Cambridges Healthtech Institute´s 22th International Molecular Med Tri-Con 2015
San Francisco, Feb 2015

Enumeration and ALK investigation of circulating tumor cells in non-small cell lung cancer (NSCLC) patients using an effective *in vivo* technology, the GILUPI CellCollector®

K. Luecke[1], L. Gasiorowski[2], S. Herold[1], G. Gallerani[3], S. Krusekopf[1], M. Masiejczyk[1], F. Fabbri[4], W. Dyszkiewicz[2], C. Schumann[5]

[1] GILUPI GmbH, Potsdam, Germany; [2] Department of Thoracic Surgery, University of Medical Sciences, Poznan, Poland ;
[3] Department of Medical and Surgical Sciences (DIMEC), and Pathology Unit, Felice Addarii Institute, University of Bologna, Italy; [4] Bioscience Laboratory, Romagna Cancer Institute - IRCCS IRST; [5] Clinic for Pneumology, Thoracic Oncology, Sleep- and Respiratory Critical Care, Klinikverbund Kempten-Oberallgaeu GmbH, Kempten, Germany

Abstract

Background: During the last decade, the options to treat lung cancer patients with individualized, targeted therapy regimens have improved enormously. A subgroup of non-small cell lung cancer (NSCLC) patients with presence of anaplastic lymphoma kinase (ALK)-rearranged can respond to ALK inhibitors. Unfortunately, the tumor tissue based ALK analysis is not always possible and represents only a single-snapshot in time. Liquid biopsy - isolating and analyzing circulating tumor cells (CTCs) from the blood of lung cancer patients may be an easy and minimal invasive method for confirming the presence of rearrangements and identifying patients eligible for ALK specific treatments. Further, changes in the molecular profile of patients could be detected by the use of liquid biopsy. Currently, CTCs are isolated *in vitro* from limited volumes of patient blood samples. To overcome this limitation, an effective device, the GILUPI CellCollector® was used to isolate CTCs *in vivo*, directly from the bloodstream of lung cancer patients. **Patients and Methods:** The efficiency of the GILUPI CellCollector® was investigated and compared to the current standard method by CTC enumeration. In total, 62 lung cancer patients (NSCLC and SCLC) were screened for CTCs in a single device application. In parallel, blood samples were analyzed with the CellSearch® system. CTC enumeration was conducted by immunofluorescence microscopy using both technologies. In a second set of patients CTC enumeration and ALK characterization using the GILUPI CellCollector® is conducted. The downstream diagnostic of isolated CTCs is performed by immunofluorescence microscopy with specific ALK-antibodies and break-apart fluorescence in situ hybridization (FISH) test. **Results:** The higher effectiveness of the GILUPI CellCollector® could be shown in the first set of patients. The successful isolation of CTCs was significantly more frequent with the GILUPI CellCollector® (73%) compared to CellSearch® (29%). Preliminary data of the second set of patients showed the possible detection of ALK rearrangements directly on the device.

GILUPI CellCollector® - an *in vivo* CTC isolating method

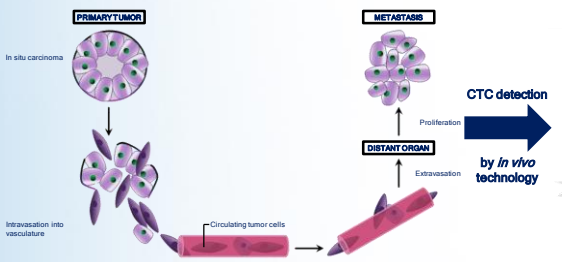

Figure 1: CTCs and cancer progression, Schematic model of metastatic model. Modified from Paris *et al.*, 2013.

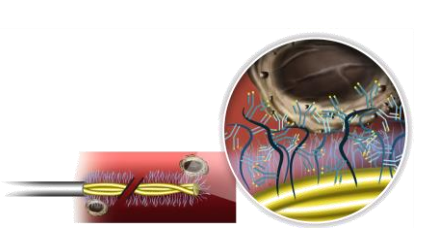

Figure 2: The functionalized surface of the stainless steel wire consists of a gold layer and a hydrogel incorporated with antibodies against epithelial cell surface marker EpCAM. During the application the 40 mm special coated tip comes into direct contact with the blood circulation.

