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In vivo isolation of circulating tumor cells in different clinical settings for multiple downstream applications

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Abstract

Background: Usually, the development of metastasis is the final cause of cancer related death and the detection of metastasis at an early stage and during treatment monitoring is of utmost importance for optimal clinical management and for improving patients' prognosis. Circulating tumor cells (CTCs) are prerequisites for formation of metastasis and therefore are a promising surrogate marker for monitoring disease progression and response to therapy. Detection and characterization of these rare cells could provide a powerful approach for early disease diagnosis as well as therapy monitoring. The GILUPI CellCollector® offers medical personnel at any point-of-care with the unique opportunity to enrich CTCs *in vivo.* Primary goal was to select clinical settings with focus on therapy relevant targets detected on CTCs by using this *in vivo* device.

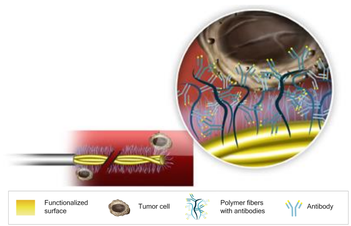
Method: The GILUPI CellCollector® are medical devices, which screen a large volume of blood for CTCs directly in the vein of cancer patients. We selected four clinical studies with the following initial applications: 26 in Prostate Cancer (PC) and 62 in Lung Cancer (LC) patients. More than 400 follow up applications already took place (not discussed here). First, a PC study with detection of androgen receptor splice variant AR-V7 on CTCs. Second, a non-small cell lung cancer (NSCLC) study with detection of EML4-ALK chromosomal rearrangement on CTCs. Third, a screening study for different groups of patients with pulmonary disease to demonstrate the clinical utility of CTCs combined with low-dose computed tomography (LDCT) in screening for early stage LC in high risk patients (He et al., 2017). And fourth, a LC study with detection of PD-L1 expression on CTCs. CTC enumeration was conducted by immunofluorescence microscopy to investigate the detection rate of CTCs in the selected studies. Further 3D-immuno-FISH analysis was performed to detected EML4-ALK rearrangement. Immunostaining was used for PD-L1 and AR-V7 detection. And NGS was used to screen cancer-related genes for existing mutations.

Results: In this analysis, CTCs could be isolated in the blood of prostate and lung cancer patients. Overall detection rate of the GILUPI CellCollector® at baseline visits is 62%. Therapy relevant characteristics, AR-V7, PD-L1 and EML4-ALK rearrangements were clearly detectable down to a single cell level. The combination of LDCT and CTCs could be used as screening for early stage lung cancer in high risk patient groups.

Conclusion: The GILUPI CellCollector® overcomes blood volume limitations and thereby increases CTC detection rates. Overall they were applied to more than 3.000 cancer patients. They allow CTC enumeration, molecular characterization, and biomarker expression analysis. Future implementation into clinical practice may improve early detection, prognosis and therapy monitoring of cancer patients. Besides enumeration, captured CTCs are ready for molecular characterization and will help to establish more personalized treatment regiments.

GILUPI CellCollector® – an *in vivo* CTC isolation technology

Figure 1: Technology
The functionalized surface of the stainless steel wire consists of a gold layer and a hydrogel which bears covalently bound antibodies against the epithelial cell surface marker EpCAM.



Insertion into peripheral arm vein of a patient

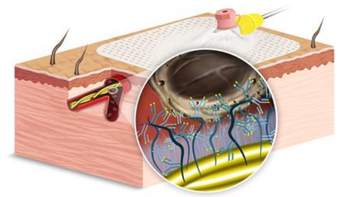


Figure 2: Device application
Insertion of the GILUPI CellCollector® through an indwelling cannula into a peripheral arm vein for 30 min. During the application the 40 mm long functionalized tip comes into direct contact with the blood circulation and captures CTCs via EpCAM binding.

Methods and Results Prostate Cancer

Study Design

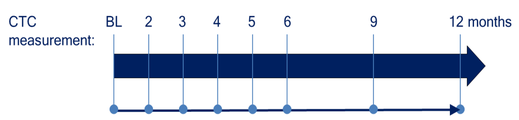


Figure 3: Schematic representation of the study design.

