Liquid Biopsy in Breast Cancer
Ready for Prime Time?

...work in progress

Dr PN Mainwaring
Disclosures; Payment, Research, Patents

• Companies; AZ, Genentech, Janssen, Pfizer
• Previous AIBN/UQ affiliation; patents licensed from AIBN@UQ
• Co-Founder XING Technologies
  • Paid research diagnostic companies; will not discuss technology specifics
What do our patients need?

3. **Is there a problem?**
   - Screening, Early detection, Prognosis, Prediction, Response, Toxicity, Relapse
   - Metastatic therapy, Neoadjuvant therapy, Early disease therapy

2. **Are there solutions?**
   - History, clinical, imaging, histopathology, **molecular pathology**

1. **Technology**
   - Laboratory Validity: available, cost-effective, accurate; sensitivity, specificity, reproducible
   - Clinical Validity: single/multiple aberration(s), specific panel, general panel, WES, WGS
   - Clinical Utility: prediction; MOS/MOR, estimating prognosis, toxicity

   More – multi-ome; pathways analysis, systems biology, pG
What is cfDNA/ctDNA?

- Normal cells **5 mutations per genome per cell division**; NB ~40 trillions cells; 2 billion bases
- > **10-fold increase** in advanced disease, but the fraction of mutant DNA templates is still < **10%** of the total templates
- Only clonal proliferations of tens of millions of cells with the identical mutation, can be detected at frequencies above **0.01%** of cfDNA in plasma which is ~ 1,500 Genome Equivalent/mL (GE/mL)
- Half-life of circulating cfDNA is **less than 1 hour** (range 15min-2hr), due to rapid clearance
- Depends on the anatomic **location, volume intra/inter-tumoural heterogeneity** of the tumour;
  - Other sources; mitochondrial DNA, extra-chromosomal DNA, microbiome DNA, bacterial DNA, exosomal DNA, CTC/CSCs
  - Other states including pro-inflammatory diseases and autoimmune disorders, such as liver cirrhosis, emphysema, as well as pregnancy and intense physical exercise
  - Age, body mass index, sex, and physiological or ethnical parameters might also modify cfDNA/ctDNA concentrations
- Approx **3-9 ng of cell-free DNA/mL** of plasma from normal individuals and patients with early-stage cancer; screening is challenging
CHIP; Somatic evolution

Associated with
- Aging
- All cause mortality
- MDS (TET2), AML
- Inc risk cardiovascular disease
- Inc risk cerebrovascular disease
- Overlap solid & liquid panels

Genovese NEJM 2014
GeJaiswal NEJM 2014 & 2017
Cull Exp Hematol 2017
Kennedy TIC 2019
Calvillo-Argüelles JAMA Cardiol 2019
Technology
3 easy steps

• Pre-analytical; Guidelines, kits, DIY
• Analytical; Guidelines, kits, DIY
• Post analytical; Open season? ML/DL
Ultra-deep sequencing

100% VAF: ~10,000 genomic equivalents are found in 10ml of blood

1% VAF: ~100 tumor genomic equivalents (typical detection limit of most ctDNA assays)

0.01% VAF: ~1 tumor genomic equivalents
## Technologies

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Optimal Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanger sequencing</td>
<td>&gt; 10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Next-generation sequencing</td>
<td>2%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Quantitative PCR</td>
<td>1%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>ARMS</td>
<td>0.10%</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>CapSeq TECSeq, ddPCR</td>
<td>0.01% or lower</td>
<td>ctDNA, rare variants in tumor tissue</td>
</tr>
<tr>
<td>BEAMing, PAP, Digital PCR, TAM-Seq</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Diaz JCO 2014
Orthogonal Comparison of Four Plasma NGS Tests With Tumor Suggests Technical Factors are a Major Source of Assay Discordance

Daniel Stetson, MS¹; Ambar Ahmed, MS¹; Xing Xu, PhD²; Barrett R.B. Nuttall, MS¹; Tristan J. Lubinski, PhD¹; Justin H. Johnson¹; J. Carl Barrett, PhD¹; and Brian A. Dougherty, PhD¹

Range of sensitivity (38% to 89%)
Positive predictive value (36% to 80%)

<table>
<thead>
<tr>
<th>Vendor</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>Sensitivity (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>38</td>
<td>55</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>10</td>
<td>2</td>
<td>89</td>
<td>63</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>23</td>
<td>6</td>
<td>68</td>
<td>36</td>
</tr>
</tbody>
</table>

Most discordance in our cross-vendor study was observed below 1% VAF
68% resulted from technical discordance
SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing—Based Oncology Panels

A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

Lawrence J. Jennings, Maria E. Arcila, Christopher Gorliess, Suzanne Kamel-Reid, Ira M. Lubin, John Pfeiffer, Robyn L. Temple-Smolkin, Karl V. Voelkerding, and Marina N. Nikiforova

Multilaboratory Assessment of a New Reference Material for Quality Assurance of Cell-Free Tumor DNA Measurements


CANCER-ID (https://www.cancer-id.eu/) analytical stds
CANCER-ID (https://www.cancer-id.eu/) pre-analytical stds
<table>
<thead>
<tr>
<th>Study design</th>
<th>No. patients enrolled in the study/translational sub-study</th>
<th>Disease and stage</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparing the responses of patients who received physician-driven treatment to CTCs-based treatment for first-line chemotherapy (HT vs. CT)</td>
<td>778</td>
<td>MBC</td>
<td>CTCs</td>
</tr>
<tr>
<td>Changing therapy vs. maintaining therapy in patients with persistently increased CTCs</td>
<td>598</td>
<td>MBC</td>
<td>CTCs</td>
</tr>
<tr>
<td>Adjuvant trastuzumab for 6 cycles vs. observation</td>
<td>63</td>
<td>EBC</td>
<td>CTCs</td>
</tr>
<tr>
<td>AR-V7+ CTC status as a biomarker of resistance to HT</td>
<td>118</td>
<td>mCRPC</td>
<td>CTCs (and ctDNA)</td>
</tr>
<tr>
<td>Fulvestrant plus anastrozole vs. fulvestrant plus placebo vs. exemestane alone</td>
<td>161 (63 ESR1+)</td>
<td>LABC or MBC</td>
<td>ctDNA</td>
</tr>
<tr>
<td>Fulvestrant plus palbociclib vs. fulvestrant plus placebo</td>
<td>360 (91 ESR1+)</td>
<td>LABC or MBC</td>
<td>ctDNA</td>
</tr>
<tr>
<td>Exemestane plus everolimus vs. exemestane plus placebo</td>
<td>550 (238 PIK3CA+)</td>
<td>LABC or MBC</td>
<td>ctDNA</td>
</tr>
<tr>
<td>Fulvestrant plus buparlisb vs. fulvestrant plus placebo</td>
<td>587 (200 PIK3CA+)</td>
<td>LABC or MBC</td>
<td>ctDNA</td>
</tr>
<tr>
<td>Alpelisib plus fulvestrant vs. fulvestrant plus placebo</td>
<td>549 (186 PIK3CA+)</td>
<td>MBC</td>
<td>ctDNA</td>
</tr>
<tr>
<td>SoFEA: fulvestrant plus anastrozole vs. fulvestrant plus placebo vs. exemestane alone</td>
<td>383 (115 ESR1+)</td>
<td>LABC or MBC</td>
<td>ctDNA</td>
</tr>
</tbody>
</table>

Rossi Ca Res 2019
Cytosine methylation; Epigenetics/Fragmentation

Aberrant DNA methylation events occur early in cancer development, sometimes before the acquisition of SNVs.

Thus, such changes could represent an ideal marker of early-stage cancer & give T-o-O

Exceedingly complex +++

Snyder Cell 2016; Dor Lancet 2018; Chen CCR 2019
Advanced Breast Cancer Treatment Guidance

- Quantitative & Qualitative assessments
- Response/Resistance
  - De Novo
  - Secondary
  - ? Threshold MAF %; [aberration]/RECIST
- Surrogate for PFS/OS
  - Disease volume, heterogeneity, truncal clones, phylogenetic evolution, signatures
- Combination therapy
  - Strategies therein; chemotherapy, targeted therapy, immunotherapy
- Pharmacogenomics
- Toxicity; CTCAE; irAE

Unanswered Questions

Some evidence emerging
levels of ctDNA increased at one or more consecutive time points, on average 5 months (2 to 9) before the establishment of progressive disease by means of imaging.
Table 2. Mutation Frequency of PIK3CA or ESR1 by Line of Therapy

