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 beeing 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. 42 applications of the FSMW were performed. Clinical results from 37 applications (5 failed down streaming analysis) indicate a sensitivity of 86,5% 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.

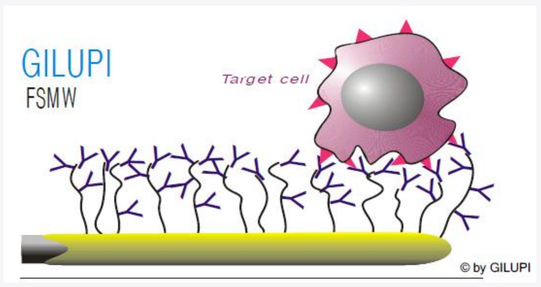


Figure 1: EpCAM antibodies bound to a hydrogel coating of the wire mediate specific binding of EpCAM expressing target cells.

Patient Population

Table 1: Number of patients included in the studies in Poznan (Poland). The application of the FSMW is preoperatively

Title study	Total No. patients	Inclusion characteristics
FSMW EpCAM-Breast	36	Subjects suffering from breast cancer (diagnosed) • 30 patient with single application of the FSMW • 6 patients with double application of the FSMW

There were no AEs. All patients showed very good biocompatibility and no side effects.

Immunocytochemical analysis

a) Example 1 **b) Example 2**

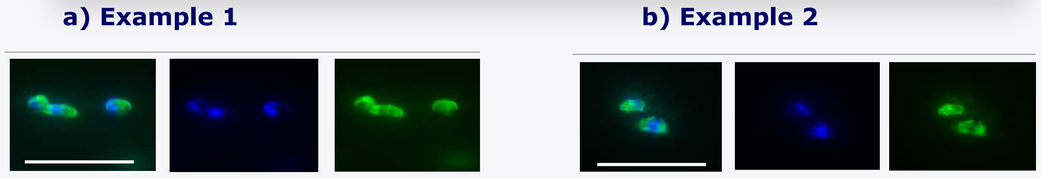


Figure 2: Immunocytochemistry analysis of CTCs captured *in vivo* with the FSMW in the blood of breast cancer patients. The CTCs were identified and enumerated via positive EpCAM and DAPI staining (respective green and blue staining in top panels, incl. overlay), size and morphological characteristics. The white scale bar corresponds to 50µm.

Results *in vivo* captured CTCs

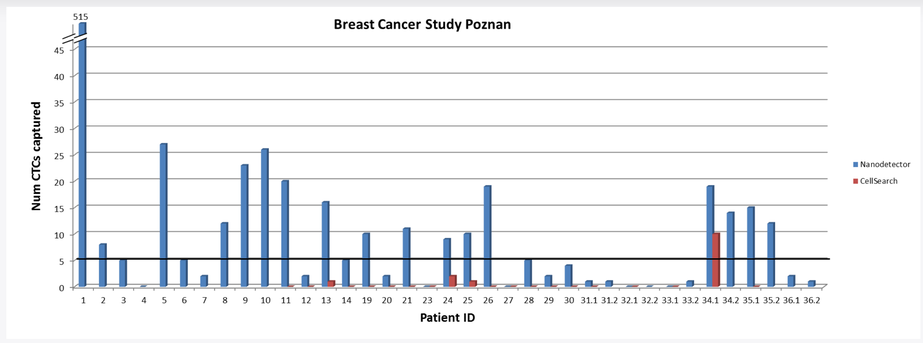


Figure 3: Comparison of the number of CTCs captured in breast cancer patients with the FSMW *in vivo* and the Cell Search® method *in vitro*.

Table 2: Results of CTCs captured *in vivo* with the FSMW in the blood of breast cancer patients.

a) Single application

PID	Tumor stage	Result FSMW *in vivo* Gilupi
001	T4N0M0	515
002	T3N1M0	8
003	T1N1M0	5
004	T1N1M1	2
005	T2N1M0	27
006	T4N2M0	21
007	T1N1M0	2
008	T4N+M0	12
009	T2N3M0	23
010	T1N3M0	26

b) Single application Reference: CellSearch

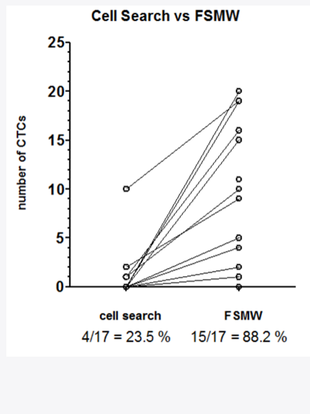
PID	Tumor stage	Result CellSearch Veridex	Result FSMW Gilupi
011	T2N1M0	0	20
012	T2N1M0	0	2
013	T2N0M0	1	16
014	T1N1M0	0	5
015	T1N0M0	0	n.a.
016	T1N0M0	0	n.a.
017	T2N0M0	0	n.a.
018	TxN0M0	0	n.a.
019	T1N0M0	n.a.	10
020	T2N2M0	0	2

PID	Tumor stage	Result CellSearch Veridex	Result FSMW Gilupi
021	T2N3M0	n.a.	11
022	TxN0M0	n.a.	n.a.
023	T2N0M0	n.a.	0
024	T4N0M0	2	9
025	T1N1M0	1	10
026	T1N0M0	0	19
027	T1N0M0	0	0
028	T1N0M0	0	5
029	T4N1M0	0	2
030	T1N0M0	0	4

c) Double application Reference: CellSearch (Investigation of the precision of the FSMW)

PID	Tumor stage	Result CellSearch Veridex	Result FSMW Gilupi	Deviation in %
031.1	TxN0M0	0	1	0
031.2			1	
032.1	T2N3M1	0	0	0
032.2			0	
033.1	TxN3M0	0	0	100
033.2			1	
034.1	TxN0M1	10	19	26,3
034.2			14	
035.1	T2N0M0	0	15	20
035.2			12	
036.1	T1N0M0	n.a.	2	50
036.2			1	

- reached precision of the FSMW up to 100% compliance of the values



- in 100% of paired samples:

  FSMW >= Cell Search

Figure 4: Comparison of the methods, the FSMW *in vivo* and the Cell Search® method *in vitro*.

Table 3: Distribution of the disease stages

Stage	T	N	M	Total application number	Positive tested for CTC's	Detection rate (%)
IA	T1	N0	M0	6	5	83,3
IIA	T1	N1	M0	4	4	100
	T2	N0	M0	3	2	66,6
IIB	T2	N1	M0	3	3	100
IIIA	T2	N2	M0	1	1	100
	T3	N1	M0	1	1	100
IIIB	T4	N0	M0	3	3	100
	T4	N+	M0	1	1	100
	T4	N2	M0	1	1	100
IIIC	Tx	N3	M0	1	1	100
	T1	N3	M0	1	1	100
	T2	N3	M0	2	2	100
IV	Tx	N0	M1	1	1	100
	T1	N1	M1	1	1	100
	T2	N3	M1	1	0	0
NS	Tx	N0	M0	1	1	100

- results from 31 subjects were included into the analysis (5 failed downstream analysis)
- CTCs could be detected in all tumor stage, including early stages

  (except in one patient with T2N3M1 were the FSMW was tested after chemotherapy)

Summary

- CTCs *in vivo* captured with the FSMW resulted to **86,5% detection rate** in breast cancer patients
- CTC detection rate with the FSMW is **5 times** higher than CTC capturing rate compared with the FDA-approved Cell Search analysis, in **100%** of paired samples: **FSMW >= Cell Search**
- 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)

Also founded by: