

The CellCollector™ technology

In vivo isolation of circulating tumor cells by the CellCollector™ system



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We Collect the Cells you search for

Unmet medical need in oncology:

- Access to tumor cells not only at the time of diagnosis
- Besides the established analysis of the primary tumor
- Biopsies from bone marrow or metastases?

Disadvantages:

- Risk in the procedure
- Limited acceptance
- High cost

CTC isolation as a liquid biopsy:

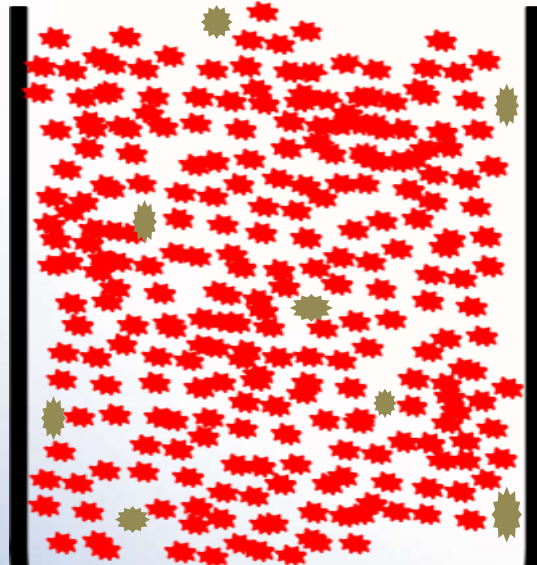
- Prognostic and predictive biomarker
- Assessment of treatment efficacy
- Comprehensive biomarker analysis

Disadvantage of current standard methods:

- Current standard diagnostic approaches for the extraction of CTCs are *ex vivo* methods
 - inherent limitation is the rather small blood sample they depend on

Difference between *in vivo* and *in vitro*

In vivo by GILUPI



1000 -1500 ml

In vitro methods

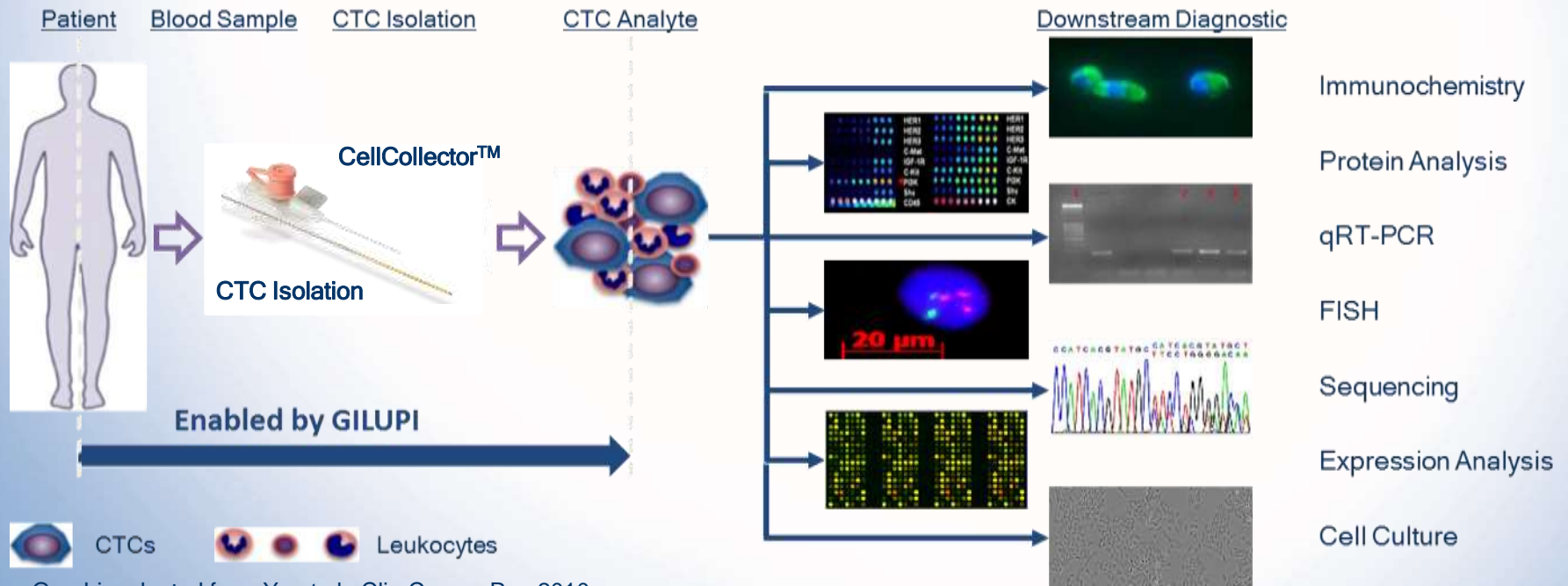


5-10 ml

The solution:

