

In vivo Isolation of Circulating Tumor Cells

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Abstract

Circulating tumor cells (CTCs) are mostly isolated *in vitro* from small volumes of patient blood samples. In order to circumvent this limitation we developed a functionalized and structured medical wire (FSMW) for *in vivo* application which enables the capture of CTCs from the patient's blood stream with high sensitivity. The medical wire is being inserted in a patient's vein for thirty minutes. Enumeration and characterization of those CTC will serve to improve and monitor clinical cancer treatment.

The interaction of target CTCs with the FSMW is mediated by an antibody directed against the epithelial cell adhesion molecule (EpCAM), an epithelial cell surface antigen which is expressed by many carcinomas. In our clinical study, we successfully isolated EpCAM-positive tumor cells originating from breast cancer patients. CTCs were isolated and identified by performing immunocytochemical staining against commonly used tumor markers. 54 applications of the FSMW were performed. Whereas 29 included healthy volunteers had no CTC on the FSMW, clinical results from 49 applications indicate a sensitivity of 89,7% and a significant higher CTCs capturing rate compared to the FDA-approved CellSearch[®] method.

Functional Structured Medical Wire

The biological functionalization of the wire is achieved using an antibody against the epithelial tumor marker EpCAM.

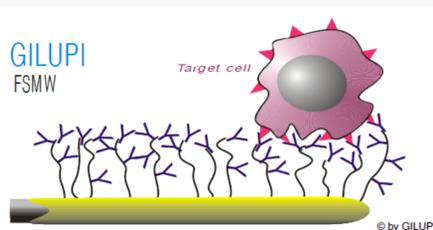


Figure 1: EpCAM antibodies bound to a hydrogel coating of the wire mediate specific binding of EpCAM expressing target cells.

Immunocytochemical analysis

a) Example 1
Her-2 negative CTC

b) Example 2
Her-2 positive CTC

b) Example 3
PBMC

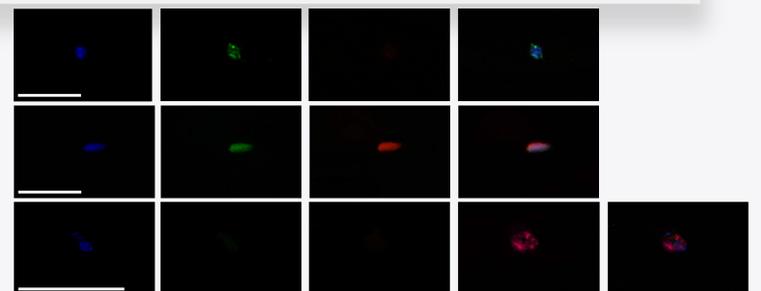


Figure 2: Immunocytochemistry analysis of CTCs captured *in vivo* with the FSMW in the blood of breast cancer patients. The CTCs were identified and enumerated via positive Cytokeratine, or EpCAM and Hoechst staining (respective green and blue staining in top panels, incl. overlay), size and morphological characteristics. HER-2 staining (orange) was taken as a possible therapeutic decision marker. The white scale bar corresponds to 50µm.

Study Design

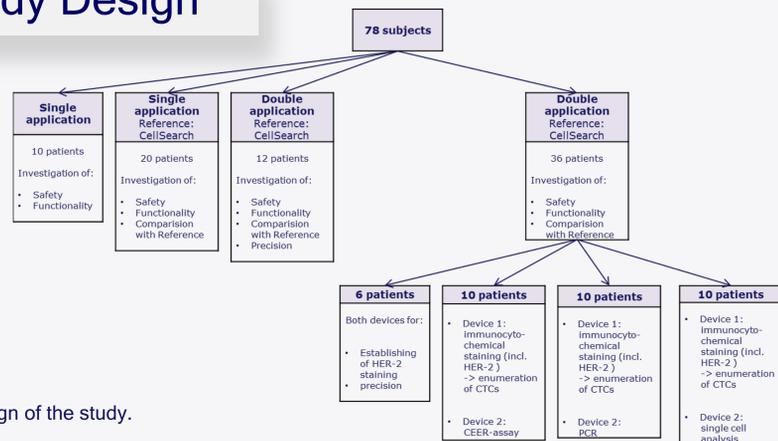


Figure 3: Design of the study.

Removing CTCs

Figure 6: Removing of a stained breast cancer cell line with a micromanipulator after immunocytochemistry analysis.



Patient Population

Title study	Total No. patients	Inclusion characteristics
FSMW EpCAM-Breast	42	Subjects suffering from breast cancer (diagnosed) • 30 patient with single application of the FSMW • 12 patients with double application of the FSMW

There were no AEs. All patients showed very good biocompatibility and no side effects. 29 included healthy volunteers had no CTC on the FSMW.

Results *in vivo* captured CTCs

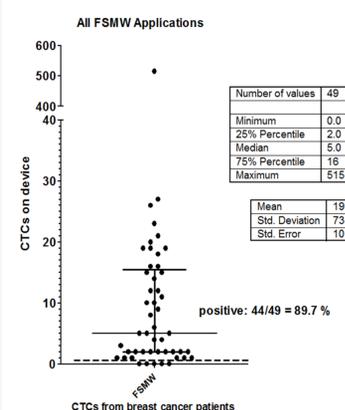


Figure 4: Results of CTCs captured *in vivo* with the FSMW in the blood of breast cancer patients.

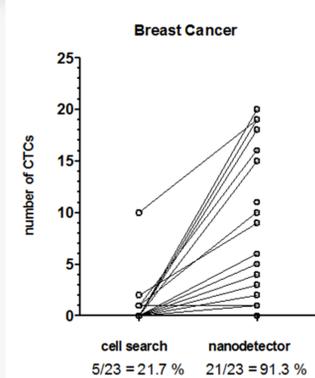
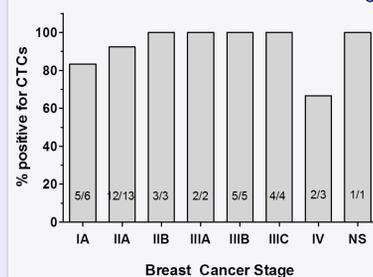


Figure 5: Comparison of the methods, the FSMW *in vivo* and the CellSearch[®] method *in vitro*.

Table 1: Distribution of the disease stages



- results from 37 subjects were included into the analysis (5 failed downstream analysis)
- CTCs could be detected in all tumor stage, including early stages

Summary

- CTCs *in vivo* captured with the FSMW resulted to **89,7% detection rate** in breast cancer patients, **specificity is 100%**.
- CTC detection rate with the FSMW is **5 times higher** than CTC capturing rate compared with the FDA-approved CellSearch[®] analysis,
- in **100% of paired samples: FSMW >= CellSearch[®]**
- double application of the device in the same patient indicates very good precision
- detection of CTC's could be shown in **all occurred tumor stages** (especially as well early stages)

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