Detection of CTC

**CTC-Trap**

EpCAM+ CTC are strongly associated with poor prognosis for patients. We investigated the presence of EpCAM- CTC in metastatic prostate cancer (mPC) and metastatic breast cancer (mBC) patients and related their presence with overall survival.

**Cell Line Recovery**

Prostate (PC3) and breast (MDA-MB-231) cancer cell lines were used for spiking in healthy controls at 3 separate occasions and processed at 6 different sites to validate the procedure.

<table>
<thead>
<tr>
<th>EpCAM antigens per cell</th>
<th>EpCAM+ CTC recovery</th>
<th>Unaccounted EpCAM- CTC recovery</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PC3</strong></td>
<td>1.0x10^4</td>
<td>27%</td>
<td>18 µm</td>
</tr>
<tr>
<td><strong>MDA-MB-231</strong></td>
<td>1.5x10^4</td>
<td>26%</td>
<td>16 µm</td>
</tr>
</tbody>
</table>

**Breast Cancer Patients**

- Mean EpCAM+ CTC: 13 CTC (n=22, min 0-max 208)
- Mean EpCAM- CTC: 8 CTC (n=22, min 0-max 35)
- EpCAM+ CTC ≥5 = 32%
- EpCAM- CTC ≥5 = 36%
- Using EpCAM+ and EpCAM- for ≥5 CTC: detection of patients increases to 64%

**Prostate Cancer Patients**

- Mean EpCAM+ CTC: 124 CTC (n=106, min 0-max 3300)
- Mean EpCAM- CTC: 3 CTC (n=93, min 0-max 24)
- EpCAM+ CTC ≥5 = 53%
- EpCAM- CTC ≥5 = 28%
- Using EpCAM+ and EpCAM- for ≥5 CTC: detection of patients increases to 70%

**Evaluation of CTC**

A collection of 100 objects were independently reviewed by 7 operators to determine consensus of scoring EpCAM+ and EpCAM- CTC. For 19 of the objects operators were divided whether the object was a CTC or not. For 5 objects all operators were in complete agreement it definitely being a CTC.

**Patient Population**

Patients from 6 clinical sites in the European CTC-Trap consortium were enrolled (a) in the study for detection of EpCAM+ CTC (by CellSearch) (b) and EpCAM- CTC (by filtration) (c), followed by quantification (d) and review analysis with the program ACCEPT (e).

**Conclusion**

In a multicenter study EpCAM+ and EpCAM- CTC were detected in mPC and mBC patients.

EpCAM+ CTC are associated with poor overall survival in mBC patients and EpCAM- CTC are not.

The clinical value of EpCAM- CTC in mPC remains questionable.