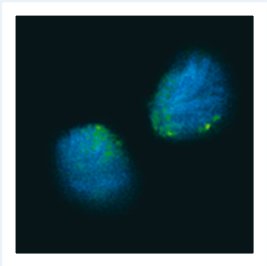
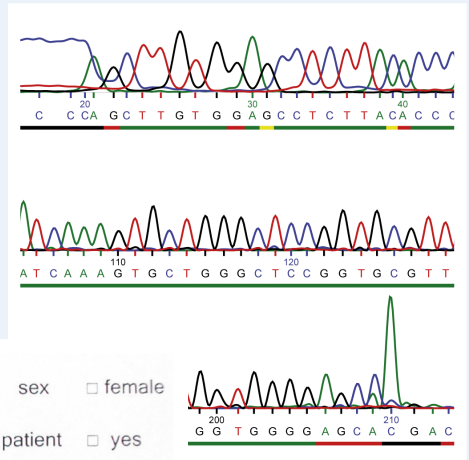
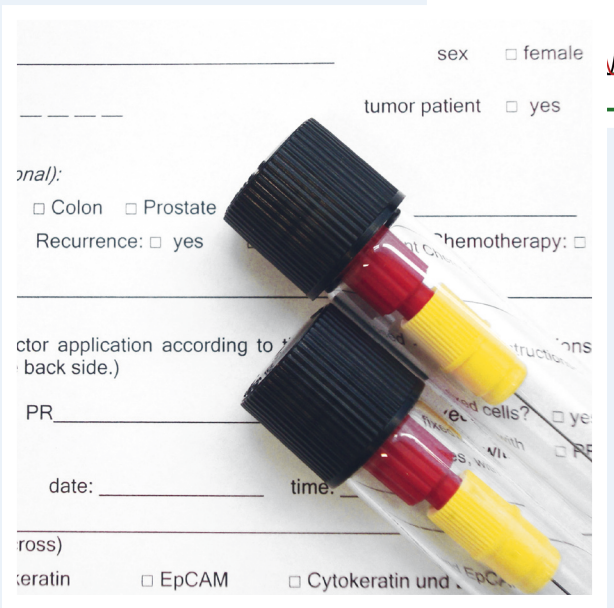
GILUPI
NANOMEDIZIN







We collect the cells you search for

GILUPI CellCollector®

In vivo Isolation of Circulating Tumor Cells
Detection & Characterization of Circulating Tumor Cells

CE Available for distribution and sale in countries accepting CE mark

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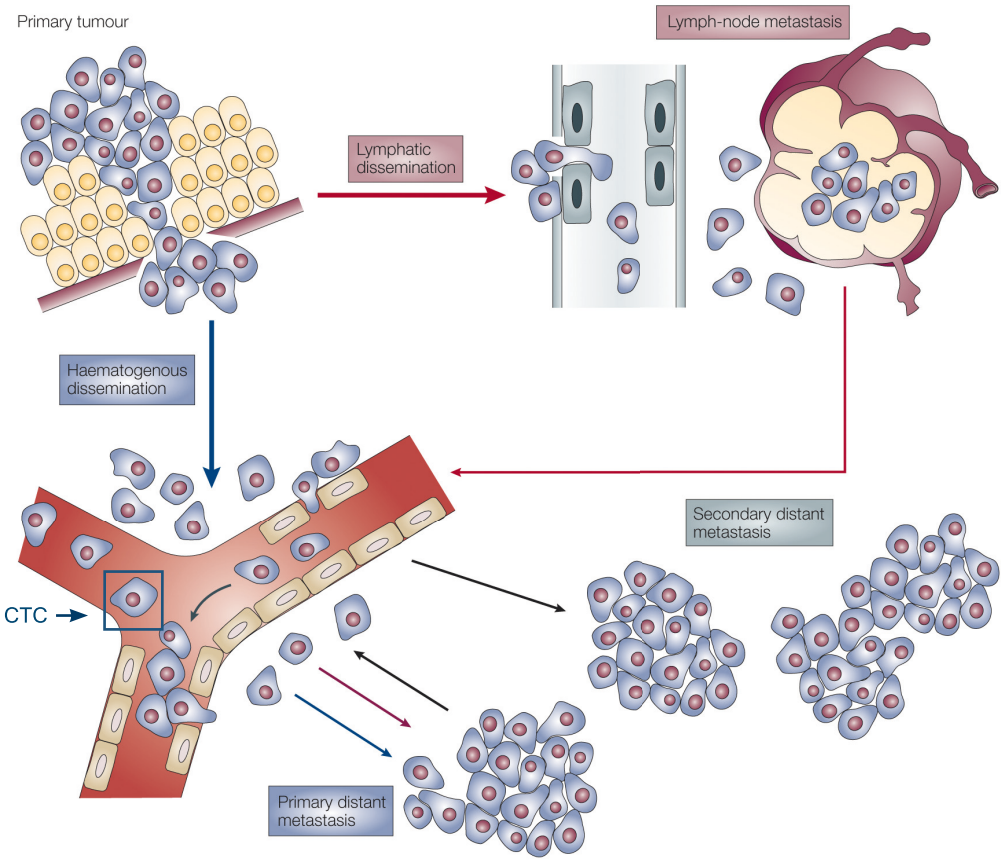
Circulating Tumor Cells

