62%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dawson et al, NEJM 2013
ctDNA is more sensitive than Cell Search CTC Assay

<table>
<thead>
<tr>
<th></th>
<th>CTC</th>
<th>ctDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated (≥5)</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Detected (1–4)</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Not detected (0)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>1</td>
</tr>
</tbody>
</table>

ctDNA sensitivity, 29/30 (97%)
CTC sensitivity (detected, >0), 26/30 (87%)
CTC sensitivity (elevated, ≥5), 18/30 (60%)

Samples

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated (≥5)</td>
<td>45</td>
<td>1</td>
<td>46</td>
</tr>
<tr>
<td>Detected (1–4)</td>
<td>28</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>Not detected (0)</td>
<td>33</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>20</td>
<td>126</td>
</tr>
</tbody>
</table>

ctDNA sensitivity, 106/126 (84%)
CTC sensitivity (detected, >0), 76/126 (60%)
CTC sensitivity (elevated, ≥5), 46/126 (37%)

Median ratio of ctDNA copy numbers (per 3.75 ml of plasma) to number of CTCs (per 7.5 ml of whole blood) = 133 (interquartile range, 51–528)

r²=0.61
P<0.001
Comparison of Circulating Biomarkers to Monitor Tumor Dynamics

Dawson et al, NEJM 2013
ctDNA is prognostic of survival

Dawson et al, NEJM 2013
Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial

According to Mutation Status A, Superior overall survival (OS) is shown for patients without mutation (MT) in ESR1 (wild-type [WT]) compared with those with D538G and/or Y537S mutation. B, Overall survival results are shown for WT or D538G alone or Y537S alone or both D538G and Y537S (double MT).
Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial

Kaplan-Meier Curves for Effect of Addition of Everolimus to Exemestane

**A**, Progression-free survival (PFS) for patients without ESR1 mutation (wild-type [WT]) or with the D538G mutation (D538G). **B**, Results for patients without ESR1 mutation (WT) or with the Y537S mutation (Y537S). Addition of everolimus (EVE) was associated with improved progression-free survival (PFS) for patients with WT or D538G mutation but not for those with Y537S mutation. PBO indicates placebo.
ctDNA in clinical trials: use as a companion biomarker

BELLE-2 Met the Primary Endpoint for PFS Improvement in the Full Population

- A similar PFS improvement was observed in the main population (HR 0.80 [95% CI: 0.68–0.94]; one-sided P value 0.003)
- Follow-up for OS analysis is ongoing, with a pre-specified target of 588 deaths in the full population
  - At the time of primary PFS analysis, OS data were immature (281 deaths in the full population), with a trend in favor of the buparlisib arm

<table>
<thead>
<tr>
<th></th>
<th>Full Population (N=1147)</th>
<th>Buparlisib + Fulvestrant n=576</th>
<th>Placebo + Fulvestrant n=571</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, months (95% CI)</td>
<td>6.9 (6.8–7.8)</td>
<td>5.0 (4.0–5.2)</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.78 (0.67–0.89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-sided P value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Buparlisib Plus Fulvestrant Resulted in Higher Response Rates in Patients With ctDNA PIK3CA Mutations

Overall Response Rate, %

- Full Population: Buparlisib + fulvestrant 11.8, Placebo + fulvestrant 7.7
- PI3K Pathway Activated (Archival Tissue): Buparlisib + fulvestrant 10.6, Placebo + fulvestrant 8.2

- PIK3CA Mutant (ctDNA): Buparlisib + fulvestrant 18.4, Placebo + fulvestrant 3.5
- PIK3CA Non-mutant (ctDNA): Buparlisib + fulvestrant 11.6, Placebo + fulvestrant 10.6
PFS Improvement in the PI3K Activated Group Was Not Statistically Significant

- Buparlisib + fulvestrant (n/N=116/188)
- Placebo + fulvestrant (n/N=144/184)

<table>
<thead>
<tr>
<th>PI3K Activated Group (N=372)</th>
<th>Buparlisib + Fulvestrant (n=188)</th>
<th>Placebo + Fulvestrant (n=184)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, months (95% CI)</td>
<td>6.8 (4.9–7.1)</td>
<td>4.0 (3.1–5.2)</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.76 (0.60–0.97)</td>
<td></td>
</tr>
<tr>
<td>One-sided P value*</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

Keck Medical Center of USC

Baselga et al, SABCS 2015
ctDNA in clinical trials

Buparlisib Plus Fulvestrant Produced a Clinically Meaningful PFS Improvement in Patients With ctDNA PIK3CA Mutations

<table>
<thead>
<tr>
<th>ctDNA PIK3CA Mutant</th>
<th>Buparlisib + Fulvestrant n=87</th>
<th>Placebo + Fulvestrant n=113</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, months</td>
<td>7.0 (5.0–10.0)</td>
<td>3.2 (2.0–5.1)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.56 (0.39–0.80)</td>
<td></td>
</tr>
<tr>
<td>One-sided nominal P value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Accuracy of ctDNA vs tissue

