GILUPi
MEDICINE. PERSONALIZED.

IN VIVO ISOLATION OF CIRCULATING TUMOR CELLS

ADVANCING DIAGNOSTICS FOR PRECISION MEDICINE

LIQUID BIOPSY THROUGH CTC ANALYSIS

To emphasize the difference to conventional biopsies from solid tissue, the sampling of circulating DNA, RNA or tumor cells from blood is called "Liquid Biopsy". In contrast to locally confined tissue biopsy samples, CTCs (circulating tumor cells) represent the heterogeneity of all cancerous lesions within the body. The collection of CTCs from the patient's blood is usually a minimally invasive procedure and can be performed repeatedly. Thus, CTC investigation can be used in combination with current diagnostic methods as a real-time marker for tumor staging, monitoring of disease progression and therapy responsiveness.

CTCs – a link between primary tumor and metastasis

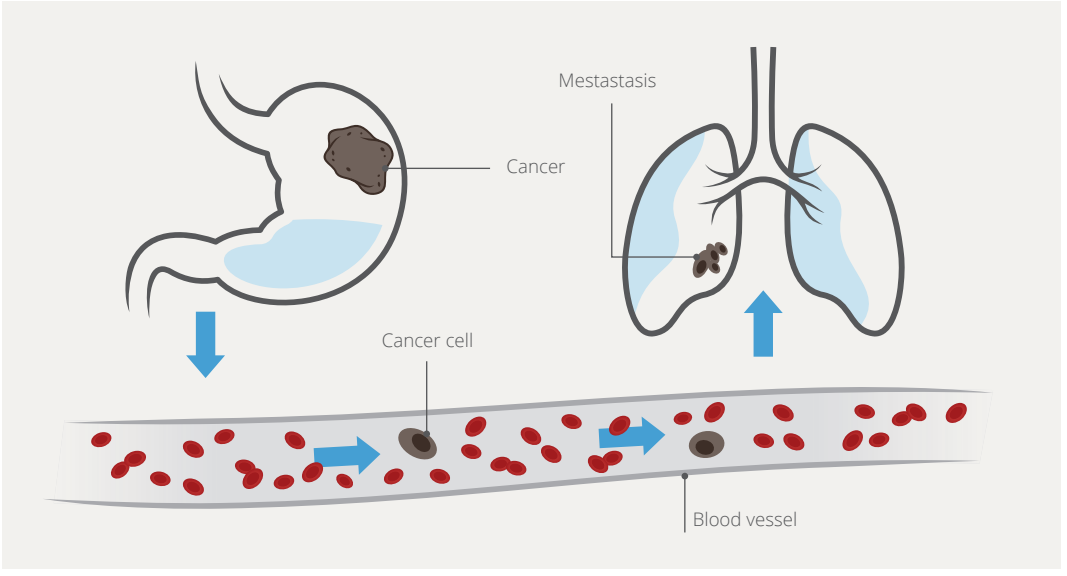


Figure 1: *Development of distant metastasis from a primary tumor site through dissimination of CTCs.*

The spread of invasive tumor cells from the primary tumor and the development of distant metastases is caused by single tumor cells or cell clusters which detach from the primary tumor. They circulate in the blood and the lymphatic system to finally leave at a distant location, with the potential to initiate metastatic growth.

GILUPI CellCollector® TECHNOLOGY

CTC detection is challenging due to the extremely low frequency of CTCs amongst millions of leucocytes and billions of red blood cells. The GILUPI CellCollector® enables *in vivo* tumor cell isolation with optimal sensitivity and selectivity for subsequent analysis of clinically relevant parameters.

Most solid cancers are of epithelial origin and the cells emanating from these tumors express epithelial cell surface markers such as the epithelial cell adhesion molecule (EpCAM). This molecule is used as a marker for positive CTC-enrichment by the GILUPI CellCollector®.