CTCs - a link between primary tumor and metastasis



Source: Klaus Pantel & Ruud H. Brakenhoff, Dissecting the metastatic cascade; Nature Reviews Cancer 4, 448-456 (June 2004)

Cancer related death is usually caused by the spread of invasive tumor cells from the primary tumor and the development of distant metastases. Single tumor cells or cell cluster detach from the primary tumor by down-regulation of cell-cell interactions; they can enter the blood and/or the lymphatic system and will finally leave the bloodstream at an anatomically different location which can lead to the initiation of metastatic growth.[1] The single tumor cells found in the patient's blood are called circulating tumor cells (CTCs).

Today, tumor staging is mainly based on tumor size, metastatic lymph node involvement, and evidence of overt distant metastasis obtained by imaging technologies. However, these staging procedures are not sensitive enough to detect early tumor cell spread as a key event in tumor progression. To fill this gap, CTCs have become part of the tumor staging procedure proposed by the American Joint Committee on Cancer (AJCC)[2] and treatment decisions might be based on these cells in addition to the information obtained from the current procedures.

Liquid biopsy through CTC analysis

Compared to the investigation of the primary tumor or a biopsy taken from a distant metastasis, the investigation of blood is relatively simple, less invasive and can be performed repeatedly. Due to these benefits, CTC investigation can be used in addition to current procedures as a real-time marker for staging, disease progression and therapy responsiveness. Furthermore, the molecular analysis of CTCs can provide highly valuable information for clinicians and researchers as early detection and treatment of the disease and its metastatic spread can reduce cancer mortality dramatically.[3]

CTC enumeration and characterization can assist in

- Malignancy detection at an early stage
- Finding treatments tailored to the patient by patient stratification through companion diagnostics
- Treatment monitoring in real time
- Identification of optimal treatments and investigation of drug susceptibility by *ex vivo* culturing of CTCs on an individual level