The CellCollector™ technology

CTCs as Liquid Biopsy for Comprehensive Biomarker Analysis



Graphic adapted from Yu et al., Clin Cancer Res 2010

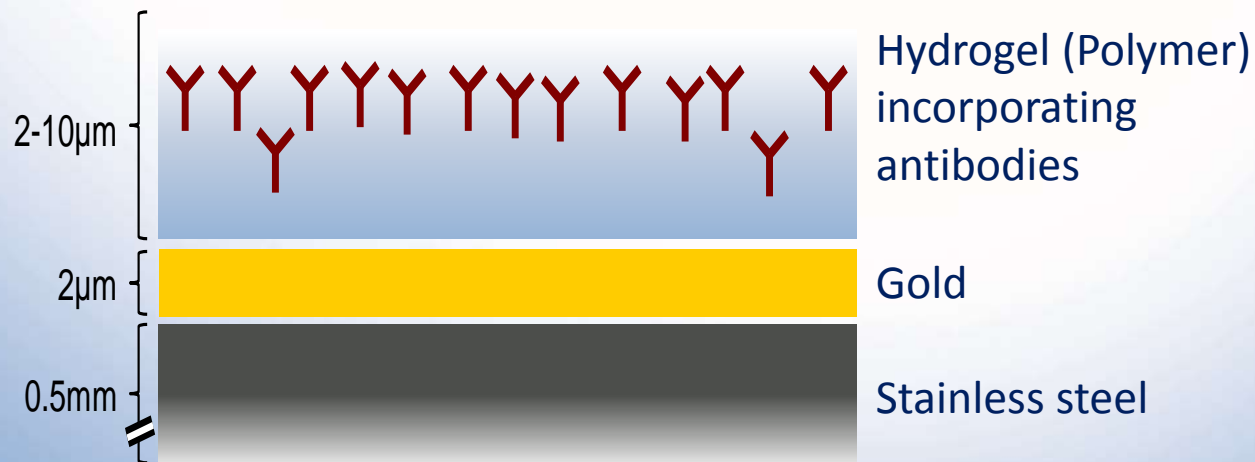
CTC's generated by GILUPI technology can be used with all common downstream diagnostic techniques

Description of the CellCollector™ I

- CE approved medical device (class IIa)
- Detektor CANCER01, Type FSMW EpCAM
(functional structured medical wire)
- Intended use: Isolation of EpCAM-positive CTCs from
peripheral blood

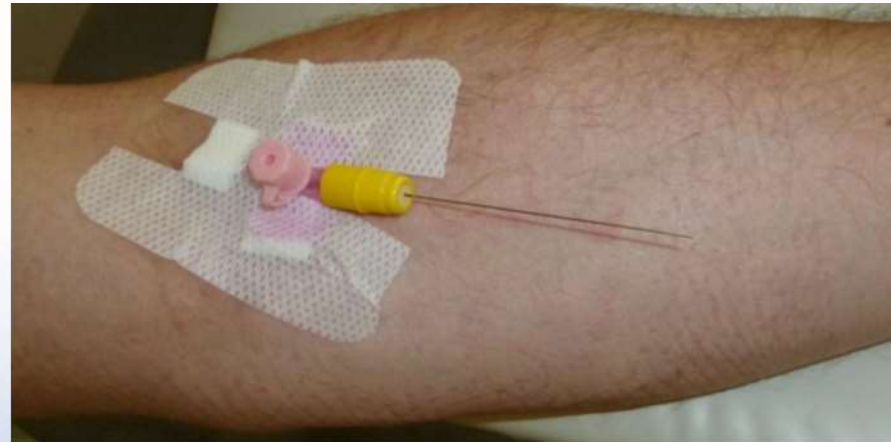
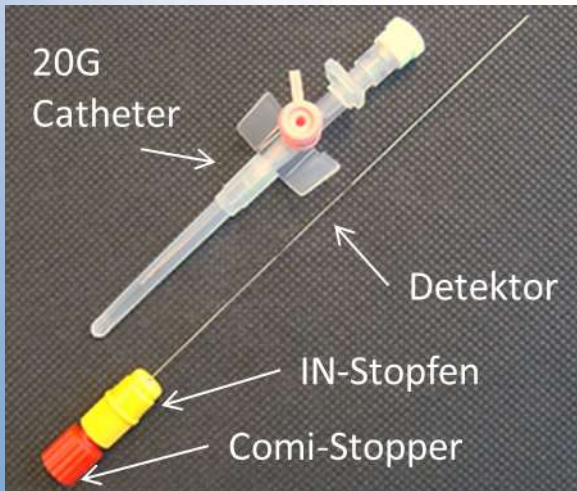
Description of the CellCollector™ II

- Bioreactive wire:
 - Flexible, stainless steel
 - 16 cm long
 - Rounded ends
- Two layer coating:
 - Gold
 - Hydrogel
- Incorporated in Hydrogel: specific antibodies targeting the antigen expressed by the searched cells



Application of the CellCollector™ I

- Use of a standard Venflon system for the application
 - 20G catheter
- By using the catheter system the CellCollector™ is inserted into the patients vein, the device is fixed by Luer-Lock system



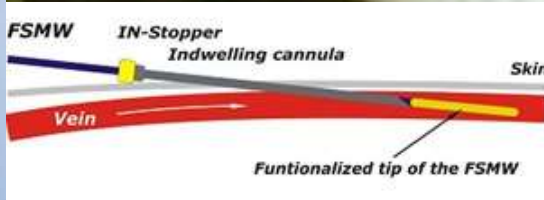
Application of the CellCollector™ II



Insertion into patient's Vein at the doctor's office



Exposure time 30 min



CellCollector™

Target cell

Hydrogel (1 - 5 μm) functionalized with antibodies to epithelial cell surface marker EpCAM, attached to a 0.2 μm thick gold layer

