APPLICATIONS OF LIQUID BIOPSY IN BREAST CANCER

Dr. Monica Arnedos
Head of breast cancer research
Institut Bergonié, Bordeaux, France
Honoraria as invited speaker: Novartis
Honoraria for participation in Advisory Board: Puma Biotechnology, AstraZeneca, AbbVie
Honoraria to institution for masterclass: Roche, Genomic Health
Honoraria to institution for preceptorship: Novartis
Institutional financial interest for research as a coordinating PI: Novartis and Puma Biotechnology
INTRODUCTION

Distinguished panel of experts

Applications of Liquid Biopsy in Breast Cancer
INTRODUCTION

Learning objectives

- Understand how to integrate liquid biopsy in clinical trials for breast cancer patients in terms of concepts of patient identification to surrogates of standard endpoints
- Understand the use of liquid biopsy profiling in breast cancer for prognosis, biomarker discovery and patient stratification
- Understand the use of liquid biopsy dynamics to capture tumour evolution and drug resistance in breast cancer
APPLICATIONS OF LIQUID BIOPSY IN BREAST CANCER

Programme

Welcome & Introduction
Dr. Monica Arnedos, Breast Unit, Institut Bergonié, Bordeaux, France

Integrating liquid biopsies in clinical trials and clinical practice for breast cancer
Dr. Michail Ignatiadis, Medical Oncology Department, Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium

The use of liquid biopsy profiling for prognosis, biomarker discovery and patient stratification in breast cancer
Dr. Pedram Razavi, Breast Medicine Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, US

The use of liquid biopsy dynamics to capture tumour evolution and drug resistance in early and advanced breast cancer
Prof. Nicholas Turner, Breast Unit, Royal Marsden; Institute of Cancer Research, London, UK

Q&A

Concluding remarks
Dr. Monica Arnedos, Breast Unit, Institut Bergonié, Bordeaux, France
INTEGRATING LIQUID BIOPSIES IN CLINICAL TRIALS AND CLINICAL PRACTICE FOR BREAST CANCER

Prof. Michail Ignatiadis

Medical Oncology Department
Jules Bordet Institute, Université Libre de Bruxelles
Brussels, Belgium
DISCLOSURES

Honoraria as invited speaker: Novartis
Honoraria for participation in Advisory Board: Novartis
Honoraria for participation in the Independent Monitoring Committee: Seattle Genetics
Institutional financial interest for research as a coordinating PI: Pfizer, Roche, Natera
Non-remunerated activities as a member of Board of Directors and officer: EORTC
LIQUID BIOPSY

Other tumour derived or associated factors (including extracellular vesicles, TEPs, miRNAs, stromal or immune cell subsets, proteins and metabolites)

tDNA

CTC cluster

CTC

Blood draw contains diverse tumour cells and products shed from multiple tumour sites

1. Early cancer detection

2. Surveillance for micrometastatic disease

3. Treatment selection and response monitoring in patients with metastatic disease

WEBINAR SERIES
OUTLINE

Integrating liquid biopsies in:

- clinical trials
- clinical practice
OUTLINE

Integrating liquid biopsies in:

- clinical trials
- clinical practice
Trastuzumab versus observation for HER2 nonamplified early breast cancer with circulating tumor cells (EORTC 90091-10093, BIG 1-12, Treat CTC): a randomized phase II trial

M. Ignatiadis1,2,*, S. Litière3, F. Rothe4, S. Riethdorf5, C. Proudhon6, T. Fehm6, K. Aalders3, H. Forstbauer7, P. A. Fasching5, E. Brain9, P. Vyhlídek10, E. Guardiola11, R. Lorenz12, K. Pantel9, K. Tryfonidis6, W. Janni13, M. Piccart1, C. Sotiriou1,2, B. Rack13 & J.-Y. Pierga5,14

- Is there a role for trastuzumab as adjuvant treatment in HER2 non-amplified breast cancer?
Retrospective analyses of the NSABP B31 and NCCTG N9831 trials\textsuperscript{1,2} suggested that women with breast cancer that was HER2-positive by local test but HER2-negative after central pathology review had similar benefit from adjuvant trastuzumab compared to women with HER2-positive tumours by both local and central test.

Trastuzumab might benefit women with HER2 non-amplified breast cancer by targeting HER2-low expressing stem cells\textsuperscript{3} or HER2-overexpressing Circulating Tumour Cells (CTCs)\textsuperscript{4}.