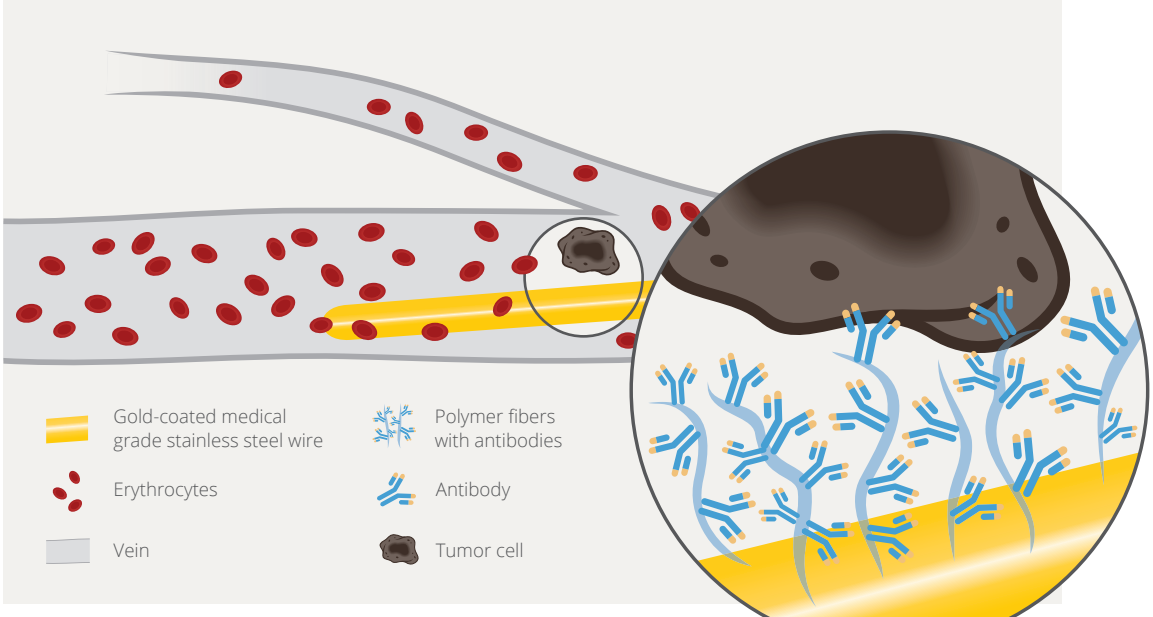


Figure 2: *GILUPI CellCollector® in patient's vein with schematic representation of functionalized surface.*

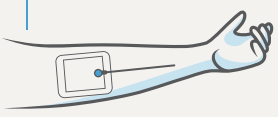
The GILUPI CellCollector® type Detektor CANCER01 (DC01) consists of a stainless steel wire with a 2 cm functionalized surface area at the tip (gold and hydrogel coating with incorporated anti-EpCAM antibodies). The product is a CE marked medical device. It is also approved by the Chinese Food and Drug Administration (CFDA) for applications in China.

APPLICATION PROCEDURE FOR CTC COLLECTION

The GILUPI CellCollector® application is comparable to venous blood sampling. The access into the arm vein of the elbow bend is made via a 20 G venous indwelling cannula. The GILUPI CellCollector® remains in this position for 30 minutes. Depending on the desired subsequent diagnostic procedure, a specific post-treatment is required. Immediate post-treatment of the GILUPI CellCollector®, such as washing and fixation, depends on subsequent diagnostic procedures. After isolation, the cells bound to the GILUPI CellCollector® are available for different diagnostic procedures, for example immunofluorescence staining, PCR, *in situ* padlock probe technology, sequencing, or gene expression analysis. Since the cells captured by the GILUPI CellCollector® are vital, they could be cultured for further diagnostics.

Workflow

1



Application

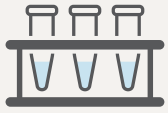
The access into the arm vein of the elbow bend is made via a 20 G venous indwelling cannula.

2

CTC collection

The GILUPI CellCollector® remains in the arm vein of the elbow for 30 min.

3



Post-treatment

Depending on the desired subsequent diagnostic procedure, a specific post-treatment of the product is required.