Part of the wire which remains inside the puncture cannula

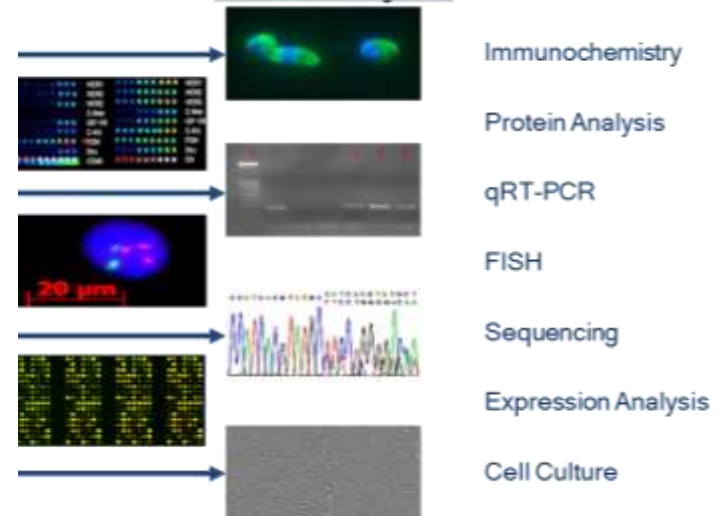
20 mm long gold-coated tip of the stainless steel wire which is in direct contact with the blood circulation



Result



Downstream Diagnostic



Main features:

The CellCollector™ technology

- *In vivo* isolation of circulating tumor cells
- An increased cell capture efficiency and a pure cell isolation can be reached

Main features:

- High efficiency, low risk, lower cost
- Use of various commercial downstream diagnostic approaches
- Simple integration into outpatient / inpatient hospital logistics
- Application possible at any hospital or doctor's office, regardless of its laboratory equipment

Clinical Data of the system

In vivo isolation of circulating tumor cells by the CellCollector™ system

S. Herold¹, M.R.Hoda², L. Gasiorowski³, P. Nowaczyk⁴, K.Müller¹, P.S. Kim⁵, T. Krahn⁶, W. Dyszkiewicz³, N.G. Morgenthaler¹, D. Murawa⁴, G.Theil², K. Luecke¹

¹ GILUPI GmbH, Potsdam, Germany

² Department of Urology and Kidney Transplant Center, Martin-Luther-University Halle-Wittenberg, Germany

³ Department of Thoracic Surgery, Medical University, Poznan, Poland

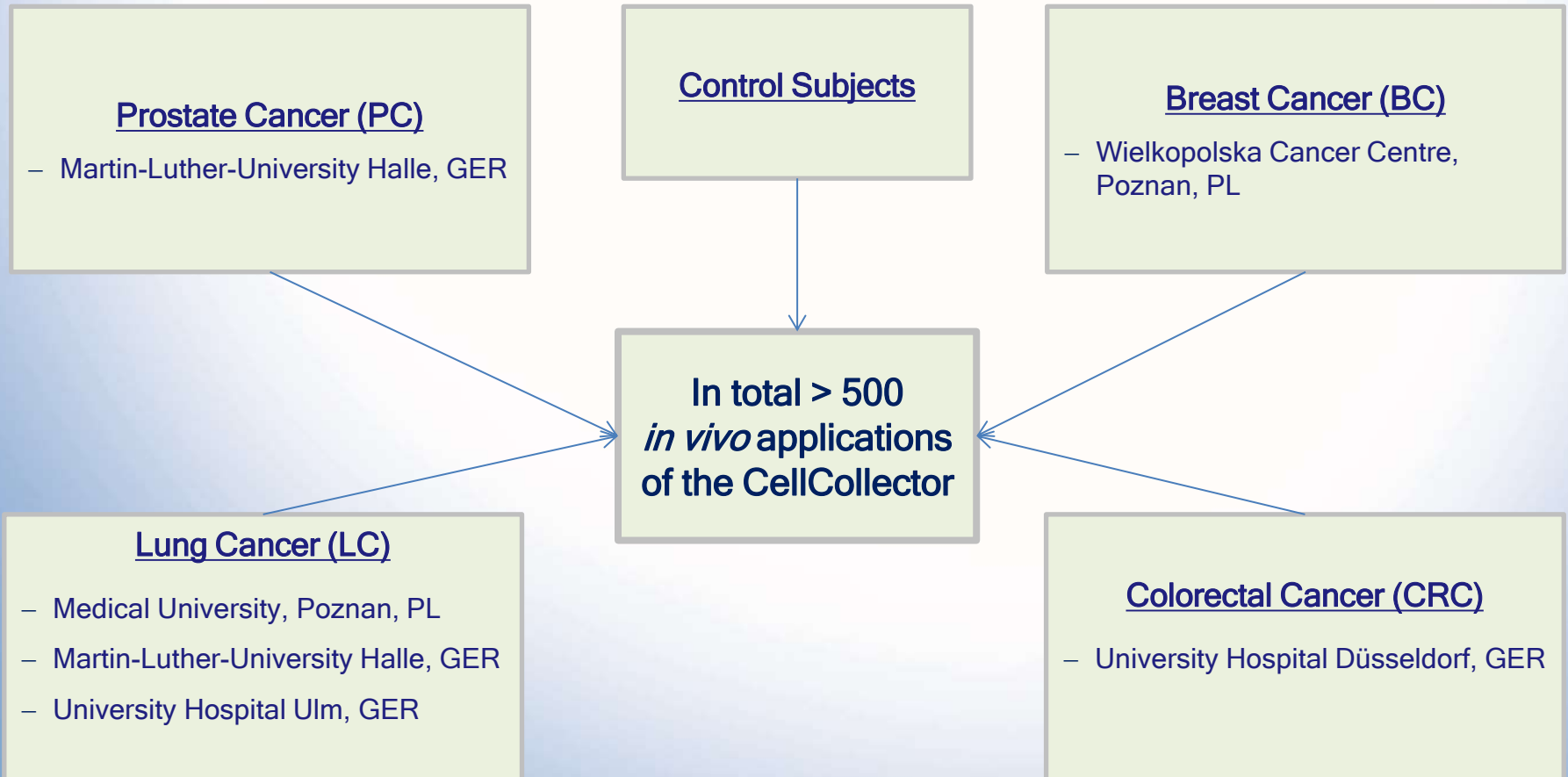
⁴ Wielkopolska Cancer Centre, Poznan, Poland