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patients With ≥ 1 Genetic Alteration(^a)</th>
<th>First-line(^b)</th>
<th>Second-line + Early Relapsers(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, mutant/ N, total</td>
<td>Freq. %</td>
<td>n, mutant/ N, total</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>207/588</td>
<td>35</td>
<td>100/303</td>
</tr>
<tr>
<td>ESR1</td>
<td>83/588</td>
<td>14</td>
<td>13/303</td>
</tr>
</tbody>
</table>

**Figure 2. PFS in Patients With ≥ 1 Genetic Alteration Detected in Baseline ctDNA**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Events</th>
<th>Median PFS (95% CI)</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribociclib</td>
<td>400</td>
<td>183</td>
<td>19.09 (16.43-23.03)</td>
<td>0.68 (0.54-0.85)</td>
</tr>
<tr>
<td>Placebo</td>
<td>200</td>
<td>119</td>
<td>14.55 (10.84-17.48)</td>
<td></td>
</tr>
</tbody>
</table>
ESR1 mutations detected in 30% (151/383) baseline samples

**PFS**

- Wild type + E: 106/121 (4.8mth [3.7, 6.2])
- Wild type + F: 120/147 (4.1mth [3.6, 5.5])
- Mutant + F: 69/73 (3.9mth [3.0, 6.0])
- Mutant + E: 40/42 (2.4mth [2.0, 2.8])

**OS**

- Wild type + E: 70/121 (79% [71, 85])
- Wild type + F: 78/147 (81% [74, 87])
- Mutant + F: 46/73 (80% [68, 87])
- Mutant + E: 28/42 (62% [45, 75])

Interaction p = 0.02

Turner et al. SABCS 2018
**PIK3CA ctDNA Analysis and Alpelisib**

**De Novo Sensitivity/Resistance**

**ctDNA PIK3CA mutant**

Plasma ctDNA analysis - **HR 0.55**

Retrospectively analysed with a PCR assay not optimised for ctDNA analysis

Sensitivity ~55%

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**Number of patients still at risk**

- **Alpelisib + ful**: 92 87 80 77 68 61 54 52 44 43 41 38 34 31 29 24 23 19 18 16 9 8 6 2 2 1 1 1 0
- **Placebo + ful**: 94 90 58 53 42 41 37 34 30 30 26 22 20 19 18 14 11 10 9 6 5 2 2 1 1 1 0

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*Juric et al. SABCS 2018*
Longitudinal

Acquired Sensitivity/Resistance

Custom NGS assay; 87 genes
Detection of BRCA reversion mutations in \textit{BRCA1/2} mutant cancers

Detection of 9 different mutations in plasma after prior treatment with carboplatin and PARP inhibitor

Acquired Sensitivity/Resistance

Custom NGS

Weigelt et al CCR 2017
HER2 copy number in CRC

PLASMA COPY NUMBER (pCN) IS INFLUENCED BY TISSUE COPY NUMBER + TUMOR FRACTION – RETROSPECTIVE COHORT

Siravegna ESMO 2019
Is a single genomic biomarker enough in a disease dominated by CNV/other ‘omes’?
[ctDNA] & response to ICPI in NSCLC lesson to be applied in TNBC?
PIK3CA mu in HER2 Neoadjuvant therapy

De Novo Sensitivity/Resistance

- PIK3CA wt
- PIK3CA mutant

$p_{interaction 1} = 0.036$

$p_{interaction 2} = 0.189$

- Overall: $P < 0.001$
- HR-ve: $P = 0.125$
- HR+ve: $P < 0.001$
- Trastuzumab: $P = 0.343$
- Laptinib: $P = 0.389$
- T+L: $P < 0.001$

$n = 967$
$n = 424$
$n = 543$
$n = 315$
$n = 251$
$n = 401$

Loibl Annals 2016
Post-neoadjuvant therapy; RctDNA vs. pCR

residual tumor size (ypT)

- T0
- Tis
- T1–3

? Δ
? level

ctDNA fraction

0.04%
0.03%
0.02%
0.01%
0.005%
0.001%

Residual disease
pathCR

After neoadjuvant therapy

targeted digital sequencing (TARDIS)

Pretreatment
Post NAT

McDonald STM 2019

Do not duplicate or distribute without permission from author and ESO.
ctDNA in the Adjuvant setting

- Molecular residual disease (MRD)
- Molecular relapse (MR)
- “2 sides of the same coin”
Relationship ctDNA & clinical parameters

![Graph showing ctDNA positivity across different molecular subtypes and tumor sizes.]