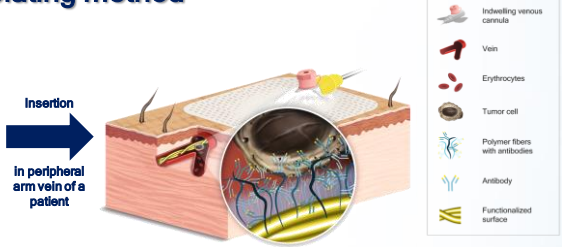

Figure 3: Insertion of the GILUPI CellCollector® through an 20 gauge indwelling cannula in a peripheral arm vein.

Results

Patient recruitment in several clinical trials

NSCLC Poznan
- Started in January 2010 and was finished in 2012
  - 48 NSCLC patients
  - 12 non-cancer subjects

NSCLC + SCLC Ulm
- Started in January 2012
  - 50 lung cancer patients all stages with follow up visits 3 months after systemic therapy initiation

NSCLC + SCLC Ulm
- Started in March 2013
  - 20 lung cancer patients all stages with monthly follow up visits over 3-4 months after systemic therapy initiation

Immunocytochemical analysis

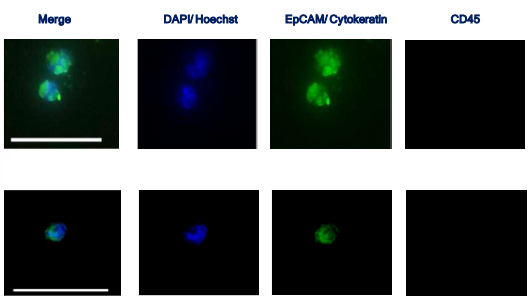

Figure 4: Immunocytochemistry analysis of CTCs captured *in vivo* with the GILUPI CellCollector® in the blood of NSCLC patients. The CTCs were identified and enumerated via positive EpCAM and/or Cytokeratin and DAPI staining and negative CD45 staining (respective green, blue and red staining in top panels, incl. overlay), size and morphological characteristics. CTC examples represent 3 different NSCLC patients. The white scale bar corresponds to 50µm.

CTC enumerations

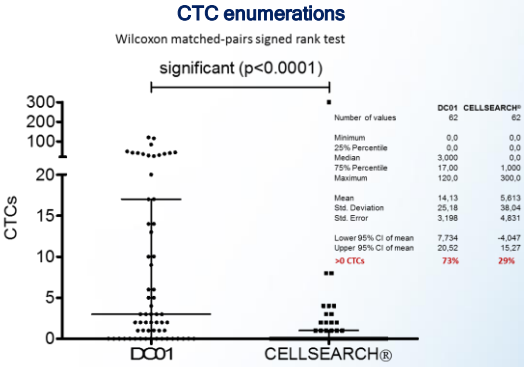

Figure 5: Results of CTC enumerations with the *in vivo* GILUPI CellCollector® DC01 compared to the CELLSEARCH® method of samples received of the described studies. Data from first visits with direct comparison of both methods was used for this analysis.

Further diagnostic approaches

ALK staining and FISH

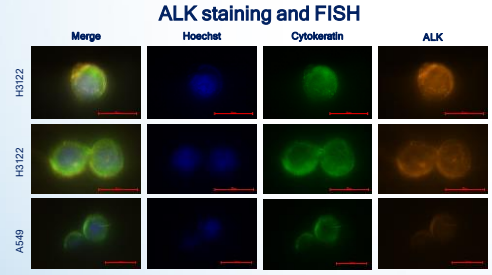

Figure 6: Lung cancer cells were captured by the GILUPI CellCollector® (*in vitro*). Cells were identified by immunocytochemistry staining for epithelial markers (CKs), possible therapeutic target (ALK), and nuclear counterstaining using Hoechst33342. Specificity of the staining was confirmed using Alk high expressing cells (H3122) and Alk low expressing cells (A549). The red scale bar corresponds to 20µm

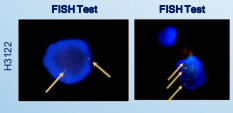

Figure 7: Lung cancer cells were captured by the GILUPI CellCollector® (*in vitro*). ALKs rearrangements were identified using FISH Test. 2 or more orange-only signals could be detected.

Summary

- Summarized detection rate of **73 %** for *in vivo* captured CTCs with the GILUPI CellCollector® in patients with NSCLC and SCLC patients
- Thus, the successful isolation of CTCs was significantly more frequent with the GILUPI CellCollector® compared to CellSearch® with detection rate of 29%.
- Due to this high CTC detection 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. It could also help to establish personalized treatment regimens, e.g. based on the characterization of ALK .
- Besides enumeration, the method may allow the molecular analysis of the CTCs, resulting in personalized treatment regiments

Also funded by: Europäische Union „Investition in Ihre Zukunft" Europäischer Fonds für regionale Entwicklung