- Matched tissue for sequencing is often not available:
  - Carpenter et al, Clin Cancer Research 2016 lung cancer: ctDNA NGS on 102 patients but only 49% successful NGS of matching tumor tissue.
  - Guardant 360 ASCO 2016 presentation by Zill et al: 2.6% (386/15,000) patients included had matched tumor tissue for comparison.
- Allelic frequencies in plasma tumor DNA derived from low-level subclonal variants present in a metastatic site are extremely low (lower than current sensitivity of NGS).
Feasibility of A Prospective Study of Molecular Profiling of Tissue and Blood in Metastatic Triple Negative Breast Cancer

- Single institutional study with the goal of molecularly profiling of tumor tissue and plasma DNA with hybrid capture based NGS
- Goal: perform molecular profiling on a recent biopsy and blood, convey results within 28 days.
- Met interim analysis for futility, study stopped.
- Did show high concordance of mutations found in liquid biopsy with tissue biopsy, but it was challenging to perform NGS of prospective tissue biopsies rapidly enough to impact clinical decision making.
Are CTCs the ideal circulating biomarker?

- CTCs are prognostic in all stages of breast cancer
- Deep sequencing revealed matching mutation with tumor sub clones – represents heterogeneity
- Intact cells could be resistant clones (DNA not in ctDNA) – isolation and characterization could give valuable information for therapeutic decisions
- Can be used for functional assays (DNA, RNA, protein)
- Can be cultured to evaluate drug resistance *in vitro* or *in vivo*
CTCs may be used for functional assays

Yu et al, Science 2014
CTCs Exhibit Dynamic Changes in EMT Composition

Yu et al, Science 2013
CTC as a liquid biopsy: N of one design

Yu et al, Science 2013
CTCs may be classified by intrinsic subtype

Lang et al, Breast Cancer Research and Treatment 2015
Hierarchical clustering of 192 differentially expressed genes in CTCs, Mets and PB.

<table>
<thead>
<tr>
<th>sample</th>
<th>Histology</th>
<th>primary biomarkers</th>
<th>site of met</th>
<th>met biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>79412</td>
<td>IDC</td>
<td>ER/PR+,HER2-</td>
<td>pericardial effusion</td>
<td>ER/PR+,HER2-</td>
</tr>
<tr>
<td>79556</td>
<td>IDC</td>
<td>triple negative</td>
<td>pleural effusion</td>
<td>ER-, PR/HER2 unknown</td>
</tr>
<tr>
<td>79555</td>
<td>IDC</td>
<td>triple negative</td>
<td>pleural effusion</td>
<td>triple negative</td>
</tr>
<tr>
<td>78536</td>
<td>ILC</td>
<td>triple negative</td>
<td>bone</td>
<td>triple negative</td>
</tr>
<tr>
<td>38978</td>
<td>ILC</td>
<td>ER+,PR/HER2-</td>
<td>LN</td>
<td>ER+,PR/HER2-</td>
</tr>
<tr>
<td>68185</td>
<td>IDC</td>
<td>triple negative</td>
<td>cerebrospinal fluid</td>
<td>unknown</td>
</tr>
<tr>
<td>80540</td>
<td>IDC</td>
<td>triple negative</td>
<td>skin</td>
<td>unknown</td>
</tr>
<tr>
<td>81103</td>
<td>IDC</td>
<td>ER/HER+,PR-</td>
<td>pleural effusion</td>
<td>unknown</td>
</tr>
</tbody>
</table>
CTCs may be used as a liquid biopsy predicting response to targeted therapies

Ring et al, AACR 2016
Potential Disadvantage of CTCs

- Rare ‘events’ – isolation technically challenging, profiling may be more costly if necessary to also profile blood background
- Sampling bias of captured cells – affinity based, size based selection
- Single-cell/ low cell number sequencing challenging (heterogeneity observed could be biological or technical bias)
A Word of Caution about Amplification Bias for Both CTCs and ctDNA Assays
Optimizing for advantages of both CTCs and ctDNA as companion biomarkers

- CTCs may prove to be better at discovering novel targets and frequency of multiple known targets in multi-institutional cohort studies.
- ctDNA of defined targets may be useful in clinical trials, but when resistance emerges it may be necessary to utilize CTCs as a functional assay.
- Comparison of both to tissue biopsies in relation to therapeutic response is a critical issue to be addressed to move the field forward.
# Acknowledgements

## Lang Lab
- Tania Porras, MS; Lab manager
- Pushpinder Kaur, PhD; research fellow
- Dany Barrak, MD (surgery resident, research fellow)
- Alex Ring, MD; Ph. D student
- Victoria Forte, MD USC Heme Onc fellow; research fellow

## UCSF
- Janet Scott, Ph.D.
- John Park, MD
- Laura Esserman, MD, MBA
- Denise Wolf, Ph.D.
- Laura Van’t Veer, Ph.D.
- Mark Magbanua, Ph.D.
- Thea Tlsty, Ph.D.

## BD Biosciences
- Emily Park, Ph.D.
- Chip Lomas
- Tom Frei, Ph.D.

## MD Anderson
- Debu Tripathy, MD

## USC
- Michael Press, MD, Ph.D
- Terry Church, MA
- Barish Poole
- Stephen Sener, MD
- Lora Barsky, MS
- Vasu Punj, Ph.D.
- Min Yu, MD, Ph.D
- Gabriel Zada, MD
- Naveed Wagle, MD
- Susan Groshen, PhD

---

**Vasu Punj, PhD**
Co-investigator
Bioinformatics