CTCs by CellSearch® is the only liquid biopsy biomarker with demonstrated clinical validity for its association with worse outcome in early breast cancer\textsuperscript{5}.

“TREAT CTC” DESIGN

- **R**: Randomisation
- **T**: Trastuzumab
- **O**: Observation

**CTC Blood tests:**
Women with HER2-negative breast cancer after (neo) adjuvant Chemo & surgery

Centrally confirmed ≥1 CTC/15mL by CellSearch®

W0

W18

EORTC
European Organisation for Research and Treatment of Cancer
The future of cancer therapy

ESMO
European Society for Medical Oncology
Webinar Series
OBJECTIVES

Primary objective
• To evaluate whether 6 cycles of trastuzumab eliminate CTC in patients with HER2-negative primary BC

Secondary objective
• To compare clinical outcomes between the trastuzumab and observation arms
MODELING CTC DETECTION AT WEEK 18 IN THE CONTROL ARM

- Probability that 1 CTC is found in 15 ml of blood when CTCs are actually present in a given patient at a concentration of 1CTC/7.5ml (Poisson distribution)\(^1\) : 80%

- CellSearch® assay efficiency\(^1\) : 80%

- Inter-reader variability\(^2\): 88%

- Effect of endocrine treatment on CTCs\(^3\)

Key assumption: CTC detection at week 18 in the observation arm will be 30%

With 156 planned evaluable CTC-positive patients, the study was designed to detect with 82% power a decrease in CTC detection rate at week 18 from 30% in the observation arm to 15% in the trastuzumab arm (1-sided alpha of 10%).

Assuming that 10% of randomised patients will not be evaluable at week 18 (e.g. due to a failed test, lost to follow-up) and that at baseline 8% of patients with HER2-negative primary tumour will have detectable CTCs, and will be available for randomisation, 2175 patients have to be screened for CTCs.
An interim analysis for futility and superiority was planned after half of the required number of evaluable patients were randomised, however the interim look was performed sooner than planned upon the recommendation of an independent data monitoring committee (IDMC), and led to the premature closing of the study accrual due to futility.
Patients CTC-positive (after central image review): 95

Patients randomised: 63

- Consent withdrawal: 4
- Primary tumour HER2-positive: 6
- Other 22

Trastuzumab: 31  Observation: 32

Trastuzumab: 29  Observation: 29

Eligible for the primary endpoint
## BASELINE CTCs

<table>
<thead>
<tr>
<th>N of CTCs</th>
<th>Trastuzumab N = 31</th>
<th>Observation N = 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21 (68%)</td>
<td>18 (56%)</td>
</tr>
<tr>
<td>2</td>
<td>3 (10%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>3</td>
<td>2 (6%)</td>
<td>4 (12%)</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>5+</td>
<td>5 (16%)</td>
<td>3 (10%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At least one CTC HER2+/3+</th>
<th>Trastuzumab N = 31</th>
<th>Observation N = 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>25 (81%)</td>
<td>23 (72%)</td>
</tr>
<tr>
<td>yes</td>
<td>6 (19%)</td>
<td>9 (28%)</td>
</tr>
</tbody>
</table>
## CTCs AT WEEK 18

| CTC test at week 18 | Trastuzumab  
| N = 31 | Observation  
| N = 32 |
|---|---|
| Negative | 24 (77%) | 25 (78%) |
| Positive | 5 (16%) | 4 (13%) |
| Missing | 2 (7%) | 3 (9%) |

Primary analysis (N = 58) evaluable patients:

**One-sided Fisher exact test p-value = 0.765**

The corresponding OR = 1.30 with one-sided 90% CI [0, 3.32]
Invasive disease free survival

NO DIFFERENCE IN iDFS

CONCLUSIONS

The Treat CTC trial raised concerns about the reproducibility of the CTC CellSearch assay in the early breast cancer setting

The trial failed to demonstrate that trastuzumab could decrease the CTC detection rate in women with HER2 non-amplified breast cancer following (neo)adjuvant chemotherapy and surgery

The Treat CTC trial results are in line with the NSABP-B47 study

Although clearly a negative trial, the ‘Treat CTC’ trial introduced for the first time the use of liquid biopsy as a tool for precision medicine in early breast cancer
ctDNA DETECTION USING PERSONALISED ASSAYS SEEMS MORE REPRODUCIBLE
# ctDNA RELAPSE IN EARLY BREAST CANCER