Selected CTC enumeration and AR-V7 detection

Patient	V1	V3	V4	V5	V6	V7	V8
1	1 CTC	8 CTCs	10 CTCs	1 CTC	44 CTCs (partially organized in clusters)	6 CTCs	3 CTCs
2	2 CTCs 1x ARV7 +	0 CTCs	0 CTCs	2 CTCs 1x ARV7 +	2 CTCs	2 CTCs	2 CTCs

Figure 4: CTC enumeration data and AR-V7 detection hormono-Naïve patients with Metastatic Prostate Cancer under therapy with Enzalutamide

CTC images

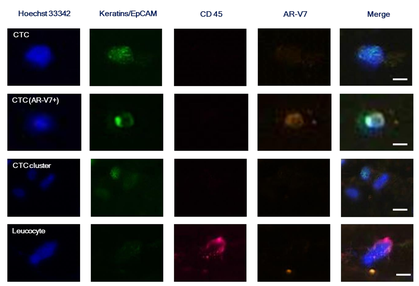


Figure 5: Representative images of CTCs and CTC clusters isolated with the GILUPI CellCollector® and one leukocyte. Tumor cells were identified as EpCAM- and/or pan-keratin-positive (green) and CD45-negative (red) events. Hoechst33342 (blue) was used for nuclear counterstain. And anti - AR-V7 (orange) was used for AR-V7 detection. Scale bars: 10 µm.

Methods and Results Lung Cancer

Study Design

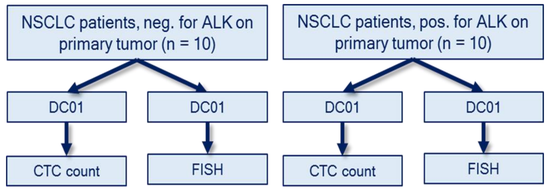


Figure 6: Schematic representation of the study design.

Immuno-DNA FISH assay using the functionalized wire

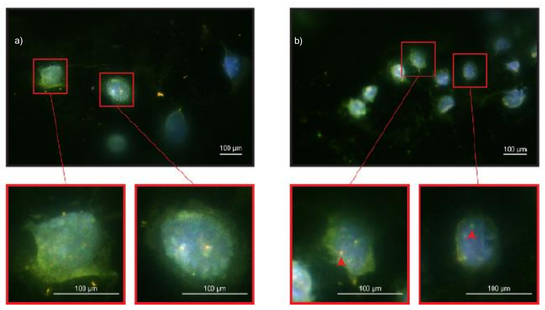


Figure 7: Representative image of NCI-H1975 cells on the wire. Although the 3D shape of the cells on the wire make difficult to acquire fully in-focus images, EpCAM signal is well visible in all the cells. (b) Representative image of NCI-H3122 cells on the wire.

Study Design

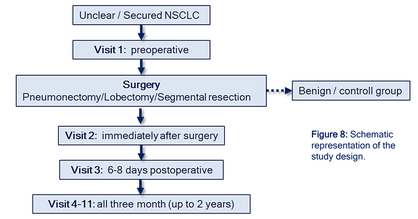
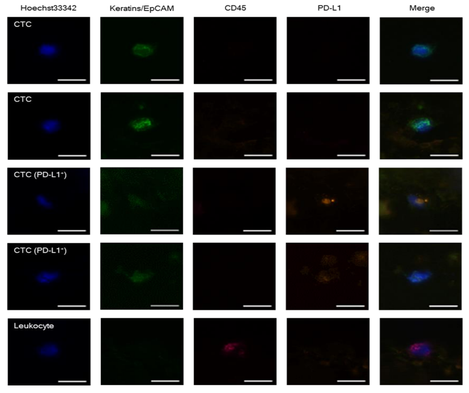


Figure 8: Schematic representation of the study design.

CTC enumeration and ALK detection



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Summary

- **CTCs** can be **captured** ***in vivo*** with the GILUPI CellCollector®.
- The GILUPI CellCollector overcomes blood volume limitations and thereby increases CTC detection rates.
- More than 3.000 applications in cancer patients. Method allows CTC enumeration, molecular characterization, and biomarker expression analysis.
- Future implementation into clinical practice may improve early detection, prognosis and therapy monitoring of cancer patients. Besides enumeration, captured CTCs are ready for molecular characterization and will help to establish more personalized treatment regiments.

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