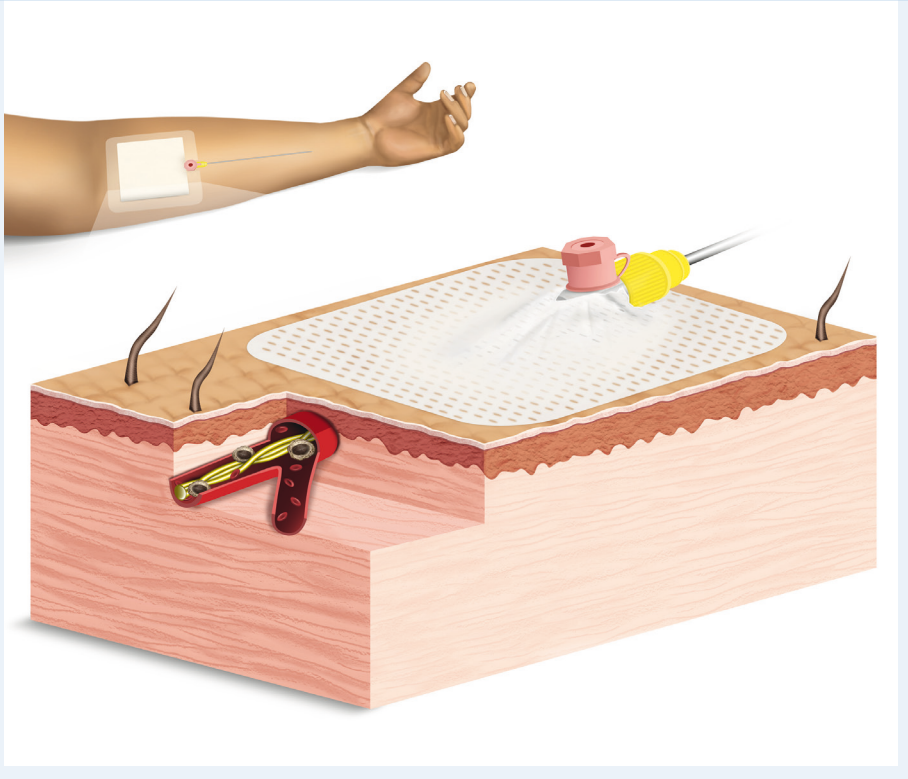
GILUPI CellCollector®

First *in vivo* platform for circulating tumor cell isolation

CTC detection is challenging due to their extremely low frequency amongst millions of leucocytes and billions of red blood cells. The advanced GILUPI technology aims at the detection and characterization of CTCs combined with the proof of the malignant origin of the collected cells. The tumor cells are captured *in vivo* with high sensitivity and selectivity providing compelling and improved diagnostic value for personalized medicine, early diagnosis as well as treatment and clinical monitoring.

The GILUPI CellCollector® favors "positive enrichment" of CTCs, which is based on the fact that the largest group of cancers is of epithelial origin and cells spread from these tumors express epithelial cell surface markers like EpCAM, the epithelial cell adhesion molecule.[4] During the *in vivo* application, the functionalized surface comes into direct contact with the circulating blood and rare cells are bound to the device.

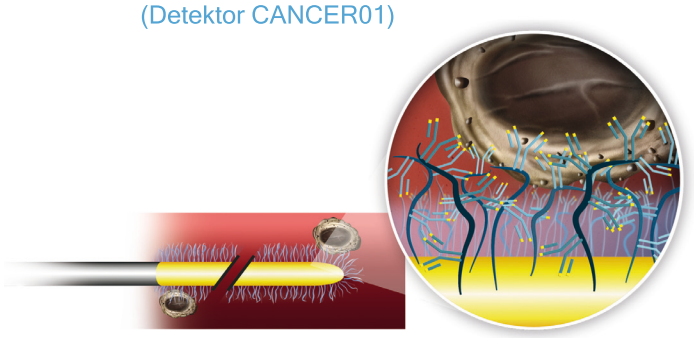
In vivo CTC technology - Screening of a larger blood volume



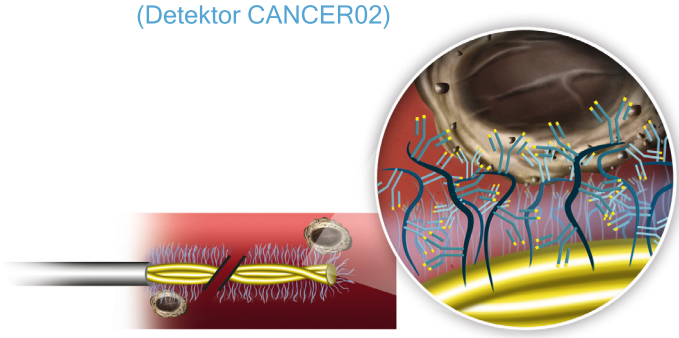
Screening for CTCs in up to 1000ml blood volume

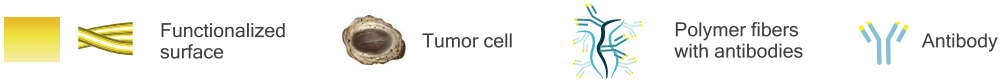
Unique characteristics

GILUPI CellCollector® DC01 (Detektor CANCER01)