## Table 2 | Studies of ctDNA-based monitoring of treatment outcomes in patients with early-stage breast cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Disease subtype</th>
<th>Setting of ctDNA monitoring</th>
<th>ctDNA technology used</th>
<th>Number of patients included</th>
<th>Number of patients with evaluable ctDNA results</th>
<th>Median lead time from ctDNA relapse to clinical relapse (months)</th>
<th>DFS/RFS*</th>
<th>pCRb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olsson et al. (2015)171</td>
<td>All</td>
<td>Adjuvant</td>
<td>ddPCR</td>
<td>20</td>
<td>20</td>
<td>11</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Riva et al. (2017)102</td>
<td>TNBC</td>
<td>Neoadjuvant</td>
<td>ddPCR</td>
<td>46</td>
<td>38</td>
<td>NR</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Chen et al. (2017)153</td>
<td>TNBC</td>
<td>Adjuvant</td>
<td>134-gene NGS panel</td>
<td>38</td>
<td>33</td>
<td>&lt;8</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Garcia-Murillas et al. (2015 and 2019)136,137</td>
<td>All</td>
<td>Neoadjuvant and/or adjuvant</td>
<td>ddPCR</td>
<td>225</td>
<td>144</td>
<td>10.7</td>
<td>Yes</td>
<td>NR</td>
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<tr>
<td>Rothe et al. (2019)154</td>
<td>HER2+</td>
<td>Neoadjuvant</td>
<td>ddPCR</td>
<td>119</td>
<td>69</td>
<td>NR</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Coombes et al. (2019)145</td>
<td>All</td>
<td>Adjuvant</td>
<td>Signatera assay</td>
<td>50</td>
<td>49</td>
<td>8.9</td>
<td>Yes</td>
<td>NA</td>
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<tr>
<td>McDonald et al. (2019)144</td>
<td>All</td>
<td>Neoadjuvant/adjuvant</td>
<td>TARDIS</td>
<td>33</td>
<td>33</td>
<td>NR</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>Zhang et al. (2019)135</td>
<td>All</td>
<td>Adjuvant</td>
<td>68-gene NGS panel 136-gene NGS panel</td>
<td>102</td>
<td>102</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
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<tr>
<td>Radovich et al. (2020)136</td>
<td>TNBC</td>
<td>Post-neoadjuvant</td>
<td>FoundationACT or FoundationOne Liquid CDx</td>
<td>196</td>
<td>142</td>
<td>22.8</td>
<td>YES</td>
<td>NA</td>
</tr>
</tbody>
</table>

ctDNA, circulating tumour DNA; ddPCR, droplet digital polymerase chain reaction; DFS/RFS, disease-free survival or relapse-free survival; NA, not applicable; NGS, next-generation sequencing; NR, not reported; pCR, pathological complete response; TNBC, triple-negative breast cancer. *Association between ctDNA detection during follow-up surveillance after neoadjuvant and/or adjuvant chemotherapy and surgery and unfavourable DFS/RFS. **Association between ctDNA detection before and during the administration of neoadjuvant chemotherapy and a lower pCR rate.
ctDNA RELAPSE: A NEW INDICATION FOR DRUG DEVELOPMENT

Table 3 | ctDNA relapse as a new indication for clinical drug development in breast cancer

<table>
<thead>
<tr>
<th>Treatment setting</th>
<th>Metastatic</th>
<th>Adjuvant</th>
<th>Neoadjuvant</th>
<th>Post-neoadjuvant*</th>
<th>ctDNA relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curability</td>
<td>Uncurable</td>
<td>Curable</td>
<td>Curable</td>
<td>Curable</td>
<td>Unknown</td>
</tr>
<tr>
<td>Target</td>
<td>Overt metastatic disease</td>
<td>Treatment-naive MRD</td>
<td>Primary tumour and treatment-naive MRD</td>
<td>Treatment-resistant MRD</td>
<td>Treatment-resistant MRD</td>
</tr>
<tr>
<td>Methods for direct monitoring of treatment response</td>
<td>Imaging-based (e.g. by RECIST)</td>
<td>None</td>
<td>Pathological assessment of resected tumour tissue (i.e. for a pCR)</td>
<td>None</td>
<td>ctDNA elimination</td>
</tr>
<tr>
<td>Key trial end points</td>
<td>PFS and OS</td>
<td>iDFS and OS</td>
<td>pCR rates, EFS and OS</td>
<td>iDFS and OS</td>
<td>iDFS and OS</td>
</tr>
<tr>
<td>Example treatment</td>
<td>Trastuzumab</td>
<td>Trastuzumab</td>
<td>Trastuzumab or pertuzumab</td>
<td>Trastuzumab emtansine (T-DM1)</td>
<td>CDK4/6 inhibitors for HR⁺/HER2⁻ disease&lt;sup&gt;153&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ctDNA, circulating tumour DNA; EFS, event-free survival; HR, hormone receptor; iDFS, invasive disease-free survival; MRD, minimal residual disease; OS, overall survival; pCR, pathological complete response; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors. *Given immediately after surgery in those without a pCR.
# ctDNA Trial Designs in Early Breast Cancer