⁵ Prometheus Therapeutics & Diagnostics, San Diego, USA

⁶ Bayer Pharma AG, Berlin, Germany

Pooled clinical data of the CellCollector™

Data collective: pooled data form clinical applications of the method



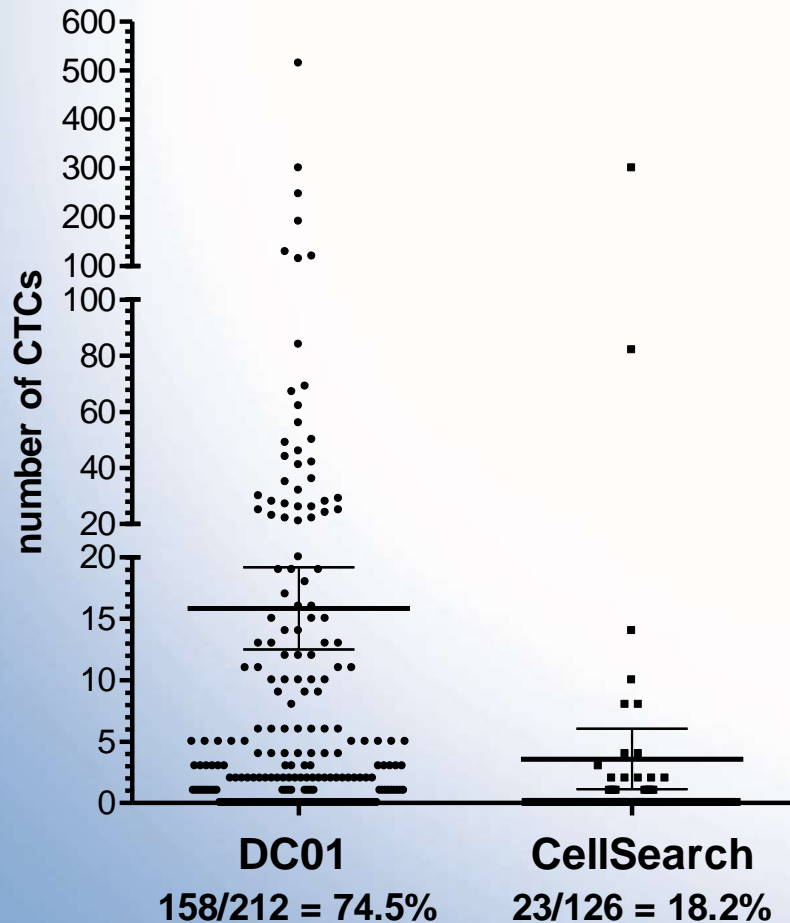
Safety of the device

- Application of the CellCollector was **well tolerated**, with **no product related adverse events by 100%** of the subjects (more than 500 applications)
- More than 95 % of subjects described the discomfort as “similar to a blood draw”

Efficacy of the device

- CellCollector detection rate **74,5%** (158 of 212)
- median (range) CTCs: 3 (0-515)
- mean 15 CTCs
- similar for early and late stage cancer patients

Current clinical data of the CellCollector™



	DC01 CANCER	CellSearch CANCER
Number of values	212	126
Minimum	0.0	0.0
25% Percentile	0.0	0.0
Median	3.000	0.0
75% Percentile	12.00	0.0
Maximum	515.0	300.0
Mean	15.85	3.587
Std. Deviation	48.63	27.65
Std. Error	3.340	2.464
Lower 95% CI of mean	9.270	-1.289
Upper 95% CI of mean	22.44	8.463
Sum	3361	452.0

Results of CTC enumerations with the Detektor CANCER01 compared to the CellSearch® method

Immunocytochemistry analysis of captured CTCs with the CellCollector™ I:

- Quadruple staining:
 - Identified and enumerated via positive staining:
 - Cytokeratin / EpCAM
 - Hoechst (nucleus)