GILUPI CellCollector® DC02 (Detektor CANCER02)

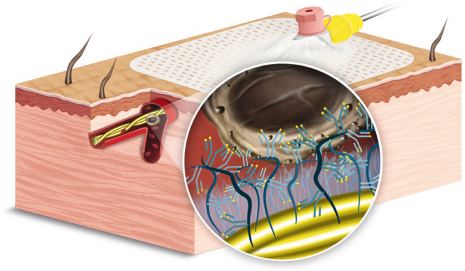




The GILUPI CellCollector® is available in two different product versions, Detektor CANCER01 (DC01) recommended for CTC enumeration and Detektor CANCER02 (DC02) recommended for further downstream analysis methods. Both products consist of a stainless steel wire, which has a functionalized surface at one end (gold and hydrogel coating with incorporated anti-EpCAM antibodies). The first generation product DC01 has a smooth, 2 cm long functionalized tip. The next generation product DC02 has a longer, 4 cm functionalized tip which consists of three thin twisted stainless steel wires for improved haemodynamic characteristics. Both products are CE marked medical devices meeting all requirements of the Medical Devices Directive (MDD) and comply with clinical and diagnostic requirements to be applied in cancer patients. Next-generation products will focus on improved structural characteristics and on the incorporation of different antibodies or antibody combinations for detection of further CTC subtypes (e.g. stem-cell like cells). We also offer customized products.

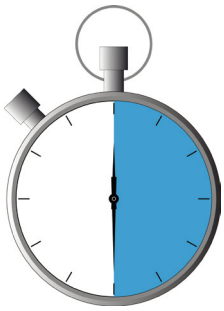
Workflow

1. *In vivo* CTC isolation in 30 minutes



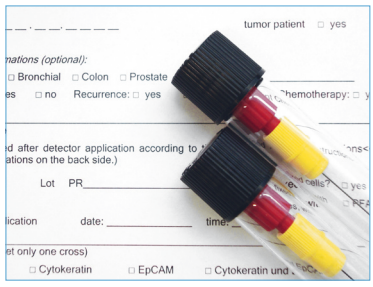
Product application via standard catheter system

2. Sample preparation



Preparation of isolated cells for subsequent analyses

3. Open for various downstream applications



- Immunofluorescence analysis
- Mutation analysis
- Fluorescence in situ hybridization (FISH)
- *Ex vivo* cell culturing

and more

Clinical application in solid tumors

Confirmed CTC isolation in

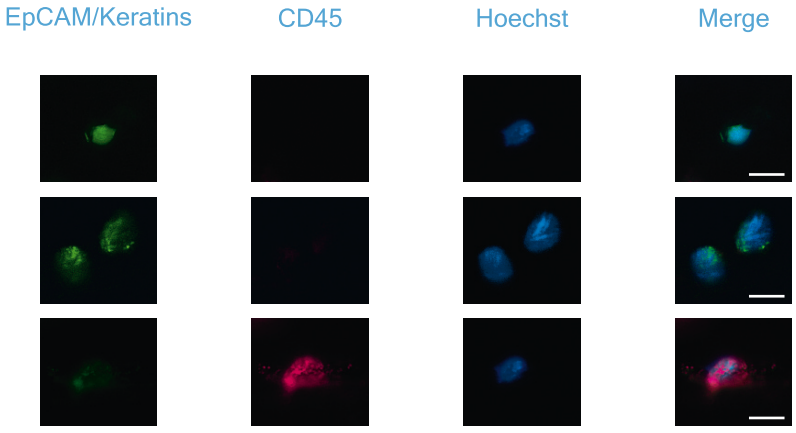
- Lung cancer
- Breast cancer
- Prostate cancer
- Colorectal cancer
- Renal cancer
- Pancreatic cancer
- Neuroendocrine tumors