## Design 1
- Randomization
- ctDNA profiling and ctDNA-guided treatment
- No ctDNA profiling; primary tumour-guided treatment

## Design 2
- ctDNA testing
- Randomization
- Experimental treatment
- Standard treatment or observation

## Design 3
- ctDNA testing
- Randomization
- Experimental treatment
- Standard treatment or observation

## Design 4
- ctDNA testing
- Experimental treatment

## Design 5
- Comprehensive profiling of tissue and/or plasma ctDNA to identify druggable aberrations
- Aberration A
  - Experimental treatment A
- Aberration B
  - Experimental treatment B
- Aberration C
  - Experimental treatment C
- Aberration D
  - Experimental treatment D
- Aberration ...
  - Experimental treatment ...

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OUTLINE

Integrating liquid biopsies in:

- clinical trials
- clinical practice
ROADMAP FOR INTEGRATION OF A LIQUID BIOPSY ASSAY INTO CLINICAL PRACTICE

Development and validation of the assay
- Ensure analytical validity
- Establish clinical validity
- Demonstrate clinical utility

Regulatory approval
- Incorporation into guidelines
- Reimbursement

Incorporation into clinical workflow
- Invest in laboratory and human resources
- Train physicians in application of the test and interpretation of the findings
- Create standard operating procedures for application in different clinical scenarios

WEBINAR SERIES
# MODELS OF MOLECULAR PATHOLOGY ANALYSIS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Centralized</th>
<th>Decentralized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target service area</td>
<td>Central laboratory for several academic or community hospitals, at the regional, national or international level</td>
<td>Laboratory for one hospital</td>
</tr>
<tr>
<td>Resource requirements</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Wet laboratory workflow</td>
<td>Complex</td>
<td>Simple</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>Often needed</td>
<td>Often not needed (e.g. when a single-gene assay is used)</td>
</tr>
<tr>
<td>Tumour tissue assays</td>
<td>Multigene panels (can be PCR-based or NGS-based assays, such as Oncotype Dx, FoundationOne CDx, MSK-IMPACT, UW-OncoPlex and others)</td>
<td>ER protein expression and ALK translocation by IHC and FISH, respectively</td>
</tr>
<tr>
<td>ctDNA assay</td>
<td>Multigene assays, usually NGS-based (such as Archer, Avenio, FoundationOne Liquid CDx, Guardant360 CDx, Oncomine, MSK-ACCESS, Signatera, UW-OncoPlex CT and many others)</td>
<td>Single-gene PCR-based assays (for example, EGFR or PIK3CA assays)</td>
</tr>
</tbody>
</table>
| CTC assay               | CTC characterization assays (for example, Oncotype Dx AR-V7 
 | CTC detection assays (CELLSEARCH)                                             |                                      |

CTC, circulating tumour cell; ctDNA, circulating tumour DNA; ER, oestrogen receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing.
TEN TOP PRIORITY AREAS FOR LIQUID BIOPSY RESEARCH

- Standardization of preanalytical variables
- Use of artificial intelligence to improve liquid biopsy assays
- Data sharing and international academic–private collaboration
- Incorporation of liquid biopsy assays into the clinical workflow
- Evaluation of clinical benefit of targeted therapies when the relevant molecular aberrations are detected in plasma cell-free DNA but not in tumour tissue
- Demonstration of the value of circulating tumour cells (CTCs) as a complementary tool to circulating tumour DNA (ctDNA) in precision medicine
- Evaluation of CTCs and ctDNA as complementary adjuncts to standard imaging assessments
- Use of liquid biopsy to optimize treatment with cancer immunotherapy
- Improvement in overall survival through the detection and subsequent treatment of ctDNA relapse during follow-up surveillance after therapy for early stage disease
- Cancer diagnosis leading to improved overall survival without compromising quality of life
The use of liquid biopsy profiling for prognosis, biomarker discovery and patient stratification in breast cancer

