ELCC 2015
Geneva, 15th-18th April

# Enumeration & molecular characterization of circulating tumour cells in lung cancer patients using the GILUPI CellCollector®, an effective in vivo device for capturing CTCs

N. Scheumann[1], T. Gorges[2], N. Penkalla[3], B. Nowack[1], T. Schalk[3], S. Riethdorf[2], K. Lücke[1], K. Pantel[2], T. Krahn[4], C. Schumann[5]

1 GILUPI GmbH, Potsdam/Greifswald, Germany; 2 Tumour Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 3 Pneumology, Ulm Medical University, Ulm, Germany; 4 Global Biomarker Research, Bayer Pharma AG, Berlin, Germany; 5 previously Ulm, now: Clinic for Pneumology, Thoracic Oncology, Sleep- and Respiratory Critical Care, Klinikverbund Kempten-Oberallgaeu GmbH, Kempten, Germany; contact: nicole.scheumann@gilupi.com

## Abstract

**Aim**: Liquid biopsy - isolating and analysing circulating tumour cells (CTCs) from the blood of lung cancer patients - can provide additional information on prognosis, treatment efficacy and molecular tumour evolution. Currently, CTCs are isolated in vitro from limited volumes of patient blood samples. To overcome this limitation, an innovative device, the GILUPI CellCollector®, was used to isolate CTCs in vivo. Here, we conducted a comprehensive study deploying this effective device, to monitor CTC counts before initiation of chemotherapy and 12 weeks after in 50 lung patients. Further, we investigated the reproducibility of results, the correlation of the clinical response to CTC number changes, and mutations in CTCs of patients with known mutations in the primary tumour. **Patients and Methods**: 50 lung cancer patients (48 NSCLC and two SCLC) were screened for CTCs by two subsequent device applications before therapy initiation and 12 weeks after (n = 185 applications in total). Additionally, blood samples were analysed with the CellSearch· system. The CTC count change before and after therapy was correlated with clinical response. To analyse cancer specific mutations in CTCs captured with the GILUPI CellCollector®, digital PCR (dPCR) was performed. **Results**: Applying the GILUPI CellCollector®, CTCs were isolated from 78% of the patients during the pre-therapy visit. Overall, successful isolation of CTCs was significantly more frequent with the GILUPI CellCollector® (58%) compared to CellSearch® (28%). Furthermore, by using the GILUPI CellCollector® for CTC quantification before and after therapy initiation, an indication for responsive and non-responsive treatment outcomes was seen. Further, we were able to detect KRAS and EGFR mutations in CTCs known to be present in the primary tumour biopsy material. **Conclusions**: The GILUPI CellCollector® overcomes blood volume limitations of other diagnostic approaches and thereby increases the diagnostic sensitivity of CTC isolation. It allows enumeration and molecular analysis of CTCs which might help to monitor therapy efficacy and improve treatment strategies.

## GILUPI CellCollector® - an *in vivo* CTC isolation technology

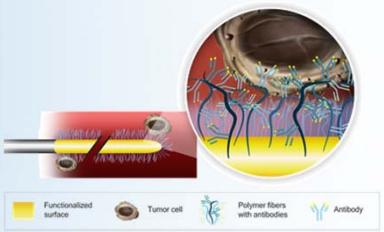



**Figure 1: The device. -** The functionalized surface of the stainless steel wire consists of a gold layer and a hydrogel which bears covalently bound antibody against epithelial cell surface marker EpCAM.

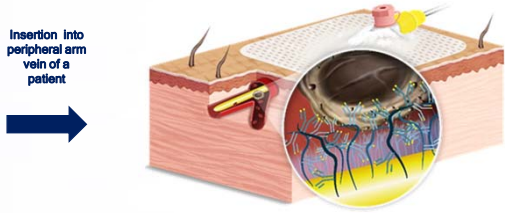



**Figure 2: The device application. -** Insertion of the GILUPI CellCollector® through an 20 gauge indwelling cannula into a peripheral arm vein for 30 min. During the application the 20 mm long functionalized tip comes into direct contact with the blood circulation and captures CTCs via EpCAM binding.