CTC detection & characterization

- Baseline CTC status
- Before, during and after therapy

Downstream applications

Immunofluorescence analysis

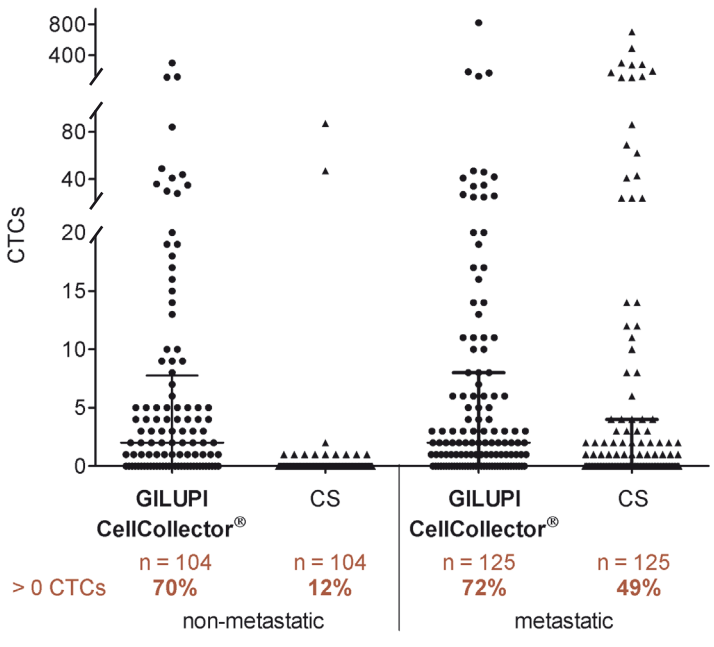


Captured cells on the GILUPI CellCollector® are stained with fluorescence labeled antibodies to EpCAM/Keratins (green color), CD45 (red color) and the nuclear dye Hoechst (blue color). Specific (positive, CTCs) cells are positive for EpCAM and/ or Keratins and negative for CD45. The scale bar corresponds to 20µm.

Quantitative detection of CTCs by immunofluorescence analysis

Immunofluorescence microscopy is a powerful technology for determining the presence and subcellular localization of a protein or an antigen in a cell. For this purpose specific fluorescently labeled antibodies are used which bind to the target, and thereby allowing visualization and examination under a fluorescence microscope.
CTCs are identified by positive staining for Keratins and/ or EpCAM and nucleus; negative selection is done via staining for CD45 (marker for blood cells, e.g. leucocytes). Immunofluorescence analysis is successfully employed by GILUPI for quantitative detection of CTCs captured with the GILUPI CellCollector® in various cancer entities like breast, lung, prostate, colorectal and neuroendocrine cancer.

High CTC detection – a clinical excerpt



The *in vivo* applied GILUPI CellCollector® DC01 shows a significantly higher CTC detection frequency of 70% in non-metastatic patients and a higher detection frequency of 72% in metastatic patients, compared to the *in vitro* system CELLSEARCH® (CS) (n= 229, pooled data from various cancer entities).

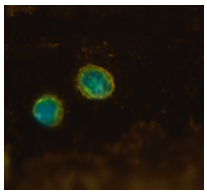
CTCs and their value for targeted therapies

Options to treat cancer patients with individualized, targeted therapy regimens have improved enormously over the last years. Knowledge of the molecular make-up of the cancer cells is an indispensable prerequisite for the effective use of targeted therapies.

Examples for possible downstream applications with the GILUPI CellCollector®:

Detection of HER2/neu overexpression in CTCs

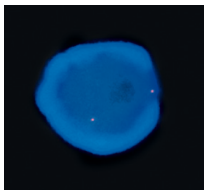
In breast cancer, different tumor subtypes vary in terms of biology, histology, therapeutic options, patterns of dissemination and metastasis as well as individual prognostic outcome.[6] Especially for HER2 positive patients, regular re-testing of remaining cancer cells or CTCs for their HER2 status is important for the adequate choice of targeted therapies (e.g. trastuzumab or lapatinib).



HER2 positive CTC

Detection of *EML4-ALK* translocation in CTCs

Between 2-7% of patients with non-small cell lung cancer (NSCLC) harbor a translocation of the *ALK* gene (European Union).[7] Lung cancer patients with a proven *EML4-ALK* translocation can benefit from targeted therapies like crizotinib or ceritinib and other *ALK* inhibitors currently under development.[8] Tumor tissue and CTCs can be tested for the *EML4-ALK* translocation with a companion diagnostic test e.g. Fluorescence in situ hybridization (FISH).