Pedram Razavi, MD, PhD
Breast Medicine Service, Department of Medicine
Memorial Sloan Kettering Cancer Center
New York, NY, US
DISCLOSURES

Honoraria for participation in Advisory Board: Novartis, AstraZeneca, Foundation Medicine, Natera, Epic Science, Inivata
Institutional funding for research: Grail Inc, Novartis, Epic Sciences, ArcherDx
Compensated advisory role: Tempus
CELL-FREE DNA (cfDNA)
CIRCULATING TUMOUR DNA (ctDNA)

- Released through apoptosis, necrosis, phagocytosis and active secretion e.g. exosomes, etc.
- Cleared through elimination of nucleosomes in the liver (liver macrophages) or by circulating nucleases and kidney
- Short half life: Minutes to hours

**cfDNA shedding**
- Depends on tissue cell turnover (proliferation and apoptosis rates)
- Increased by cell injury due to inflammation, autoimmune conditions, cytotoxic therapy, etc.
- Increased by impaired clearance
- Varies by disease burden, site, tumour biologic features including histology, tumour subtype, tumour vascularisation, and proliferation and apoptosis rates

CTDNA AS A PROGNOSTIC BIOMARKER

cT-DNA quantity and mutational profile associated with overall survival

LIQUID BIOPSY

Robust biomarker with diverse clinical utilities

ctDNA mutation detection

WES/WGS

Variant allele fraction (log scale)

<table>
<thead>
<tr>
<th>100%</th>
<th>50%</th>
<th>20%</th>
<th>10%</th>
<th>5%</th>
<th>2%</th>
<th>1%</th>
<th>0.5%</th>
<th>0.2%</th>
<th>0.1%</th>
<th>0.05%</th>
<th>0.02%</th>
</tr>
</thead>
</table>

Whole exome or genome sequencing
100-300x

Suitable to identify

- Estimate ctDNA fraction
- Mutational processes
- Global mutational landscape of disease
- Copy number alterations
- Fragmentomics and tumor expression profile

Generally, not suitable for de novo mutation calling when ctDNA fraction is relatively low.
ctDNA mutation detection

Fixed targeted panel
High depth with UMI (barcoding)

Variant allele fraction (log scale)

| 100% | 50% | 20% | 10% | 5% | 2% | 1% | 0.5% | 0.2% | 0.1% | 0.05% | 0.02% |

Whole exome or genome sequencing
100-300x

Ultra-deep coverage NGS panels (*unique molecular indexes*) >20,000x

Broad application of cfDNA, especially for assessment of TMB and MSI, monitoring tumour evolution to identify mechanisms of resistance, or early detection requires:

- Sufficient genomic coverage to address intra- and inter-patient tumour heterogeneity.
- Potential increase in sequencing and biological noise.
TUMOUR MUTATIONAL BURDEN (TMB) AND TUMOUR HETEROGENEITY

10 hypermutated samples accounted for 60% of all cfDNA mutations and 75% of subclonal mutations across the entire cohort.

ctDNA AND DETECTION OF MSI

MSI-H detected by ctDNA and subsequent response to checkpoint inhibitors

LIQUID BIOPSY
For noninvasive cancer management

Molecular profiling at diagnosis  Longitudinal follow-up  Identifying actionable resistance mechanisms

ctDNA levels

Time from start of treatment

ctDNA TO MONITOR TUMOUR CLONAL EVOLUTION
Alterations in PTEN and ESR1 promote clinical resistance to alpelisib plus AI

EARLY ctDNA DYNAMICS PREDICT OUTCOMES

PIK3CA mut dynamics predict outcomes on Palbociclib plus Fulv.

EARLY ctDNA DYNAMICS PREDICT OUTCOMES
Early ctDNA response predicts response to checkpoint inhibitors

ESCALATION ADAPTIVE TRIALS IN METASTATIC DISEASE

Primary Endpoints
- PFS
- ctDNA response (molecular response)
- Objective response rate
DE-ESCALATION ADAPTIVE TRIALS IN METASTATIC DISEASE

Primary Endpoints
- PFS
- OS
ctDNA FOR MRD DETECTION

30ng cfDNA -> 9000 haploid genome equivalents

ctDNA MUTATION TRACKING FOLLOWING NEOADJUVANT CHEMOTHERAPY PREDICTS EARLY RECURRENCE IN PATIENTS WITH EARLY-STAGE BREAST CANCER

