CTC molecular characterization: Are we ready to move forward with clinical testing?

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Breast cancer: Diagnostics / Treatment

Tumor Heterogeneity/evolution

Targeted Drugs
One Drug per Pathway: Trastuzumab

Liquid Biopsy
CellSearch®

2000
2013

Multiple Drugs per Pathway: PI3K/AKT/mTOR inh

Microfluidics
Circulating DNA
Where we stand?

• Active research for CTC molecular characterization
• >400 registered clinical trials using CTCs
• Almost all studies in metastatic disease
• Liquid biopsy: not yet in the routine clinical practice
CellSearch® (FDA-cleared)

Cristofanilli et al. NEJM 2004
CTC enumeration for treatment decision
## Ongoing trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>Question</th>
<th>Setting</th>
<th>Screen</th>
<th>Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWOG 0500 (phase 3)</td>
<td>Does an early treatment change based on elevated CTC counts after 1 cycle of chemo lead to improved OS?</td>
<td>MBC starting 1st line chemo</td>
<td>610</td>
<td>120</td>
</tr>
<tr>
<td>CirCE 01 (phase 3)</td>
<td>CTC count vs clinician choice to decide whether to administer chemo vs hormono?</td>
<td>MBC starting 3rd line chemo</td>
<td>600</td>
<td>304</td>
</tr>
<tr>
<td>STIC (phase 3)</td>
<td></td>
<td>MBC ER+/HER2- starting 1st line</td>
<td>&gt;994</td>
<td>994</td>
</tr>
</tbody>
</table>
CTC characterization using one marker (e.g HER2) for treatment decision
Lapatinib monotherapy in HER2-neg MBC

**HER2-positive CTCs**

139 Pts screened

7 (5%) Pts had >1 CTC/7.5 ml and ≥ 50% HER2+ CTCs (CellSearch®)

No response, 1 SD

**EGFR-positive CTCs**

43 Pts screened

16 (37%) Pts had >1 CTC/7.5 ml and ≥ 1 EGFR+ CTCs (CellSearch®)

No response, No SD

Pestrin et al BCRT 2012

Stebbing et al Plos One 2013
## Ongoing Trials

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<tbody>
<tr>
<td>Detect III (phase 3)</td>
<td>Can addition of lapatinib to standard treatment increase PFS?</td>
<td>M+ HER2- BC, 1 HER2+ CTC/7.5ml, 1st-3rd line</td>
<td>1428</td>
<td>228</td>
</tr>
<tr>
<td>CirCEX1 (phase 2)</td>
<td>Response rate with TDM1?</td>
<td>M+ HER2- BC, before 2(^{nd}) line, HER2+ CTCs by FISH</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>COMETI P2 (phase 2)</td>
<td>CTC endocrine therapy index?</td>
<td>M+ ER+/HER2-, starting a new ET</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
CTC characterization (beyond a single marker): A better tool (compared to cell lines & mouse models) to study Tx response / resistance?
Example 1. Can characterization of EMT on CTCs help understand and target treatment resistance?
Prevailing model of systemic treatment resistance in breast cancer

Tumor → Systemic treatment → Resistant cells (EMT / CSC-like phenotype) → Relapse
CTC Isolation

RNA ISH for a panel of Epithelial and Mesenchymal Markers

\[ E = \text{Epithelial State} \]
\[ M = \text{Mesenchymal State} \]
Contrary to the prevailing model, the E/M ratio on CTCs increases after treatment.
Increase in Mesencymal CTCs is associated with CTC clusters
The demonstration of dynamic changes in Epithelial and Mesenchymal composition of CTCs
• sheds light into the mechanisms of treatment resistance
• suggests new treatment targets and,
• can serve as a surrogate efficacy marker in trials using agents that target ‘stemness’ and EMT
Example 2. Can the study of CTCs gene expression improve drug development?
Cell lines differ from CTCs in gene expression

CTC isolation using the MagSweeper

Single Cell Gene expression of a panel of 31 selected genes

## Drug development: CTCs vs cell lines

<table>
<thead>
<tr>
<th>Differentially expressed transcripts</th>
<th>Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased in CTCs</td>
<td><em>FOXC1, KRT18, PTEN, NPTN, TGFβ1, KRT8, ZEB2, and CXCR4</em></td>
</tr>
<tr>
<td>Increased in cell lines</td>
<td><em>RRM1, AKT1, and AKT2</em></td>
</tr>
</tbody>
</table>

For early trials using e.g. an AKT-inhibitor, tailor drug dose based on PIK3CA/AKT pathway activity: CTCs or cell lines?
Example 3. Can we perform whole genome sequencing on single CTCs?
Whole genome sequencing of single cells