Zhang CCR 2019
Tumour-specific dPCR assays

Baseline detection associates with tumor subtype, tumor size and grade

Future research needed to confirm whether an independent factot

Garcia-Murillas et al JAMA Oncol 2019
Personalised 16-plex assay @ pt

Median lead time 8.9 months
All ctDNA positive patients relapsed

ctDNA status at any timepoint

HR: 35.84 (7.9 –161.32)
p-value <0.001

Coombes et al Clin Cancer Res 2019
The Continuous Individualized Risk Index Approach - Overview

Individual Prognostic Factors
- Pretreatment Risk Factors
- Interim Risk Factors
- End of Treatment Risk Factors

Pretreatment Risk Factors

Diagnosis

Treatment

EoT

Surveillance

Primary Endpoint

Individualized Risk Assessment Over Time

Naive Bayes Approach:
Individual Prediction of Survival at Fixed Primary Endpoint

Bayesian Proportional Hazards Approach:
Individual Prediction of Survival at Any Future Time Point

Time

Time

Time

Time
Increasing utility of adjuvant trials

- Use ctDNA as a surrogate for DFS/EFS
  - DFS surrogate for OS in CRC, Breast, GIST, B-ALL...
- Use ctDNA clearance as an end-point

- Both with validity caveats described – need to define sample size for each tumour type acceptable to comparable overseas regulators framework (FDA, EMA, TGA, Health Canada); Project Orbis
Guidelines

**ASCO**

"there is insufficient evidence of clinical validity and utility for the majority of ctDNA assays in advanced cancer..."
Circulating Cells

• In 1869, Ashworth, an Australian, found tumour cells similar to the primary in the peripheral blood of one patient with metastatic disease and first proposed the concept of CTCs (Bonnomet Oncogene 2012)

• CTC who have undergone EMT
• CTC shed by tumour growth
• CTM-clusters
• CTM-dormancy/niche
• CSC
Microfluidic platforms; then & now & future

CELLSEARCH
Positive selection

CTC-iCHIP
Negative selection
CTC detection techniques usually combine two steps: primary enrichment followed by CTC detection and counting.

**CTC Processing Methods**
- **Immunomagnetic**
  - Positive Selection
  - Negative Selection
- **Biophysical**
  - Size 5-10μm
  - Deformability
  - Density
  - Cell surface markers
  - Electro/hydrostatic forces

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**Peripheral blood**

- **Red blood cells**
- **White blood cells**
- **Circulating tumor cells (CTCs)**

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**Technical methods**

- **Whole blood techniques**
  - Immunocapture
  - Size based
  - Density based
- **Immunomagnetic**
- **Microfluidic i.e.**
- **Membrane**
- **Microfluidic**

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**Multiparameter analysis**
- Molecular/biomarker testing
- Culture
- Therapeutic testing

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Sundling Anat Pathol 2019; Bankó JHO 2019
CTC technique comparison; EpCam; IF; RT-PCR

<table>
<thead>
<tr>
<th>Assay</th>
<th>Blood volume</th>
<th>Enrichment method</th>
<th>Detection method</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Search</td>
<td>7.5mL/23mL</td>
<td>Ferrofluids containing EpCam antibodies</td>
<td>Immunodetection of CK 8, 18, 19 and DAPI staining, lack of CD45 detection</td>
<td>≥2 CTGs/7.5mL, ≥1 CTGs/23mL</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>20 mL</td>
<td>Manually operated density gradient centrifugation</td>
<td>RT-qPCR for CK-19 mRNA Immunodetection of CK 8, 18, 19 and DAPI staining, lack of CD45 detection</td>
<td>≥0.6 MCF-7 equivalents/5µg RNA</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>10^6 PBMCs</td>
<td>Manually operated density gradient centrifugation</td>
<td>Immunodetection of CK 8, 18, 19 and DAPI staining, lack of CD45 detection</td>
<td>≥1 CTGs/10^6 PBMCs</td>
</tr>
</tbody>
</table>