Median lead time=10.7 months to clinical relapse
PERSONALISED ctDNA TRACKING PREDICTS EARLY RECURRENCE IN PATIENTS WITH EARLY-STAGE BREAST CANCER


median=8.9 months
range: 0.5-24.0 months
IMPROVED MRD DETECTION WITH INCREASING THE NUMBER OF TRACKED VARIANTS


WEBINAR SERIES

Patients with Distant Recurrence
MRD Detected Post-op or Y1

YWBC_879 5.4 Mo. Lead Time  First Detected
YWBC_809 3.4 Mo.  MRD+
YWBC_315 18.9 Mo.
YWBC_29 3.6 Mo.
YWBC_342 34.5 Mo.
YWBC_136 39.2 Mo.

YWBC_879 5.4 Mo. Lead Time  First Detected
YWBC_809 3.4 Mo.  MRD+
YWBC_315 18.9 Mo.
YWBC_29 3.6 Mo.
YWBC_342 34.5 Mo.
YWBC_136 39.2 Mo.
ctDNA DYNAMICS ON NEOADJUVANT CHEMOTHERAPY
i-SPY2, n=84, Stage II-III

LIQUID BIOPSY TO DETECT AND INTERCEPT MOLECULAR MINIMAL RESIDUAL DISEASE

Diagram showing the relationship between ctDNA level and time, with stages including:
- Curative-intent Tx
- Escalation of Tx
- Primary Resistance
- Early Relapse
- Late Relapse
- Sensitive (Cured)
CIRCULATING TUMOUR CELLS (CTC)

- Released from the primary tumour or metastatic sites at any stage of tumorigenesis through trans-endothelial transition
- **CTCs characteristics** reflect the cancer type and stage (e.g. higher mitosis in TNBC)
- **Interactions with blood components** (e.g. platelets) are crucial for CTC survival, protection from the host immune system, and promoting metastasis potential
- Individual cells or in clusters (20–50x increase in metastatic capacity)
- ** Longer half life**: hours or days
CTC IN METASTATIC BREAST CANCER

CTC count and dynamics are strongly prognostic (SWOG-S0500)

CTC IN NEOADJUVANT SETTING
CTC count at baseline is prognostic

# CTC IN ADJUVANT SETTING

CTC count after surgery is prognostic

## Table 3. Circulating tumor cells in adjuvant therapy: main published studies and meta-analysis*

<table>
<thead>
<tr>
<th>References</th>
<th>No. of patients</th>
<th>Stage</th>
<th>Blood screened, mL</th>
<th>Detection rate, %</th>
<th>Prognostic impact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre. ACT</td>
<td>Post. ACT</td>
</tr>
<tr>
<td>Studies</td>
<td></td>
<td></td>
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<tr>
<td>Krishnamurthy et al.</td>
<td>92</td>
<td>I–III</td>
<td>7.5</td>
<td>31</td>
<td>—</td>
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<td>(33) (2010)</td>
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<tr>
<td>Franken et al.</td>
<td>404</td>
<td>I–III</td>
<td>7.5</td>
<td>18</td>
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<td>(31) (2012)</td>
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<tr>
<td>Lucci et al.</td>
<td>302</td>
<td>I–III</td>
<td>7.5</td>
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<tr>
<td>Karhade et al.</td>
<td>113</td>
<td>I–III (triple-</td>
<td>7.5</td>
<td>25</td>
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<td></td>
<td>negative)</td>
<td></td>
<td></td>
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<td>Rack et al.</td>
<td>2026</td>
<td>I–III</td>
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<td>(34) (2014)</td>
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<td>Van Dalum et al.</td>
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<td>Pooled-analysis</td>
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<td>Janni et al.</td>
<td>3173</td>
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<td>(12) (2016)</td>
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*ACT = adjuvant chemotherapy; CI = confidence interval; DFS = disease-free survival; CI = confidence interval; HR = hazard ratio; n.a. = not available; OS = overall survival; pts = patients.
COMBINED ctDNA/CTC ANALYSIS