 - Negative selection via staining:
 - CD45
 - CD16

 - size and morphological characteristics

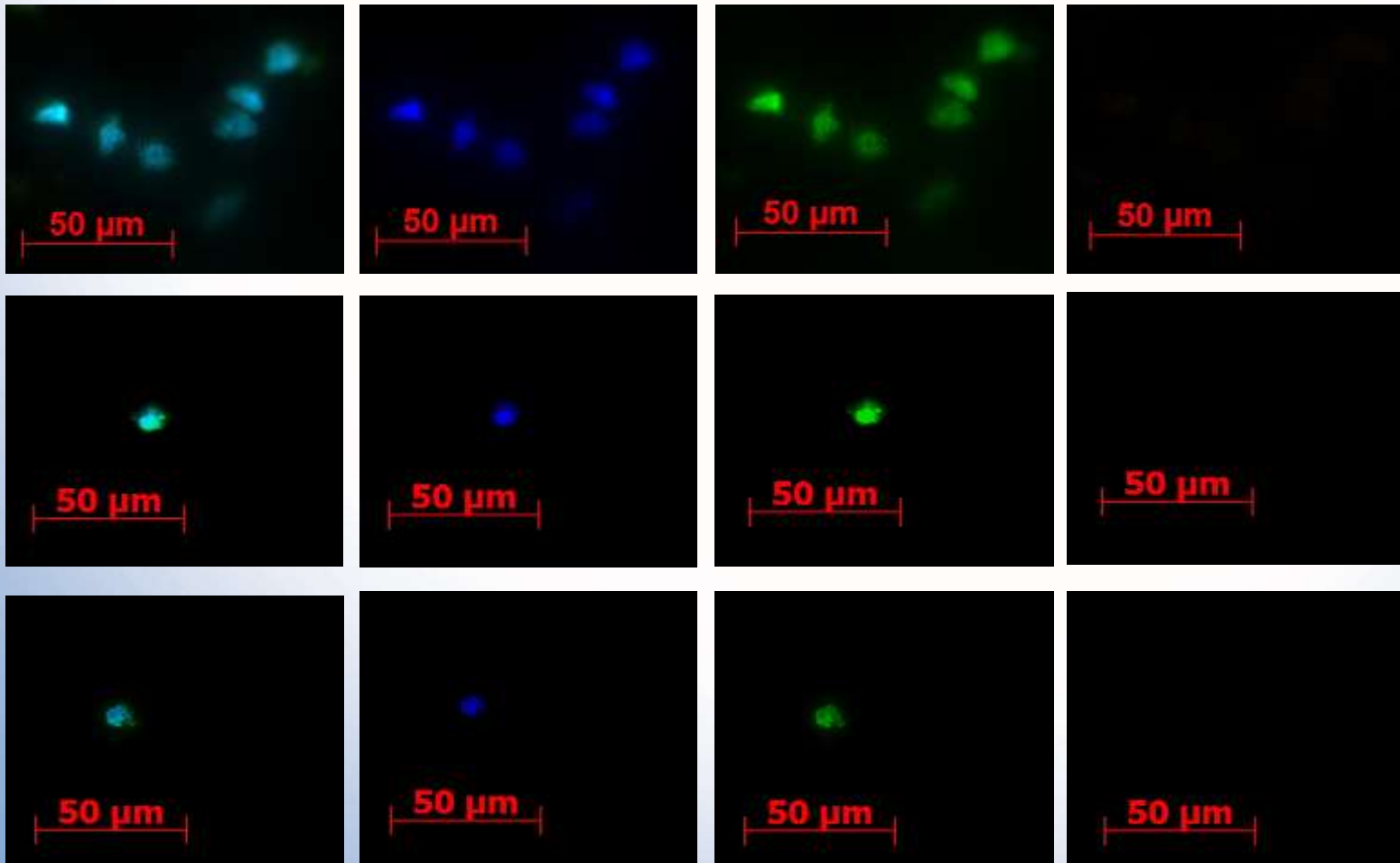
Immunocytochemistry analysis of captured CTCs with the CellCollector™ II:

