Patients with Primary Breast Cancer Treated with Primary Systemic Therapy

**Introduction**

- Tumor cells with a mesenchymal phenotype, including cancer stem cells (CSCs), are known to contribute to metastasis.
- Circulating tumor cells (CTCs) with epithelial phenotypes in peripheral blood can be detected using an anti-EpCAM antibody for capture, which may not detect CTCs undergoing epithelial-mesenchymal transition (EMT).
- We have developed an antibody-independent CTC enrichment platform, Apostream®, which does not rely on EpCAM-based capture.

**Objectives**

- Determine the clinical relevance and feasibility of measuring EMT CTCs in breast cancer patients.

**Methods**

- Blood samples from newly diagnosed breast cancer patients were prospectively collected before neoadjuvant systemic treatment (NST) (T₀), after NST (T¹), and after definitive surgery (T²) and processed using the Apostream® system.

**Results**

- Residual cells were stained with the following additional antibodies and examined on a laser scanning cytometer to identify 4 CTC subsets based on protein expression levels of various markers:
  - **Epithelial** (CK+, EpCAM+, or E-cadherin+)
  - **EMT** (β-catenin+ or vimentin+)
  - **Combined epithelial or EMT** (CK+, EpCAM+, E-cadherin+, vimentin+ or β-catenin+)
  - **CSC** (CD44+ and CD24−/−)
- Pathological complete response (pCR) to preoperative chemotherapy was correlated to CTC levels and marker expression.

**Table 1. Baseline characteristics of the patients.**

<table>
<thead>
<tr>
<th>No of patients</th>
<th>Mean age [range]</th>
<th>T stage</th>
<th>N stage</th>
<th>pCR</th>
<th>EMT CTCs</th>
<th>EMT CTCs</th>
<th>CSC CTCs</th>
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</thead>
<tbody>
<tr>
<td>47</td>
<td>51.7 (41-79)</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>0.12</td>
<td>0.2 (1-7)</td>
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**Table 2. Detection rate (≥ 1 cell) and mean number (range) of CTCs detected for each CTC phenotype.**

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<th>No of patients (%) mean (range)</th>
<th>Epithelial CTCs</th>
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<tr>
<td>T₀</td>
<td>24 (53)</td>
<td>25 (55)</td>
<td>28 (62)</td>
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<tr>
<td>T¹</td>
<td>15 (79)</td>
<td>17 (90)</td>
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<td>T²</td>
<td>21 (84)</td>
<td>18 (72)</td>
<td>22 (86)</td>
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**Conclusion & Future Perspective**

- Preliminary results indicate that Apostream® was successful in detecting EMT-CTCs in this ongoing prospective study. CTC (epithelial and EMT-CTCs) levels after chemotherapy predict pCR.
- We need to await for enrollment and follow-up data of 50 patients to be more conclusive.
- Changes in EMT CTC levels during treatment will be explored in all cohorts.

**Contact**

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**Fig. 1** Trial Design

**Fig. 2** CTC levels according to pCR status at baseline (A), after chemotherapy (B), and after surgery (C). Significant difference between pCR and non-pCR groups.

- β-catenin+ EMT-CTCs at T² are more likely to be detected at higher clinical stage (p=0.014).
- Vimentin+ EMT-CTCs at T² are more likely to be detected in HER2-negative breast cancers (p=0.016).
- Patients with a higher level of combined epithelial or EMT CTCs before surgery (T¹) are more likely to achieve pCR (p=0.038).

**Fig. 2** shows CTC levels according to pCR status at baseline (A), after chemotherapy (B), and after surgery (C).

- Significant difference between pCR and non-pCR groups.

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