Circulating Tumor Cells: Isolation, enrichment & clinical value

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Disclosure slide

I have received consultancy fees from Janssen Diagnostics,

I will discuss investigational use of trastuzumab.
Outline

• Introduction on CTCs

• Are there any data supporting the **clinical utility** of CTCs in metastatic breast cancer?

• Could CTC elimination be used as an **early signal of drug activity** in non-metastatic breast cancer?

• What is the best tissue source for **molecular characterization**: primary tumor, metastatic biopsy, ctDNA or CTC?
CTC detection technologies
**CellSearch® (FDA-cleared)**

EpCAM-positive selection

<table>
<thead>
<tr>
<th>Composite</th>
<th>CK</th>
<th>DAPI</th>
<th>CD45</th>
<th>Control</th>
</tr>
</thead>
</table>

CTCs:  
CK+/DAPI+/CD45-

Leukocyte:  
CK-/DAPI+/CD45+
CTC detection: poor outcome in metastatic breast cancer

Level I evidence that CTC detection is associated with worse prognosis in MBC

N= 177 pts, 49% (≥ 5CTCs)
HR = 4.26
p<0.0001

N= 1.944 pts, 47% (≥ 5CTCs)
HR = 2.77
p<0.0001

Cristofanilli M et al. NEJM 2004
Bidard FC et al. Lancet Oncology 2014
Are there any data supporting the **clinical utility** of CTCs in metastatic breast cancer?
CTCs drawn at baseline prior to 1\textsuperscript{st}-line chemotherapy

- CTC < 5
  - **Arm A**
    - Monitor for PFS & OS
    - 276 (46%)

- CTC ≥ 5
  - **Arm B**
    - Maintain 1\textsuperscript{st}-line chemotherapy until progression
    - 163 (57%)

CTCs drawn 3 weeks after 1\textsuperscript{st} dose of chemotherapy

- CTC < 5
  - **Randomized**
    - 123 (43%)
    - 1\textsuperscript{st} endpoint OS

- CTC ≥ 5
  - **Arm C1**
    - Maintain 1\textsuperscript{st}-line chemotherapy
  - **Arm C2**
    - Switch to alternate therapy

624 Registered
595 Eligible

29 ineligible or no screening CTC result

33 without 2\textsuperscript{nd} CTC test (death, progression, or refused)

Smerage J et al. SABCS 2013
S0500 did not meet primary endpoint

Overall Survival by Randomized Arm

- Not a failure of the CTC detection technology
- Negative answer to the scientific question
- 253 registered clinical trials in clinicaltrials.gov (e.g. STIC CTC, DETECT III, COMETI)
Could CTC elimination be used as an early signal of drug activity in non-metastatic breast cancer?
Endpoints in non-metastatic breast cancer: can we do better?

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Survival</td>
<td>Clinically relevant</td>
<td>Large trials, long f-up</td>
</tr>
<tr>
<td>Disease-free Survival</td>
<td>Clinically relevant</td>
<td>Large trials, long f-up</td>
</tr>
<tr>
<td>Pathological Complete Response</td>
<td>Small trials, short f-up</td>
<td>Prognostic, surrogacy not yet proven*</td>
</tr>
<tr>
<td>CTC elimination</td>
<td>Small trials, short f-up</td>
<td>Prognostic, surrogacy not proven</td>
</tr>
</tbody>
</table>

*Cortazar P et al. Lancet 2014*
CTCs detection: poor outcome in early breast cancer

444 pts, detection rate 40% (CK19mRNA)

2847 pts, detection rate 20% (CellSearch®)

Ignatiadis M et al. JCO 2007

Pierga JY et al. CCR 2008
Bidard FC et al. Annals of Oncology 2010
Rack B et al. Recent Results Cancer Res 2012
Lucci A et al. Lancet Oncology 2012
Franken B et al. BCR 2012
Can we use the CellSearch technology in early breast cancer (low CTC counts), in an international, multilab clinical trial?
Inter-reader variability for CTCs

8 Independent Veridex readers

22 Independent academic readers

Gallery of 272 images

Each image: CTC yes vs no

% Agreement Veridex consensus vs each academic reader

Ignatiadis M. et al BCR 2014
Conclusions of the inter-reader variability study

• Overall very good agreement between academic readers and Veridex consensus (VC) for CTC detection

• Lower agreement for images from patients with M0 disease, <5CTCs

• Continuous training, adherence to guidelines and independent image review is suggested in this setting