ALK testing by Fluorescence in situ hybridization (FISH)

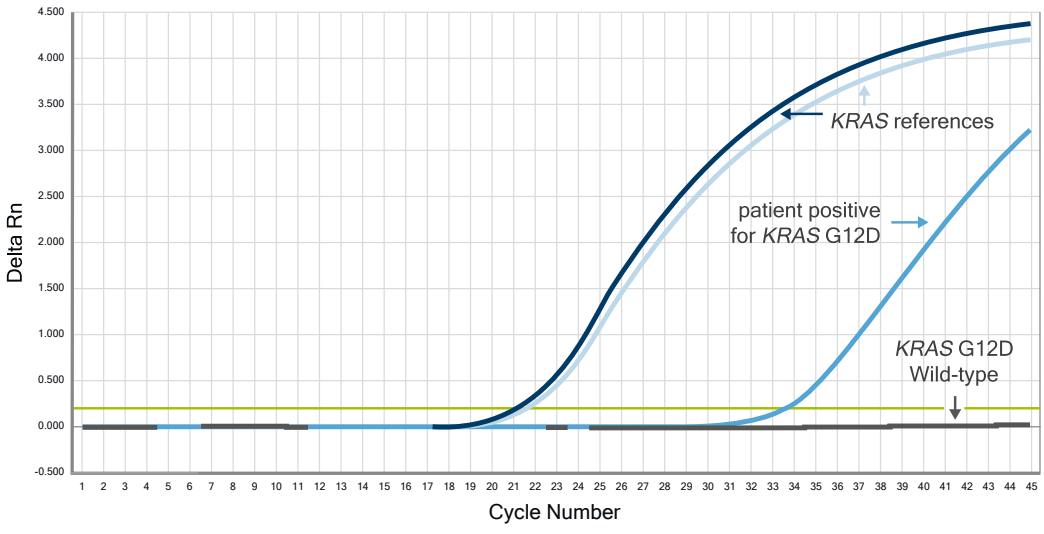
Detection of somatic mutations in CTCs

Identification of mutations in oncogenes (e.g. *EGFR* and *KRAS*) gene can provide information about a patient's responsiveness to targeted therapies (e.g. gefitinib or erlotinib).[5] Routine testing of CTCs during the treatment can give additional information concerning the usefulness of the applied therapy (e.g. resistance mutation occurrence). CTCs can be characterized on a molecular level using conventional kits. DNA isolated from CTCs can be amplified (whole genome amplification) and tested for therapy relevant mutations using e.g. mutation specific quantitative real-time PCR. In this example, a *KRAS* Mutation is detected in a lung cancer patient.

Further applications

- *Ex vivo* cell culturing
- Next generation sequencing (NGS)
- Gene expression analysis
- Protein analysis

KRAS Mutation Analysis



GILUPI CellCollector®

CTCs isolated with the GILUPI CellCollector® technology can be enumerated and characterized to assist in

- Malignancy detection at an early stage
- Finding treatments tailored to the patient by patient stratification through companion diagnostics
- Treatment monitoring in real time
- Identification of optimal treatments and investigation of drug susceptibility by *ex vivo* culturing of CTCs on an individual level

Applications

- CTC enumeration
- Immunofluorescence analysis of proteins of interest (e.g. HER2, ALK, AR-V7)
- Mutation analysis (e.g. *EGFR*, *KRAS*)
- Fluorescence in situ hybridization (FISH)
- Next generation sequencing (NGS)

Further applications are currently established (e.g. mRNA analysis)

GILUPI offers

Diagnostic Services

Quantitative detection of CTCs (immunofluorescence analysis) in an in-house diagnostics lab

Customized products

Expertise in incorporation of antibodies or antibody combinations on customized GILUPI CellCollector®

Detection of further CTC subtypes (by other antibodies than EpCAM)

References

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GILUPI CellCollector® is available for research purposes (*in vitro* only) worldwide.
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