OVERLAY

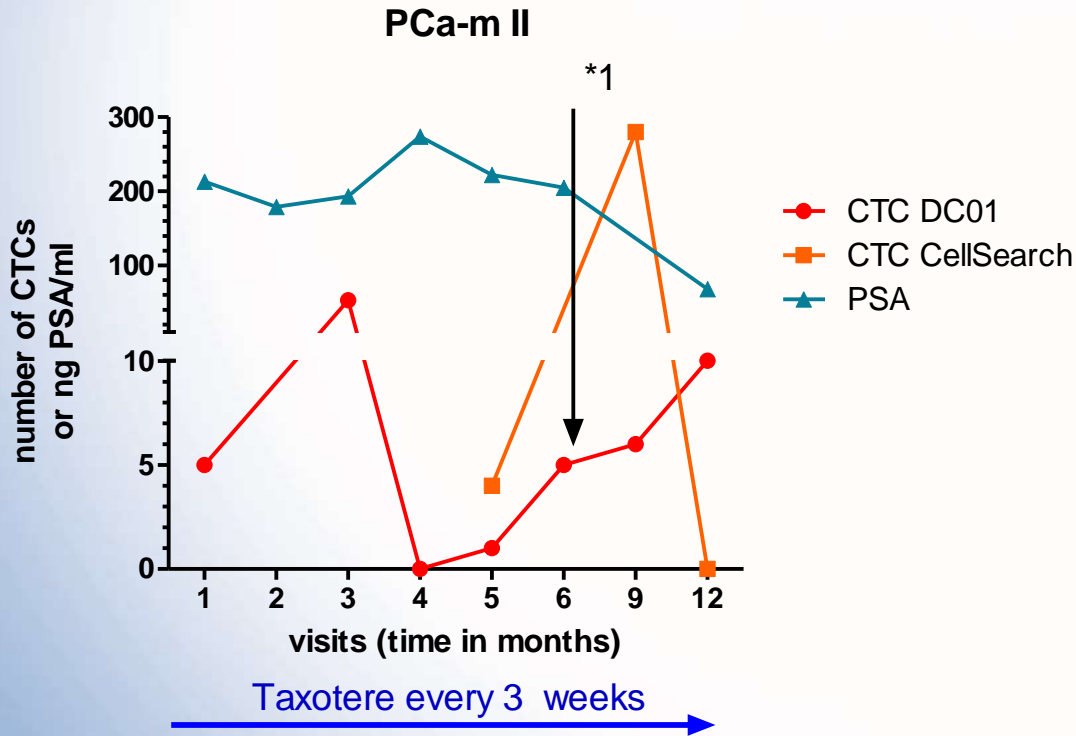
DAPI/ Hoechst

EpCAM/ CK

CD45/CD16



Example of individual CTC therapy monitoring II

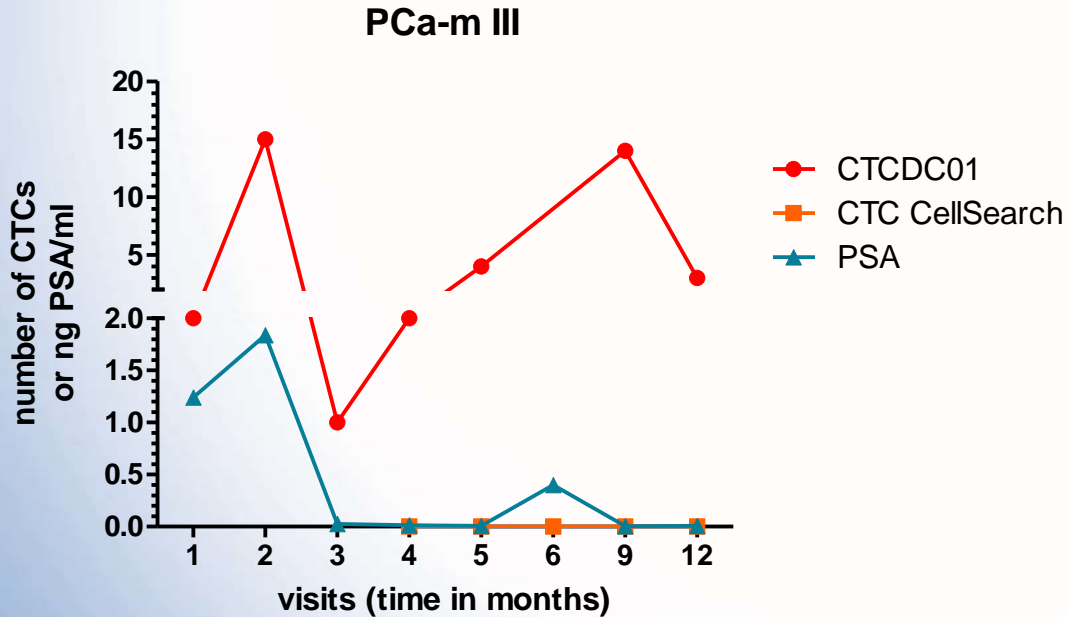


Visit	CTC DC01	CTC CS	PSA [ng/ml]
1	5	n.a.	213
2	Np	n.a.	179
3	53	n.a.	193
4	0	n.a.	274
5	1	4	222
6	5	Np	205
7	6	280	-
8	10	0	83.2

*1- start 2. chemotherapy (Cabazitaxel monthly) -3 cycle

- continuous radiotherapy
- PCa diagnosed: 1993 (+ PTx)

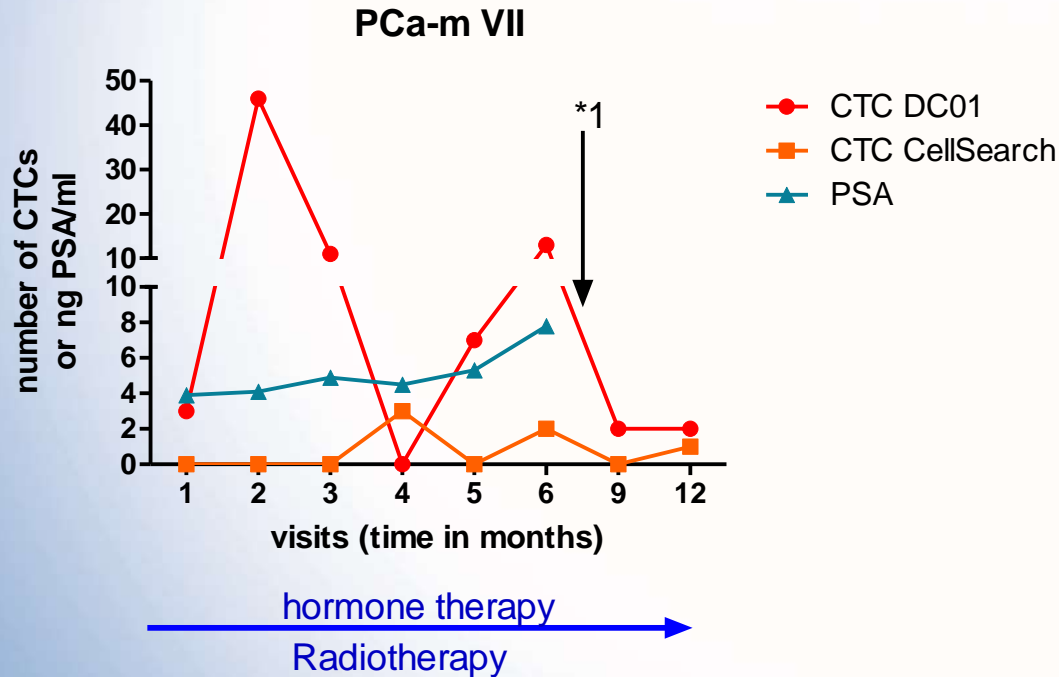
Example of individual CTC therapy monitoring III



Visit	CTC DC01	CTC CS	PSA [ng/ml]
1	2	n.a.	S1.24
2	15	n.a.	S1.84
3	1	n.a.	S0.0025
4	2	0	S0.015
5	4	0	S0.006
6	Np	0	S0.400
7	14	0	S0.005
8	3	0	S0.008

- continuous radiotherapy (no chemo- or hormone therapy)
- PCa diagnosed: 2009 (+ PTx)

Example of individual CTC therapy monitoring VII



Visit	CTC DC01	CTC CS	PSA [ng/ml]
1	3	0	3.9
2	46	0	4.1
3	11	0	4.9
4	0	3	4.5
5	7	0	5.3
6	13	2	7.8
7	2	0	-
8	2	1	-

*1- start 1. chemotherapy cycle (Docetaxel 75mg/m²)

- PCa diagnosed: 2002
- No PTx

Summary clinical data:

Safety

- *Application of the CellCollector was well tolerated with no side effects by 100% of the subjects (more than 500 applications)*

Proofed Functionality

- *CellCollector detection rate of more than 74% with a range from 0-515 CTCs*
- *Similar for early and late stages*
- *Higher or equal CTC detection rate compared to CellSearch® in nearly all samples*