**A**

<table>
<thead>
<tr>
<th></th>
<th>K coefficient</th>
<th>p-value</th>
<th>Degree of agreement</th>
<th>Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell search (cut-off ≥1) vs RT-qPCR</td>
<td>0.088</td>
<td>0.161</td>
<td>Poor</td>
<td>62</td>
</tr>
<tr>
<td>Cell search (cut-off ≥1) vs IF</td>
<td>0.078</td>
<td>0.233</td>
<td>Poor</td>
<td>61.8</td>
</tr>
<tr>
<td>Cell search (cut-off ≥2) vs RT-qPCR</td>
<td>0.072</td>
<td>0.307</td>
<td>Poor</td>
<td>73.5</td>
</tr>
<tr>
<td>Cell search (cut-off ≥2) vs IF</td>
<td>0</td>
<td>0.976</td>
<td>None</td>
<td>71.9</td>
</tr>
<tr>
<td>RT-qPCR vs IF</td>
<td>0.086</td>
<td>0.248</td>
<td>Poor</td>
<td>73.0</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th></th>
<th>K coefficient</th>
<th>p-value</th>
<th>Degree of agreement</th>
<th>Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell search (cut-off ≥2) vs RT-qPCR</td>
<td>0.281</td>
<td>&lt;0.001</td>
<td>Fair</td>
<td>63.4</td>
</tr>
<tr>
<td>Cell search (cut-off ≥2) vs IF</td>
<td>0.041</td>
<td>0.619</td>
<td>Poor</td>
<td>49.5</td>
</tr>
<tr>
<td>Cell search (cut-off ≥5) vs RT-qPCR</td>
<td>0.302</td>
<td>&lt;0.001</td>
<td>Fair</td>
<td>67.7</td>
</tr>
<tr>
<td>Cell search (cut-off ≥5) vs IF</td>
<td>0.049</td>
<td>0.607</td>
<td>Poor</td>
<td>58.1</td>
</tr>
<tr>
<td>RT-qPCR vs IF</td>
<td>0.011</td>
<td>0.902</td>
<td>Poor</td>
<td>49.5</td>
</tr>
</tbody>
</table>
MBC; CTC & Survival

C Full Set of Data

F Full Set of Data

Weeks from Baseline

Probability of Progression-free Survival (%)

No. at Risk

<5 CTC 90 87 77 69 59 52 44 39 33 26 22 16 12 5 4 2 0

≥5 CTC 87 76 48 38 34 29 24 22 17 12 9 8 4 1 1 1 0

Weeks from Baseline

Probability of Overall Survival (%)

<5 CTC

<18.0 mo

≥5 CTC

<10.1 mo

Cristofanelli NEJM 2004
CTC Clusters

A. Overall survival
B Subgroup patients

- 0 CTC: median 25.4 months (95% CI 18.3–32.3)
- 1-4 CTCs: median 21.3 months (95% CI 16.6–26)

P = 0.15

B. Overall survival
C Subgroup patients

- Clus only: median NR months (95% CI -)
- Doub & Clus: median 7.3 months (95% CI, 3.2–11)
- Doub only: median 8.4 months (95% CI, 4.1–10.5)
- No Doub & No Clus: median 14.4 months (95% CI, 11.1–17)

P = 0.01

Number at risk (number censored)

A: 89 (0) 64 (3) 40 (7) 25 (12) 12 (19) 4 (25) 0 (29)
B: 2 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0) 0 (0)
15 (0) 5 (0) 1 (0) 0 (0) 0 (0) 0 (0) 0 (0)
18 (0) 5 (0) 3 (1) 0 (2) 0 (2) 0 (2) 0 (2)
77 (0) 46 (1) 12 (4) 6 (9) 1 (12) 1 (12) 0 (12)

Paoletti CCR 2019
CTC & DTC (Bone Marrow); niche/dormant

Double-positive  Double negative

Magbanua SABCS 2018 & CCR 2019
Consensus

- Identification of two subgroups of MBC

- Stage IV_{indolent} and Stage IV_{aggressive}, independent of clinical and molecular variables.

- Thus, CTC count should be considered an important tool for staging of advanced disease and for disease stratification in prospective clinical trials.
Clinical utility of Circulating Tumor Cells (CTC) count to choose between 1st line hormone therapy & chemotherapy in ER+ HER2- metastatic breast cancer  Pre-cdk4,6i era

Results of the phase III STIC CTC trial (NCT01710605)

**Exploratory analysis: discordant groups pooled**

<table>
<thead>
<tr>
<th>Control arm</th>
<th>HT</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>N pts (%)</td>
<td>370 (47.5%)</td>
<td>105 (13.5%)</td>
</tr>
</tbody>
</table>

**HT vs CT**

303 (38.9%)

**Progression-Free Survival**

HR=0.66 [0.51-0.85] adjusted p=0.001

**Overall Survival**

HR= 0.65 [0.43-0.98] adjusted p=0.04
Prior to neoadjuvant therapy; meta-analysis CTC >1

Table 2. Multivariable survival analyses

<table>
<thead>
<tr>
<th>Variable</th>
<th>OS</th>
<th>DDFS</th>
<th>LRFI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>P†</td>
<td>P†</td>
<td>P†</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>0.72 (0.53 to 0.99)</td>
<td>0.69 (0.53 to 0.89)</td>
<td>0.70 (0.47 to 1.04)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-T2</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>T3-T4</td>
<td>1.60 (1.11 to 2.31)</td>
<td>1.56 (1.15 to 2.12)</td>
<td>1.16 (0.70 to 1.89)</td>
</tr>
<tr>
<td>T4d</td>
<td>2.85 (1.51 to 5.11)</td>
<td>2.65 (1.54 to 4.37)</td>
<td>1.69 (0.86 to 3.74)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cN0</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>cN1-3</td>
<td>1.94 (1.39 to 2.76)</td>
<td>1.63 (1.07 to 2.54)</td>
<td>1.63 (1.07 to 2.54)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>3</td>
<td>1.06 (0.76 to 1.50)</td>
<td>1.00 (reference)</td>
<td>1.53 (0.99 to 2.38)</td>
</tr>
<tr>
<td>Tumor subgroup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR+ HER2-</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>HER2+</td>
<td>1.29 (0.80 to 2.04)</td>
<td>1.05 (0.72 to 1.51)</td>
<td>2.31 (1.28 to 4.19)</td>
</tr>
<tr>
<td>HR- HER2-</td>
<td>3.92 (2.65 to 5.84)</td>
<td>2.14 (1.55 to 2.90)</td>
<td>4.35 (2.51 to 7.69)</td>
</tr>
<tr>
<td>CTC count before NCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>≥2</td>
<td>3.98 (2.81 to 5.45)</td>
<td>3.73 (2.82 to 4.90)</td>
<td>3.02 (1.88 to 4.75)</td>
</tr>
</tbody>
</table>

Bidard JNCI 2018
Early breast cancer; > 1 CTC 2 yrs post-therapy
EBC; CTC and late recurrence; > 1 CTC @ 5 yrs

AC-paclitaxel +/- bevacizumab; E5103

Table. Univariate and Multivariable Analyses of Association Among Presence of CTCs, Clinicopathologic Features, and Recurrence in Hormone Receptor-Positive Breast Cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Association With Recurrence, HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate Analysis</td>
</tr>
<tr>
<td>Age ≥50 y vs &lt;50 y</td>
<td>1.95 (0.80-4.73)</td>
</tr>
<tr>
<td>Tumor diameter &gt;2 to ≤5 cm vs ≤2 cm</td>
<td>4.05 (1.19-13.83)</td>
</tr>
<tr>
<td>Tumor diameter &gt;5 vs ≤2 cm</td>
<td>3.74 (0.75-18.53)</td>
</tr>
<tr>
<td>1-3 Positive nodes vs negative</td>
<td>1.86 (0.24-14.64)</td>
</tr>
<tr>
<td>≥4 Positive nodes vs negative</td>
<td>3.47 (0.45-26.50)</td>
</tr>
<tr>
<td>Grade III vs I and II</td>
<td>1.62 (0.70-3.75)</td>
</tr>
<tr>
<td>CTC positive vs negative</td>
<td>10.82 (4.42-26.47)</td>
</tr>
</tbody>
</table>

Abbreviations: CTC, circulating tumor cell; HR, hazard ratio.

**Figure 2. Time to Recurrence by Circulating Tumor Cell (CTC) Assay Result Among Patients With Hormone Receptor-Positive Breast Cancer**

- CTC negative
- CTC positive

No. at risk
- CTC negative: 335, 306, 211, 102, 16, 0
- CTC positive: 18, 13, 7, 3, 0, 0

RR, 10.82 (95% CI: 4.42-26.47), P<.001

Sparano JAMA Onc 2018
Biomarker Combinations
Multimodal liquid biopsy approaches for early monitoring and outcome prediction in first line metastatic hormone receptor HER2 negative breast cancer: final results of the French Breast Cancer InterGroup Unicancer (UCBG): COMET study

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# P03019

## Background

Circulating Tumor Cells (CTC) are independent markers of progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast cancer. Monitoring circulating tumor DNA (ctDNA) can detect mutation associated with resistance to treatment and its variations reflect changes in tumor burden. We prospectively monitored CTC, Circulating Endothelial Cells (CEC), serum markers and ctDNA during first line chemotherapy for MBC.