WEBINAR SERIES
CONCLUSION

- Improving technologies enabling a wider utility and implementation of liquid biopsy in personalised oncology e.g. identifying actionable alterations, MRD detection, monitoring disease response, and clonal evolution.
- It is critical to consider the strengths and shortcomings of each specific ctDNA assay.
- ctDNA/CTC baseline levels have been shown to be prognostic in metastatic breast cancer.
- Early ctDNA/CTC dynamics can predict response to therapy and be utilized for adaptive therapeutic approaches.
- Serial liquid biopsy can provide insight on mechanisms of response and resistance to therapy and can guide biomarker discovery.
- Pressing need for designing and executing adaptive clinical trials based on liquid biopsies to establish their clinical utility.
The use of liquid biopsy dynamics to capture tumour evolution and drug resistance in early and advanced breast cancer

Prof. Nicholas Turner
Breast Unit, Royal Marsden
Institute of Cancer Research
London, UK
DISCLOSURES

I have received advisory board honoraria from Astra Zeneca, Bristol-Myers Squibb, Lilly, Merck Sharpe and Dohme, Novartis, Pfizer, Roche/Genentech, GlaxoSmithKline, Zentalis pharmaceuticals, Repare therapeutics, Arvinas and research funding from Astra Zeneca, BioRad, Pfizer, Roche/Genentech, Merck Sharpe and Dohme, Guardant Health, Invitae, Inivata, Personalis, Natera
CIRCULATING TUMOUR DNA

- Cancers release circulating tumour DNA (ctDNA) into the bloodstream
- Detectable in >90% patients with metastatic cancer
- ctDNA testing may provide a current assessment of tumour genetics and allow repeated testing
- ctDNA testing may assess tumour heterogeneity to comprehensively describe the genomic landscape of advanced cancer
• Paired ctDNA exome analysis 14 patients
• Clonal selection observed in 85% patients

Acquired truncating \textit{RB1} mutations in 4.8\% of palbociclib treated patients

ESR1 mutation detected in 56% (22/39) patients
Lead time median 6.7 months (95% CI 3.7-NA) prior to clinical progression.

Two phase III studies of intervention for rising ESR1 during AI +CDK4/6
PADA1 (fulvestrant) and SERENA6 (camizestrant)
plasmaMATCH
Study outline

Primary objective
• Response rate of therapies matched to mutations in ctDNA

Secondary objective
• Frequency of targetable mutations
• Accuracy of ctDNA testing
• Proportion of patients entering a cohort
• Activity in clonally dominant vs sub-clonal ESR1 mutations

Advanced breast cancer with measurable disease
Progressed on prior therapy for ABC or relapsed within 12m adjuvant chemotherapy
Up to 2 prior lines chemotherapy for ABC

ctDNA testing
Droplet Digital PCR
Sequencing – Guardant 360 +

Actionable mutation identified

Consent for treatment cohort

Cohort A
ESR1 mutation
Extended-dose fulvestrant

Cohort B
HER2 mutation
Neratinib (plus fulvestrant in ER+ BC)

Cohort C
AKT1 mutation (ER+ BC)
Capivasertib and fulvestrant

Cohort D
AKT basket
AKT1 (ER- BC)
PTEN mutation
Capivasertib

Cohort E*
TNBC with no mutation
Olaparib and AZD6738

*Prospective from part way through recruitment (n=364), retrospective in remaining patients (n=436)
Mutational profiling of 1,500 advanced disease single site biopsies

Acquired genetic changes

Each metastasis develops a distinct mechanism of resistance
POLYCLONAL RESISTANCE IN ER POSITIVE ADVANCED BREAST CANCER

Different mechanism of resistance to endocrine therapy pre-exist in the same patient.
POLYCLONAL RESISTANCE AND OVERALL SURVIVAL IN plasmaMATCH

Which genes are more likely to be subclonal – occurring in only a single or few metastasis?

Are there specific processes that drive this process?
SUBCLONAL HETEROGENEITY: CLONAL DOMINANCE OF MUTATED GENES

- **AKT1, PIK3CA, GATA3 and TP53** alterations are frequently clonally dominant
- **ESR1, RB1, SMAD4 and KRAS** alterations are frequently subclonal

SUBCLONAL HETEROGENEITY: CLONAL DOMINANCE OF TARGETABLE GENES

• ESR1 and PIK3CA demonstrate significant variation in clonal dominance within their pathogenic mutations
  - ESR1 D538G and Y537S most clonally dominant
  - PIK3CA H1047R and L, N345K, and G1049R are most clonally dominant

**PIK3CA SUBCLONAL MULTIPLE MUTATIONS IN HR+ DISEASE**

Cancers with multiple PIK3CA mutations have been hypersensitive to PI3 kinase inhibitors.