4

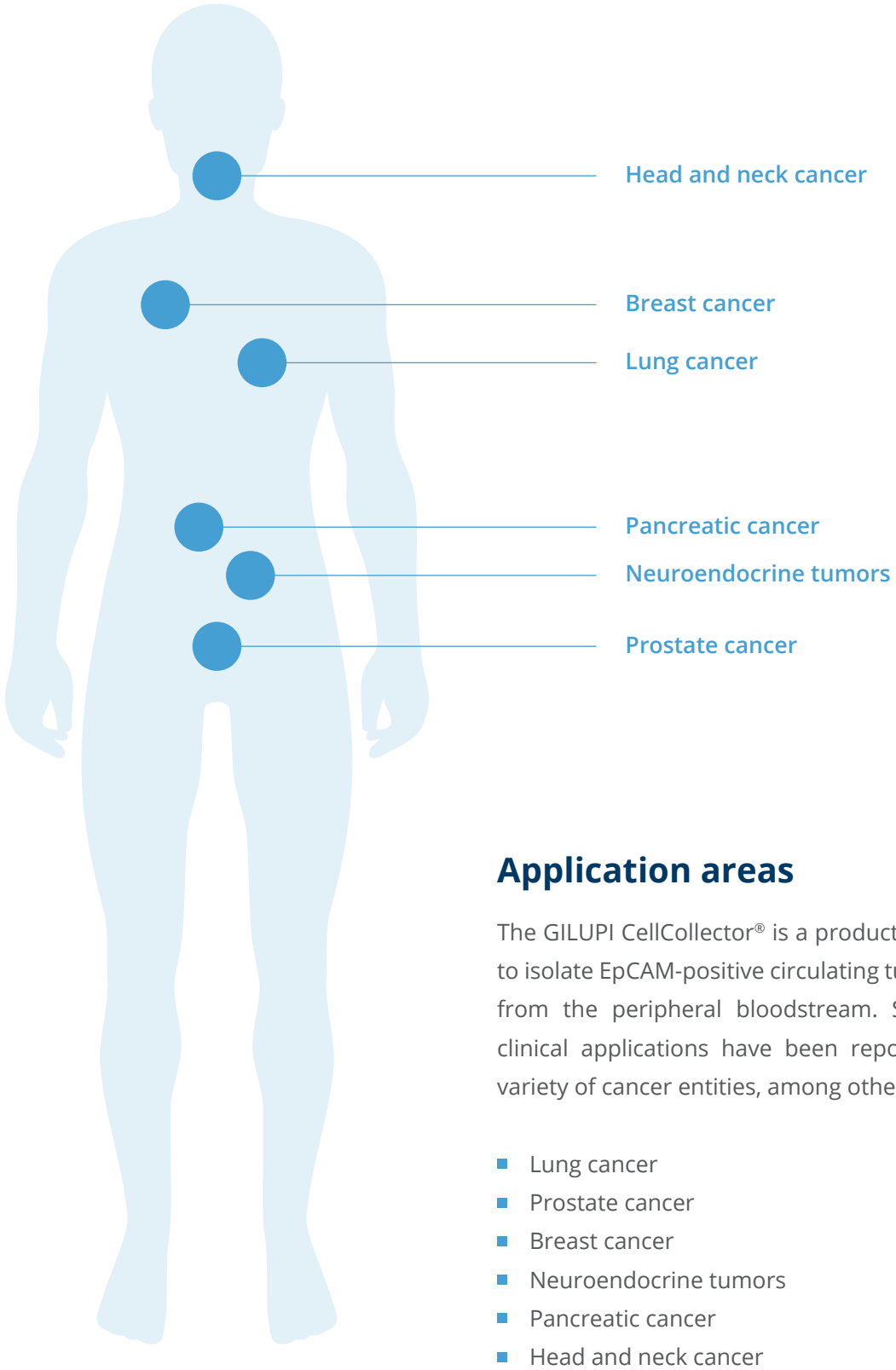
Analysis

The cells bound to the GILUPI CellCollector® are available for different diagnostic procedures.

5

Results

The data are summarized in a report, depending on the analysis method and the request of the physician.



Application areas

The GILUPI CellCollector® is a product designed to isolate EpCAM-positive circulating tumor cells from the peripheral bloodstream. Successful clinical applications have been reported in a variety of cancer entities, among others:

- Lung cancer
- Prostate cancer
- Breast cancer
- Neuroendocrine tumors
- Pancreatic cancer
- Head and neck cancer

DOWNSTREAM APPLICATIONS FOR CTC ANALYSIS

Quantitative detection of CTCs by immunofluorescence

Immunofluorescence microscopy is a powerful technology to determine the expression and subcellular localization of proteins in a cell. For this purpose, fluorescently labeled antibodies are used to bind to and to visualize specific proteins/markers (for examination). CTCs are identified by positive staining of keratins and/or EpCAM and nucleus staining, negative selection is done via staining of CD45 (marker for blood cells, e.g. leucocytes). One additional marker like HER2/neu or PD-L1 can be added to the analysis.

