**EpCAM+ circulating tumor cells in metastatic cancer patients: a multicenter study**


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**Detection of CTC**

**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.

- **EpCAM antigens per cell**
  - **PC3**: 1.0±0.9
  - **MDA-MB-231**: 1.5±1.0
  - **EpCAM+ CTC recovery**
    - Unaccounted: 27%
    - Size: 21% (18 μm)
  - **EpCAM- CTC**
    - Unaccounted: 52%
    - Size: 18% (16 μm)

**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=196, 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.

**Conclusion**

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

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).

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

EpCAM+ CTC are associated with poor overall survival in mPC, but EpCAM- CTC Care not.

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

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**CTC-Trap**

EpCAM+ CTC: DAPI+, cytokeratin+, CD45-, round, >5µm in size, DAPI-CK overlay >50% EpCAM- CTC: DAPI+, cytokeratin+, CD45-, DAPI-CK overlay

**Acceptance CTC Scoring**

- Category 1: ≥90
d- Category 2: 14–90
d- Category 3: 2–14
d- Category 4: <2

**Survival**

- EpCAM+ CTC: Overall Survival
- EpCAM- CTC: Overall Survival

- Log Rank (p=0.002)
- Presence of EpCAM+ CTC is significantly associated with poor overall survival
- Presence of EpCAM- CTC is not associated with poor overall survival