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 CTCs 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.

Study Design

Functional Structured Medical Wire

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

Immunocytochemical analysis

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

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 (respectively 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.

Figure 3: Design of the study.

Patient Population

<table>
<thead>
<tr>
<th>Title study</th>
<th>Total No. patients</th>
<th>Inclusion characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSMW EpCAM-Breast</td>
<td>42</td>
<td>Subjects suffering from breast cancer (diagnosed) • 30 patients with single application of the FSMW • 12 patients with double application of the FSMW</td>
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There were no AEs. All patients showed very good biocompatibility and no side effects. 29 included healthy volunteers had no CTC on the FSMW.

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)