Ignatiadis M. et al BCR 2014
TREATuzumab in HER2-negative Early breast cancer as secondary Adjuvant Treatment for Circulating Tumor Cells

“Treat CTC” trial
“Treat CTC” design

CTC Blood tests:
- R: Randomization
- T: Trastuzumab
- O: Observation

After (neo) adjuvant Chemo & surgery
Objectives

Primary objective
➢ To evaluate whether trastuzumab eliminates CTCs in patients with HER2-negative primary BC

Secondary objectives
➢ To evaluate feasibility, reliability, within patient reproducibility of the CTC assay
➢ To evaluate the safety of trastuzumab in these women
➢ To compare clinical outcomes between the trastuzumab and observation arms
➢ To perform translational research
Sample Size: Screen 2150 patients to randomize 174

Accrual: 92 sites, 6 countries (Austria, Belgium, France, Germany, Greece, UK), 7 academic labs

CTC Labs QC program: Q 6-month spiking experiments, central image review

Current status: 56 patients screened, 2 patients randomized (Belgium, Germany)
Challenges

CTC detection rate: Low

Few CTCs detected: 1-2 CTCs per positive sample

High Discordance: 8 patients identified as CTC-positive but not confirmed after central image review

Action: EORTC organized webex TC (central labs & Janssen Diagnostics) and a consensus was reached: 4 out of 8 patients were considered as CTC-positive

Continuous training and adherence to guidelines for image interpretation (Ignatiadis et al BCR 2014)
Minimal residual disease in early breast cancer: sensitive technologies are needed

CellSearch: validated in ~3000 pts but low detection rate, low CTC counts (1-3CTCs/7.5ml of blood)

Newer CTC detection technologies

ctDNA, digital PCR, sensitivity 0.01%

Diaz L et al. JCO 2014
Beaver JA et al. CCR 2014
What is the best tissue source for molecular characterization: primary tumor, metastatic biopsy, plasma ctDNA or CTCs?
<table>
<thead>
<tr>
<th>Primary tumor</th>
<th>Metastatic biopsy</th>
<th>Plasma ctDNA</th>
<th>CTCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cells</td>
<td>Intact Cells</td>
<td>Fragmented DNA</td>
<td>Intact cells (few)</td>
</tr>
<tr>
<td>Accessible, mostly used</td>
<td>Invasive, not always accessible</td>
<td>Non-invasive, accessible, easy to process</td>
<td>Non-invasive, accessible, laborious to isolate</td>
</tr>
<tr>
<td>DNA, RNA, protein, cell culture, xenografts</td>
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Two approaches for the study of ctDNA

**Candidate mutation**

- Mutation(s) are known or first identified in the primary tumor and then followed in plasma \(^1,2,3,4\)
- Higher sensitivity, feasible even when low disease burden
- Resistance mechanisms must be known

**Unbiased**

- Direct plasma ctDNA detection without prior analysis of tumor\(^5\)
- Lower sensitivity, high disease burden required
- Can uncover new resistance mechanisms

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Mc Bride et al. Gene Chromosomes Cancer 2010  
Dawson et al. NEJM 2013  
Forshew et al. Sci Trans Med 2012  
Heitzer et al. Cancer Res 2013  
Murtaza et al. Nature 2013
Whole exome sequencing of CTCs: a window in metastatic prostate cancer

Mapping of >99.995% of the standard exome is possible in CTCs
Conclusions (I)

- There is now level I evidence that CTC detection using CellSearch is an adverse prognostic factor in metastatic breast cancer and ongoing clinical trials are testing its clinical utility.

- The ongoing Treat CTC trial is testing CTC elimination as an early signal of trastuzumab activity in HER2-negative early breast cancer.

- The role of more sensitive CTC detection technologies or ctDNA for monitoring minimal residual disease in the early breast cancer setting should be further explored.
• **Plasma ctDNA** should be prospectively tested as a tool for **treatment selection and monitoring** in clinical trials of patients with metastatic breast cancer

• Technological advances have allowed **CTC** analysis as a ‘liquid biopsy’ to **study tumor evolution**

• **CTC analyses** offers a unique window of opportunity to **assess treatment resistance** at the cellular level
‘Liquid biopsy’ for precision medicine

CTC chip → Mutation Profile → Algorithm for best combination Tx

GEP for pathways activation

Drug Sensitivity Profile
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Women with breast cancer