Circulating Tumor Cells: Isolation, enrichment & clinical value in breast cancer

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Disclosure slide

PI of the Treat CTC (educational grants from Janssen Diagnostics and Roche)

I will discuss investigational use of trastuzumab in my presentation
Outline

• Does single cell add to bulk tumor analysis for understanding breast cancer biology?

• Will CTCs diagnostics have an impact on the way we treat breast cancer?
No two single primary tumor cells are genetically identical

Clonal: bulk tumor

Sub clonal: $\geq 2$ single cells but not in bulk tumor

Wang et al. Nature 2014
In some tumors, cells disseminate early

Klein C et al. Science 2008
Outline

- Does single cell add to bulk tumor analysis for understanding breast cancer biology?

- Will CTCs diagnostics have an impact on the way we treat breast cancer?
CTC Enrichment

**Biological properties**
- Antibody (EpCAM+) selection
- Leucocyte Depletion
- Protein Secretion
- Invasion

**Physical properties**
- Size
- Density
- Electrical Charges

Methods:
- Beads
- Microfluidics

CTC Enrichment
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
CTCs drawn at baseline prior to 1\textsuperscript{st}-line chemotherapy

CTC < 5 → Arm A
- Monitor for PFS & OS

CTC ≥ 5 → Arm B
- Maintain 1\textsuperscript{st}-line chemotherapy until progression

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

CTC < 5 → Arm C1
- Maintain 1\textsuperscript{st}-line chemotherapy

CTC ≥ 5 → Arm C2
- Switch to alternate therapy

Randomized 1\textsuperscript{o} endpoint OS

29 ineligible or no screening CTC result

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

Smerage J et al. JCO 2014

624 Registered
595 Eligible

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S0500 did not meet primary endpoint

Overall Survival by Randomized Arm
Eligible Patients

- 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</td>
<td>Small trials, short f-up</td>
<td>Prognostic, surrogacy not yet proven*</td>
</tr>
<tr>
<td>Response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTC elimination</td>
<td>Small trials, short f-up</td>
<td>Prognostic, surrogacy not proven</td>
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</table>

CTCs detection: poor outcome in early breast cancer

444 pts, detection rate 40% (CK19mRNA)

2847 pts, detection rate 20% (CellSearch®)

![Graphs showing disease-free survival](image)

Ignatiadis M et al. JCO 2007

26-30 September 2014, Madrid, Spain

Pierga JY et al. CCR 2008
Bidard FC et al. Annals of Oncology 2010
Rack B et al. JNCI 2014
Lucci A et al. Lancet Oncology 2012
Franken B et al. BCR 2012
The first reported “liquid biopsy” trial

- HER2-negative early Breast Cancer
- Patient selection based on CK19 mRNA
- Randomized phase 2 single center study
- Trastuzumab (x6) vs observation (N=75 pts)

Tilastuzumab in HER2-negative Early breast cancer as secondary Adjuvant Treatment for Circulating Tumor Cells

“Treat CTC” trial
“Treat CTC” design

T: Trastuzumab
O: Observation
R: Randomization

CTC Blood tests:
R: Randomization

After (neo) adjuvant Chemo & surgery
W0
W18

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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
What will be the role of CTCs in the era of precision medicine?
What is the best tissue source for precision medicine?

<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>
<td>DNA, RNA, protein, cell culture, xenografts</td>
<td>DNA</td>
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</table>
Ion AmpliSeq™ Cancer Hotspot Panel v2 (50 genes)

Primary/metastasis

Plasma

76% CONCORDANT
Tumor + / plasma + (n=9)

24% DISCORDANT
Tumor + / plasma – (n=2)
Tumor - / plasma + (n=2)

Rothé et al. Ann Oncol 2014
Drug sensitivity in cultured CTCs

CTC lines generated in 6 of 36 ER+ MBC patients
‘Liquid biopsy’ for precision medicine

Omics (DNA, RNA, protein)

Algorithm for best Treatment

Input

Function

Output

Drug Sensitivity

CTC Isolation

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Conclusions (I)

**Single cell analysis** is challenging our understanding of breast cancer

- No two single primary tumor cells are genetically identical
- In some tumors, cell **disseminate early** and evolve in parallel with the primary tumor
Conclusions (II)

- 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.

- CTC-derived models offer a window of opportunity to assess treatment resistance in well designed clinical trials.
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Women with breast cancer

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