Cancer Indications with shown Functionality

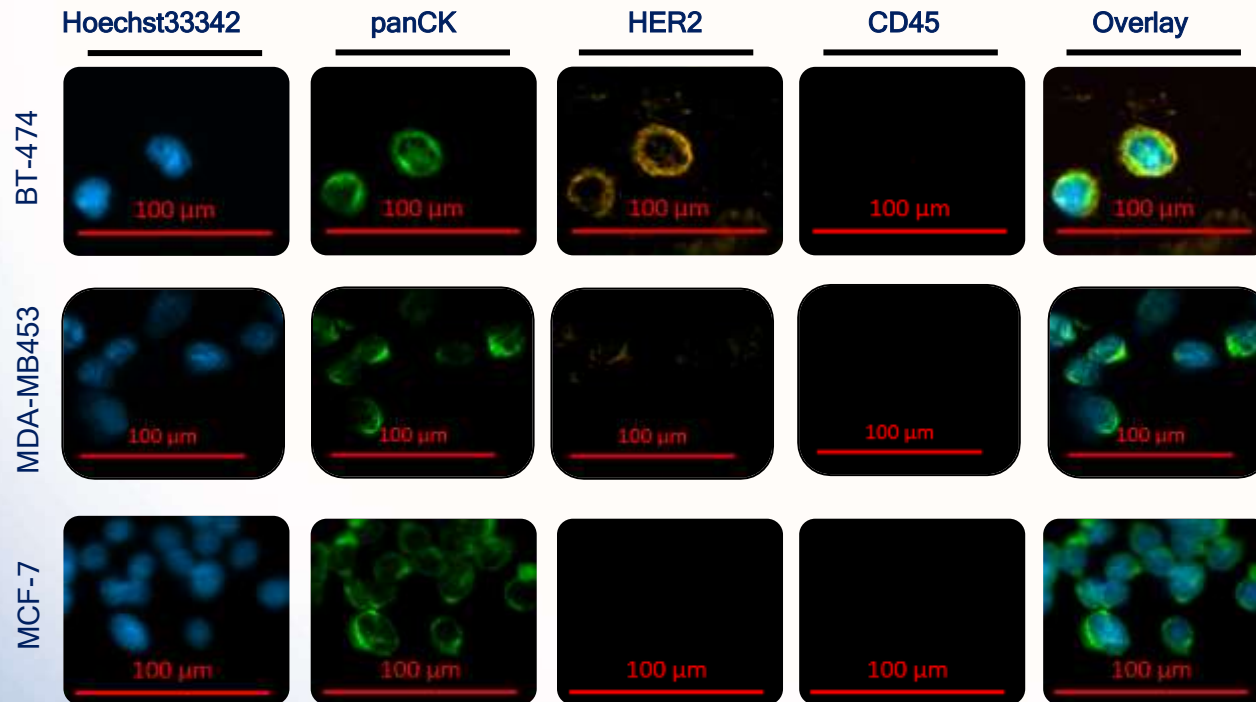
- *Bronchial Carcinoma*
- *Breast Carcinoma*
- *Colorectal Carcinoma*
- *Prostate Carcinoma*
- *Neuroendocrine Carcinoma*
- *Approved for all epithelial carcinomas*

Further diagnostic approaches I:

- Individual oncological targeted therapies will become more and more important in tomorrow's personalized medicine.
- The use of CTCs as a liquid biopsy could help to address the challenge to identify the right treatment for the specific patient
- With the CellCollector™ captured CTCs are ready for molecular characterization using immunofluorescence approaches or qPCR (among others)
 - Thus, the possibility of the establishment of more personalized treatment regimens as target e.g. on the translocation of the ALK gene, changes at the EGFR receptor or at the Androgen receptor is given

Further diagnostic approaches II:

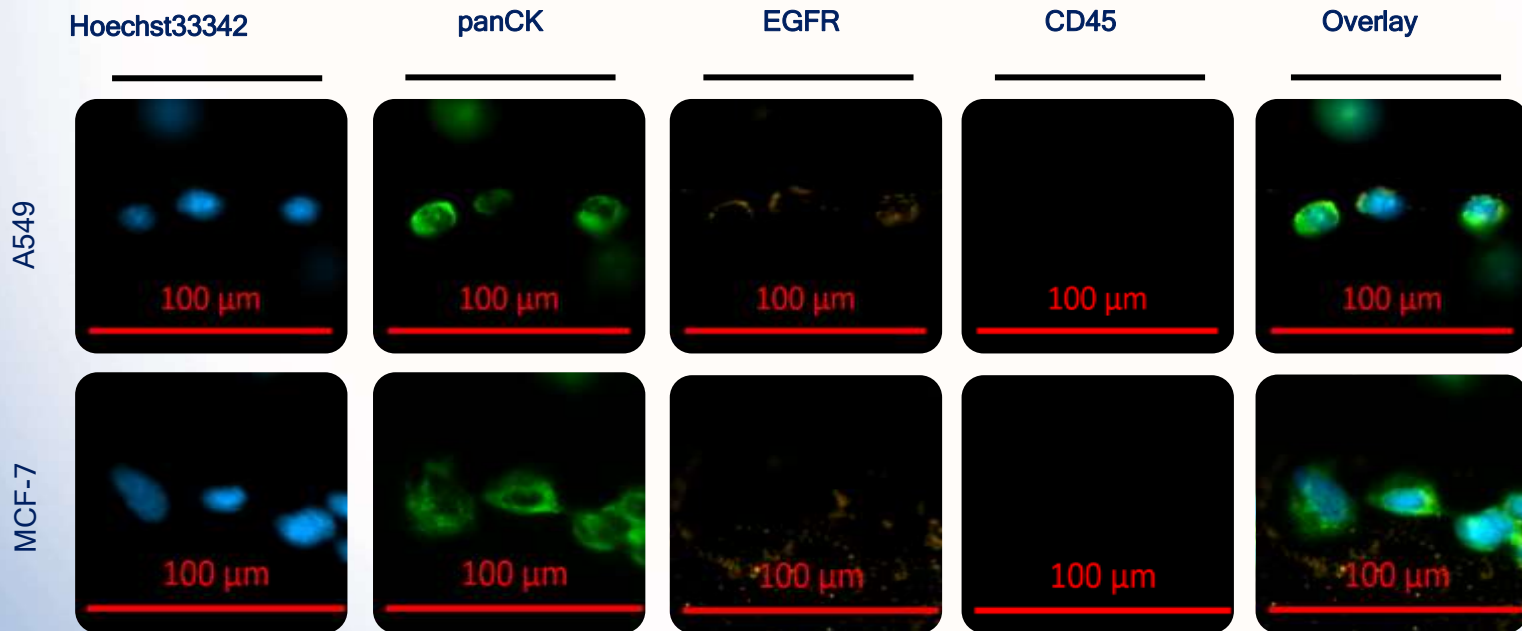
➤ HER2 staining:



Breast cancer cells (BT-474, MDA-MB-453, MCF-7) were captured by the CellCollector™ (*in vitro*). Tumor cells were identified by immunocytochemistry staining for epithelial markers (EpCAM and CKs), possible therapeutic target (HER2), and nuclear counterstaining using Hoechst33342. CD45 staining was done for negative cell selection.

Further diagnostic approaches III:

➤ EGFR staining:



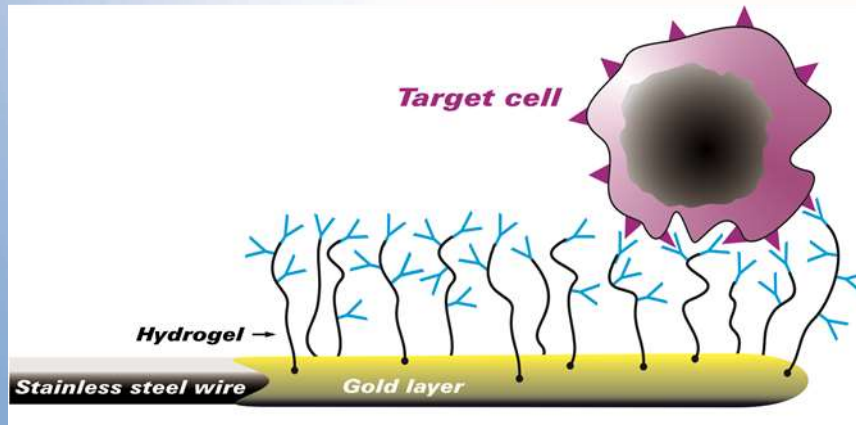
Breast (MCF-7) and Lung (A549) cancer cells were captured by the CellCollector™ (in vitro). Tumor cells were identified by immunocytochemistry staining for epithelial markers (CKs), possible therapeutic target (EGFR), and nuclear counterstaining using Hoechst33342. CD45 staining was done for negative cell selection.

Further diagnostic approaches IV:

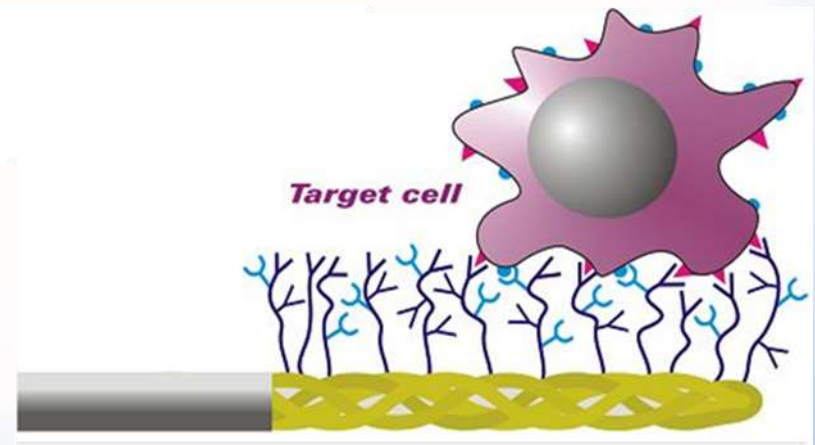
- The pre clinical proof of the HER2 and EGFR staining could be already confirmed
- For the clinical evaluation of these approach the planning of case series is nearly finished
- Preliminary results are expected in the beginning of 2014

New developments of the CellCollector:

- Modified surface structure by spiralization
 - increased surface
 - better hemodynamics
- Attached are several tumor specific antibodies



CANCE01



CANCE02

Summary

- The CellCollector™ offers a simple and efficient method to capture CTCs directly from the blood of patients
 - No expensive technical equipment needed
 - CTCs are isolated without a high background of other cells
 - Several down streaming diagnostics are possible (e.g. single cell picking, direct culturing, ICC, etc.)
- Due to a detection rate of over **75%** this new device might overcome present limitations in the enrichment of CTCs.
- CTC detection rate in lung, breast, prostate and colorectal cancer patients is higher or equal to the CellSearch® method in most cases
- The implementation of the CellCollector™ into clinical practice may improve early detection, prognosis and therapy monitoring of cancer patients
- Besides enumeration, the method may allow the molecular analysis of the CTCs, resulting in personalized treatment regiments



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SEARCH FOR**