Press Release, 20.05.2014

Molecular Mutation Analysis of Circulating Tumor Cells

GILUPI has established a method to analyze CTCs isolated with the GILUPI CellCollector™:

From a lung cancer patient with a known mutation (KRAS p.G12D, c.G35A) of the primary tumor treated at our clinical partner in Ulm (Germany), CTCs were captured at different time points during the course of the disease using the GILUPI CellCollector™.

Visual examination of the GILUPI CellCollector™ was conducted by immunofluorescence microscopy and Hoechst$^+$/Cytokeratin$^+$/EpCAM$^+$/CD45$^-$ cells were counted as CTCs (see Fig. 1).

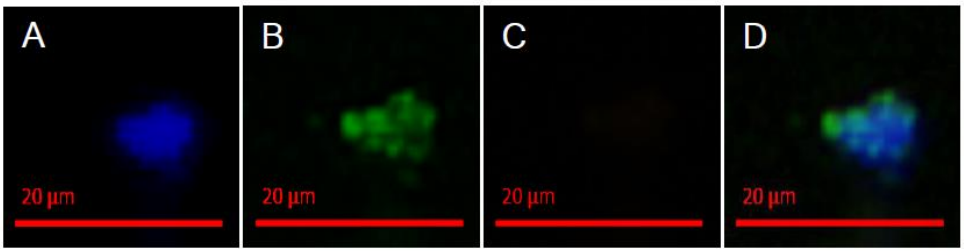

Fig.1: Immunofluorescence image of an isolated CTC
A: Hoechst33342 staining, **B**: Cytokeratin/EpCAM staining, **C**: CD45 staining, **D**: merge

All cells, including unspecifically attached blood cells, were subjected to Whole Genome Amplification (WGA) and amplified genomic DNA was further analyzed for the KRAS mutation using a mutation-specific quantitative real-time PCR.

The KRAS mutation was unambiguously detected when three and five CTCs (verified by immunofluorescence microscopy) were present on the device. Even when only one CTC was bound to the GILUPI CellCollector™, the KRAS mutation was still detectable (see Table 1).

Table 1: CTC counts and CTC KRAS G12D mutation status analysis after immunofluorescence microscopy evaluation at different time points during the course of the disease.

Time point of device application	CTC count on GILUPI CellCollector™	CTC KRAS G12D mutation detected in counted cells?
1	3	yes
2	5	yes
3	1	yes
4	1	no

These results demonstrate that somatic mutations in CTCs isolated with the GILUPI CellCollector™ can be detected down to the single cell level in a background of unspecifically attached blood cells.

GILUPI GmbH
Am Mühlenberg 11
14476 Potsdam
Germany

Tel. +49 331 58184782
Fax +49 331 58184780

info@gilupi.com
www.gilupi.com