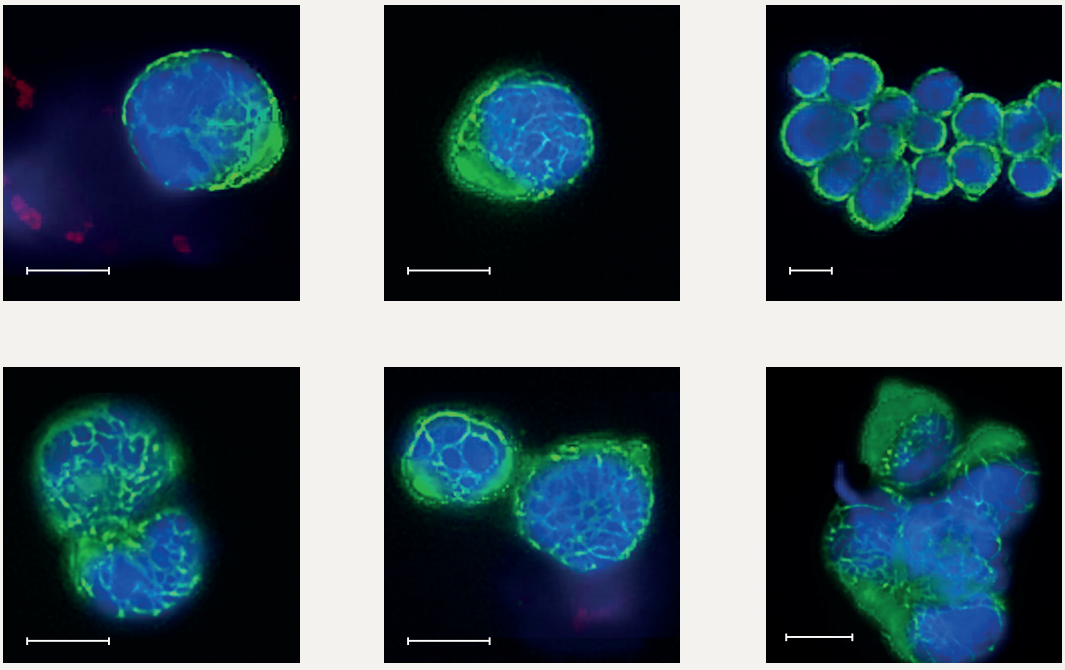


Figure 3: *Selected CTCs from a pancreatic cancer patient are stained with fluorescent-labeled antibodies to EpCAM/keratins (green), CD45 (red) and the nuclear staining (blue). Epithelial tumor cells are positive for EpCAM and/or keratins and negative for CD45. The scale bar corresponds to 10 µm.*

Quantitative detection of gene transcripts in CTCs by *in situ* padlock probe technology

After *in vivo* isolation, CTCs can be analyzed for clinically relevant targets directly on the surface of the medical device, using *in situ* padlock probe technology. This method enables *in situ* detection of RNA with enhanced sensitivity. Padlock probes are oligonucleotides which bind specifically to their target sequence e.g. AR-V7 (splice variant 7 of the androgen receptor) and are amplified by rolling circle amplification. Subsequently, the products are detected by fluorescent probes which generate quantifiable signals.

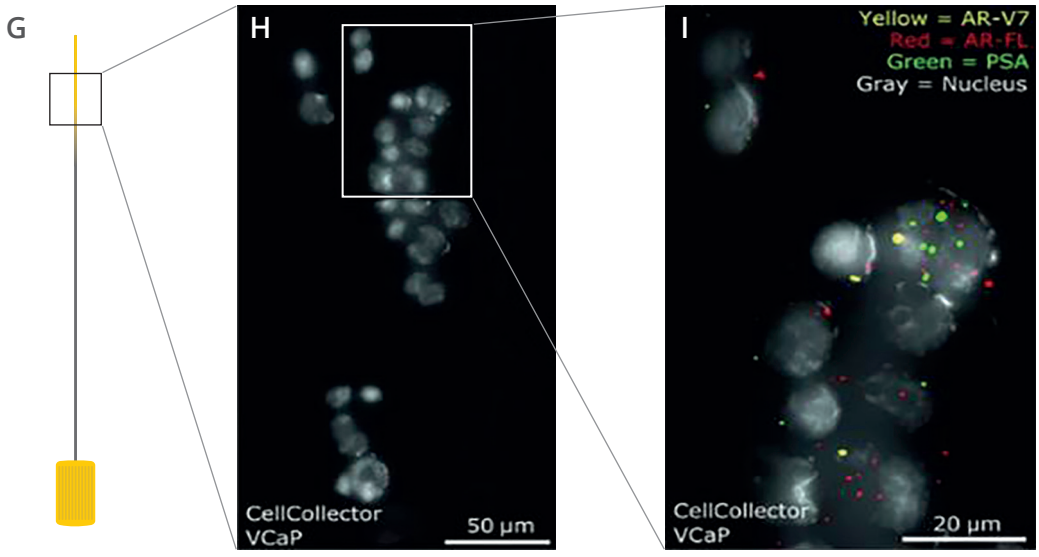


Figure 4: *In situ detection of cancer-related transcripts in prostate cancer cells on the GILUPI CellCollector®.*

Detection of somatic mutations in CTCs

The identification of mutations can provide information about a patient's responsiveness to targeted therapies. Routine testing of CTCs for resistance mutations during treatment, for example in the EGFR or KRAS genes, offers additional information about the effectiveness of the applied therapy. Isolated CTCs can be characterized on a molecular level using commercially available mutation detection kits.

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