## Patients and Methods

The French cohort COMET is a prospective study including first line HER2 negative patients receiving weekly paclitaxel and trastuzumab according to FDA approved combination. The aim of this cohort was to evaluate clinical, biological and radiological parameters associated with patients’ outcome (CTC, CEC, serum markers, ctDNA, pharmacogenomic polymorphisms, metabolic parameters, visceral fat assessed by CTscan, serum estradiol level, and quality of life).

We present here the planned analysis on patients evaluated for CTC (CellSearch), CCA IA 5.3 and ctDNA. Blood samples were obtained at baseline (BL) and during the second cycle of chemotherapy (C2).

## Study Scheme

**Bevacizumab + Paclitaxel** (weekly for 8 cycles)  
**NAC**  
**Her-2 negative**  
**Blood samples:**  
- Blood samples: Metastatic Breast Cancer  
- Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  
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- Blood samples: Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  
- Blood samples: Metastatic Breast Cancer  

## Results

**Strategy:** Targeted resequencing (custom panel 220kb)

### ctDNA Results

- **Prostatectomy:** 119 patients were analyzed, 174 had at least one somatic mutation (SNVs) detected in plasma (75%).
- **At baseline,** CTC and ctDNA levels were correlated (r=0.40, p=0.0001)
- **Despite no complete overlap,** 24 patients (12%) had no CTC (threshold 1) nor ctDNA detected at baseline.

### CtDNA at baseline

- Including Copy Number Variation analysis (CNV), the number of patients without detectable ctDNA a baseline could be reduced to 18 (8%) (analysis ongoing)

### Multivariate analysis for overall survival

- **Triple negative status, detectable ctDNA at C2, CEC ≥5 at C2 and grade 3 on primary tumor were independent prognostic factors.**

## Conclusions

- This is the largest prospective cohort assessing the respective prognostic values of early CTC and ctDNA changes in homogeneously treated first line MBC patients.
- We confirm the prognostic value of CTC detection at baseline.
- CEC and serum markers levels or variations had no prognostic values.
- Early decrease of CTC and/or ctDNA after one cycle of chemotherapy are independent marker of favorable outcome.
- Clinical utility of early ctDNA variations monitoring for MBC treatment remains to be demonstrated.

## Acknowledgements

- **Contacts**
  - Stéfane Everard – UNICANCER – stefane@unicancer.fr
  - Jean-Yves Pignot – Principal Investigator – jean-yves.pignot@curie.fr

## Comparison of predictive value of ctDNA and CTC variations

- **Overall survival according to CTC & ctDNA**
  - **Prognostic model**  
    - Clinical model + CTC: 17.08
    - Clinical model + ctDNA: 21.08
  - **Clinical model + CTC**
    - Added value of CTC knowing ctDNA: 7.83
  - **Clinical model + ctDNA**
    - Added value of ctDNA knowing CTC: 11.874

- **PFS & OS Results**
  - **Median follow-up** 53 months and median OS was 32 months.
  - *Detectable levels of CTC and ctDNA baseline were significantly associated with decreased PFS and OS.*
  - *At C2, ≥5 CTC or still detectable ctDNA were strong markers of reduced PFS & OS.*
  - *CEC and serum markers level had no prognostic value.*
Guidelines CTC

• For a widespread use of CTC/CTM detection as a diagnostic tool clinical acceptance is critical.

• The American Society of Clinical Oncology (ASCO), the National Academy of Clinical Biochemistry, the American Association for Clinical Chemistry, and the American Joint Committee on Cancer have all declined to recommend CTC/CTM assays in the detection, monitoring or staging of cancer until the benefits of the technique are clarified.
Summary

• Technologies are emerging to enable us to measure molecular aberrations in body fluids with high degrees of sensitivity & specificity

• The ultimate goal if a screening test for cancer is a long way off
  • An intermediate goal of a screening test for cancer is coming

• ‘Liquid biopsies’ for advanced cancer are coming
  BUT NEED STANDARDISATION
  • Know the technology used to understand validity

• The paradigm of laboratory validity, clinical validity and clinical utility remain the gold standard

• Watch this space but let us ensure that these are a) accurate & b) easy to use & c) easy to deliver so affordable to all