Spiking HCC38 cells

single / pool of cells

WGA Ampli1 followed by low coverage whole genome sequencing using the HiSeq 2000 ILLUMINA

Peeters et al. ISMRC 2013
Copy Number Variation single cell profiles of the HCC38 breast cancer cell line

Amplified DNA from single cells vs non amplified DNA from many cells (HCC38 cell line)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Copy Number Concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC1</td>
<td>78.8%</td>
</tr>
<tr>
<td>SC2</td>
<td>88.7%</td>
</tr>
<tr>
<td>SC3</td>
<td>76.2%</td>
</tr>
<tr>
<td>SC4</td>
<td>44.5%</td>
</tr>
</tbody>
</table>

Peeters et al. ISMRC 2013
DNA Copy Number: amplified DNA from single cells vs non amplified DNA from pool of cells

Yellow lines: amplified DNA
Red Lines: Non amplified DNA

Peeters et al. ISMRC 2013
Example 4. Is the study of CTCs suitable to capture tumor evolution?
Cancer evolution: Implications for treatment

Multiple, serial biopsies are needed to capture spatial and temporal tumor heterogeneity

Yates and Campbell et al. Nat Rev Gen 2012
Gerlinger et al. NEJM 2012
CTC analysis: a “druggable” CDK8 gain not present in primary tumor

CTC isolation using micromanipulation, WGA

Array CGH, NGS panel 68 genes

Heitzer E et al. Cancer Res 2013
Beyond CTCs: Circulating tumor DNA?
Primary Tumors (low coverage whole genome or targeted gene screen for selected mutations e.g. p53, PIK3CA)

ctDNA: Targeted approach

Targeted screen: feasible in samples with mutation present even in <1% of cDNA

Diehl et al. Nat Med 2008
Dawson et al. NEJM 2013
ctDNA: Unbiased approach

Exome sequencing: feasible if mutation present in at least 10% of cDNA
CTC or ctDNA?
Targeted or not?

Targeted approach

- Tumor or patient specific assays\(^1,2,3,4\)
- Feasible even when low disease burden in blood
- Better suited for non enriched samples (plasma cDNA)
- Lower cost

Unbiased approach

- Whole exome\(^3\) / genome sequencing
- Feasible only when high disease burden in blood
- Better suited for enriched samples (e.g. CTCs)
- Higher cost

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
Metastatic biopsies vs CTC vs cDNA
Who will be the winner?

Ongoing study
(N=10 metastatic breast cancer patients)

Ion Torrent
Ion AmpliSeq™ Cancer Hotspot Panel v2:
50 genes

Illumina HiSeq 2000
Exome Sequencing
Personal opinion

- Liquid biopsy will be the preferred option by physicians / patients for monitoring treatment resistance
- Circulating tumor DNA (apoptotic cells) to monitor known mutations that confer treatment resistance / sensitivity: Promising approach but no solid data today
- CTC molecular analysis (viable cells): a unique window to understand treatment resistance in humans
Early breast cancer: Can the use of “liquid biopsy” increase cure rates in breast cancer?
DTCs & CTCs:
 poor outcome in early breast cancer

4703 patients, detection rate 30%

2847 patients, detection rate 20% (CellSearch®)

Braun et al. NEJM 2005
Pierga et al. CCR 2008
Bidard et al. Annals of Oncology 2010
Rack et al. Recent Results Cancer Res 2012
Lucci et al. Lancet Oncology 2012
Franken et al. BCR 2012
Bone marrow DTCs display marked heterogeneity in early breast cancer: Is there a common driver?

Early breast cancer (M0 DTCs)  Metastatic breast cancer (M1 DTCs)

Klein et al. Lancet 2002
Klein Nature 2013
Trastuzumab targets CSCs in luminal breast cancer cells

MCF7: HER2 non-amplified
BT474: HER2 amplified
Effect of trastuzumab on mouse tumor xenograft depend on the timing of administration

HER2 non-amplified

HER2 amplified

Ithimakin S et al. Cancer Res 2013
The first reported “liquid biopsy” trial

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

“Treat CTC”

Screen: >2000 Pts with HER2- BC
Randomize: 174 CTC+ Pts (CellSearch®)

Blood tests:

R: Randomization
T: Trastuzumab
O: Observation

After (neo) adjuvant Chemo & surgery

Study started in Belgium
5 more countries by the end of the year

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Co-PIs: JY Pierga, C Sotiriou, B Rack, M Piccart
CTCs molecular characterization: A lot to be discovered in the coming years...
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Women with breast cancer

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