An effective in vivo Liquid Biopsy tool for the isolation of circulating tumor cells in non-small cell lung cancer

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Abstract

Circulating tumor cells (CTCs) are a possible prognostic biomarker in the analysis of tumors. Evaluation of CTCs and tumor tissue before and after therapy, at disease progression or before new treatment initiation would be informative for the selection of appropriate prognosis, treatment efficacy and therapy options. Common CTC technologies have limits particularly in detecting low frequency CTCs. The GILUPI CellCollector® screens a large volume of blood directly in the vein. Here, we demonstrate the application of the GILUPI CellCollector® in the assessment of CTCs in non-small cell lung cancer (NSCLC) patients at different tumor stages.

Results

A significantly higher isolation efficiency compared to CELLSEARCH® is shown. No CTCs could be found in controls. The overall sensitivity of the GILUPI CellCollector® is 89%. A pre-surgical isolation rate of 84% compared to 62.5% post-operative was observed.

Patients and Methods

59 NSCLC patients, stage IA to IIIB, were recruited for CTC isolation before (n=59) and after surgery (n=25). CTC enumeration was conducted by immunofluorescence (IF) microscopy followed by analysis for KRAS and EGFR mutations using digital PCR. Primary tumor tissue was analyzed for the same mutations to investigate concordance. For (n=21) CTC samples a direct comparison with CELLSEARCH® was performed.

Conclusions:

The GILUPI CellCollector® overcomes blood volume limitations of other CTC extraction approaches and thereby increases the diagnostic sensitivity of CTC isolation. It allows CTC enumeration, molecular characterization, and biomarker expression analysis, which could help guide treatment strategies and monitoring therapy efficacy.

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

![CellCollector®](image)

**Figure 1:** CTCs and cancer progression. Schematic model of materials.

**Figure 2:** The CellCollector® The functionalized surface of the stainless steel wire consists of a gold layer and a blood repellent hydrogel which bears specificity bound antibodies on the apical cell surface marker EpCAM.

**Figure 3:** The CellCollector® applications. Insertion of the GILUPI CellCollector® through on indwelling catheter into a peripheral arm vein for 30 min. During the application period the 20 min long biomarker by biomarker via direct contact with the blood circulation and isolated CTCs via dPCR sampling.

**Figure 4:** From patient cohort to molecular diagnostic. The CellCollector® was applied to patients and stained afterwards. Cells isolated with the CellCollector® were analyzed microscopically by means of immunofluorescence staining according to the staining procedure in the clinical setup. The aim was to find and to subject to WGA. Whole genome amplification took place via Multiple Displacement Amplification (MDA) technology. Primers (arrows) anneal to the template DNA and are extended at 31°C by Taq polymerase, which moves along the DNA template strand, degrading the complementary strand. After 30 min of extension DNA fragmentation takes place. This DNA is then isolated for PCR amplification and the WGA was performed and subjected to WGA. Whole genome amplification took place via Multiple Displacement Amplification (MDA) technology. Primers (arrows) anneal to the template DNA and are extended at 31°C by Taq polymerase, which moves along the DNA template strand, degrading the complementary strand. After 30 min of extension DNA fragmentation takes place. This DNA is then isolated for PCR amplification.

**Figure 5:** Detection rate of 89% for in vivo captured CTCs with the GILUPI CellCollector®

**Figure 6:** Detection of CTCs in all tumor stages could be shown.

**Figure 7:** The implementation of the GILUPI CellCollector® into clinical practice can improve early detection, prognosis and therapy monitoring of lung cancer patients.

**Figure 8:** Besides enumeration, the method allows the molecular analysis of CTCs, enabling personalized treatment management.

**Figure 9:** The same KRAS and EGFR mutations found in the primary tumors were clearly detectable when 1 to 5 CTCs were present on the CellCollector®. The analyses clearly demonstrated the specificity of our dPCR approach because no signals were seen when analyzing the G12C, L858R, or T790M mutations.

**Table 1**

<table>
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<th>Tumor stage</th>
<th>CTC count before surgery</th>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>III B</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Summary**

- Detection rate of 89% for in vivo captured CTCs with the GILUPI CellCollector®
- Detection of CTCs in all tumor stages could be shown.
- The implementation of the GILUPI CellCollector® into clinical practice can improve early detection, prognosis and therapy monitoring of lung cancer patients.
- Besides enumeration, the method allows the molecular analysis of CTCs, enabling personalized treatment management.
- The same KRAS and EGFR mutations found in the primary tumors were clearly detectable when 1 to 5 CTCs were present on the CellCollector®. The analyses clearly demonstrated the specificity of our dPCR approach because no signals were seen when analyzing the G12C, L858R, or T790M mutations.

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