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*PIK3CA* mutations in pre-treated HR+ disease:
- 24% multiple mutations

**WEBINAR SERIES**

PATTERNS OF SUBCLONAL HETEROGENEITY: MUTATIONAL SIGNATURES

- Signatures present in clonally dominant versus sub-clonal mutations
- HR+HER2- subclonal mutations enriched in APOBEC-related signatures
- TNBC subclonal mutations enriched in a time-related signature
- Exploratory analysis given targeted panel

Subclonal defined as VAF < 0.5 of maxVAF

Clonally dominant and subclonal alterations aggregated per phenotype and a bootstrap analysis for mutational signatures undertaken using deconstructsigs

*p-values calculated using Mann-Whitney U test

PIK3CA SUBCLONAL DOUBLE MUTATIONS AT APOBEC SITES IN HR+ DISEASE

PIK3CA mutations in HR+ disease:
Second mutation frequently subclonal and at APOBEC mutagenesis sites

Mean Cancer Fraction
Proportion Single Mutation
APOBEC site

APOBEC site
non-APOBEC site

HR+, HER2-
p<0.0001

TNBC
NS

Cancer fraction = VAF/patient maximum VAF
Mean clonal fraction of alterations occurring at the locus
Minimum 4 mutation occurrences for inclusion, except ERBB2 indels (all included)
Significance calculated with Fisher's Exact
Double PIK3CA mutations hyperactivate PI3 kinase signalling

TUMOURS WITH DOUBLE PIK3CA MUTATIONS ARE PI3 KINASE INHIBITOR SENSITIVITY

**Taselisib response**

**MCF10A dose response to alpelisib**

- Fraction cell viability
- Log$_2$ [alpelisib] (µM)

**Graphs**

- **D** Single PIK3CA mutant ctDNA population
- **E** Multiple PIK3CA mutant ctDNA population

**Objective response rate (%)**

- Placebo (n=80) vs. Taselisib (n=193)
- Placebo (n=23) vs. Taselisib (n=43)

DYNAMIC MONITORING OF CANCER
CHANGES IN ctDNA ABUNDANCE THROUGH THERAPY
ctDNA response followed by resistance

Weekly paclitaxel + AKT inhibitor (AZD5363)

Offers potential to personalize the duration of chemotherapy

EARLY ctDNA DYNAMICS AS AN EFFICACY SURROGATE

**BEECH – paclitaxel +/- capivasertib**

Hazard ratio 0.20 (0.083 – 0.50)

- **n=10, events = 9 (high)**
- **n=32, events = 24 (suppressed)**


**PALOMA3 – palbociclib + fulvestrant**

- **Median PFS 11.2m (95%CI 11.1 – X)**
- **Median PFS 4.1m (95%CI 3.6 – 5.5)**

**Log rank p value <0.0001**

**EARLY ctDNA DYNAMICS AS AN EFFICACY SURROGATE**
PIK3CA ctDNA DYNAMICS TO DIRECT COMBINATION THERAPY

Poor ctDNA suppression on cycle 1 day 15

Randomise

1) Palbo + fulvestrant
2) Palbo + fulvestrant + AKTi

CONCLUSIONS

• Assessment of genomic heterogeneity with ctDNA assays identifies poor prognostic groups, and potential for PI3 kinase inhibitor super-responders

• Sequential ctDNA tracking can identify resistance mutations months prior to clinical progression
  - Interventional studies testing ESR1 mutations PADA1 and SERENA6

• Early dynamic changes provide an opportunity to guide therapy
  - Intervention studies now required
APPLICATIONS OF LIQUID BIOPSY IN BREAST CANCER

Dr. Monica Arnedos
Head of breast cancer research
Institut Bergonié, Bordeaux, France
Honoraria as invited speaker: Novartis
Honoraria for participation in Advisory Board: Puma Biotechnology, AstraZeneca, AbbVie
Honoraria to institution for masterclass: Roche, Genomic Health
Honoraria to institution for preceptorship: Novartis
Institutional financial interest for research as a coordinating PI: Novartis and Puma Biotechnology
APPLICATIONS OF LIQUID BIOPSY IN BREAST CANCER

Before treatment
- Identify actionable molecular alterations
- Prognosis
- Predictive biomarkers

During treatment
- Clonal Evolution
- Anticipate response to therapy
- Predict outcome (early progression)
- For on-treatment management:
  - Escalation and de-escalation strategies
  - Personalise duration of treatment

After treatment
- To detect mechanisms of secondary resistance
- Minimal residual disease
- Identify early relapse