## Results

### Study Design

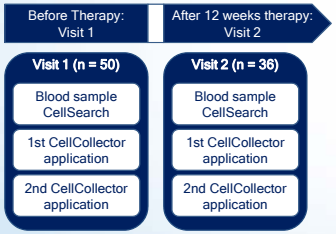



**Figure 3: Study design**. The study was designed with the first visit directly before systemic therapy initiation and the second visit 12 weeks after treatment started. Patients underwent the same CTC investigation procedures at each visit: a 7.5 mL blood draw for CellSearch analysis followed by 2 GILUPI CellCollector applications

### CTC enumeration

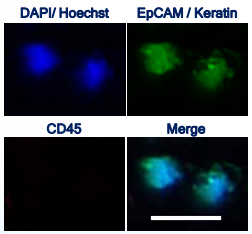



**Figure 4: Immunofluorescence analysis.** CTCs captured directly from circulating blood with the GILUPI CellCollector®. CTCs were identified via positive EpCAM/Keratin, DAPI and negative CD45, and morphological features. Scale bar corresponds to 50µm.

### Clinical correlation

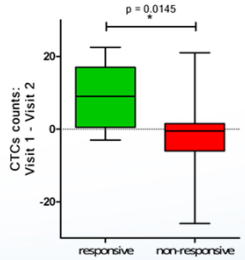
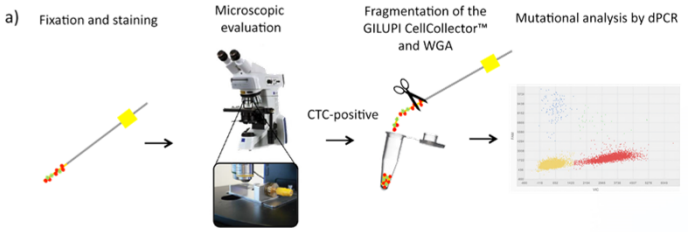
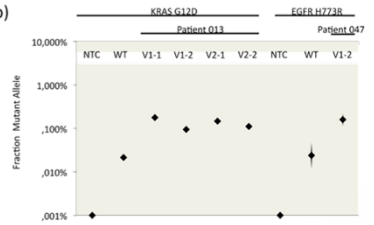



**Figure 5: Change of CTC counts under therapy**. Box-Whisker-Plot of CTC count difference between visit 1 and visit 2 shows a reduction of CTC numbers for patients responsive to therapy. Mann Whitney test confirms difference to patients with non-responsive disease.

### Molecular Analysis




c)

| Patient | Visit | # | CTC count | Blood cells | Primary tumor mutation |
|---|---|---|---|---|---|
| 013 | 1 | 1 | 3 | >500 | KRAS G12D |
| | | 2 | 5 | >1000 | KRAS G12D |
| | 2 | 1 | 1 | >1000 | KRAS G12D |
| | | 2 | 1 | <50 | KRAS G12D |
| 047 | 1 | 2 | 1 | <50 | EGFR H773R |

**Figure 6: Targeted mutation analysis of captured CTCs.**

a) Scheme of the workflow: Cells isolated with the GILUPI CellCollector® were stained according to the staining procedure in the clinical setup and analysed by immunofluorescence microscopy. The GILUPI CellCollector® was fragmented und cells were subjected to whole genome amplification. Amplified DNA was analysed by digital PCR.

b) Amplified genomic DNA was analysed by digital PCR for the mutations KRAS G12D and EGFR H773R which were known to be present in the primary tumour of the respective patients. Both mutation could be detected.

c) Respective patient material examined by digital PCR: CTC count , blood cell background and primary tumour mutation status.

## Summary

- Cumulated (pre-therapeutic und post-chemotherapy initiation) isolation rate of 58% for *in vivo* captured CTCs with the GILUPI CellCollector® in patients with NSCLC
- Successful isolation of CTCs was significantly more frequent with the GILUPI CellCollector® compared to CellSearch® which had a cumulated isolation rate of 27% (data not shown)
- Patients with responsive disease usually showed a reduction of isolated CTC number 12 weeks after chemotherapy initiation
- *KRAS* and *EGFR* mutations present in the primary tumour were also detected in the CTCs isolated with the GILUPI CellCollector®
- Due to this high CTC isolation rate, the device may overcome present limitations in the enrichment of CTCs, especially for early stages.
- The implementation of the GILUPI CellCollector® into clinical practice may improve early detection, prognosis and therapy monitoring of lung cancer patients.
- Besides enumeration, the method allows the molecular analysis of the CTCs, enabling personalized treatment management