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Somatic mutations of the EGFR, KRAS and BRAF genes: homogeneity in single cells from cell lines and heterogeneity in circulating epithalial tumor cells (CETCs) as determined using the cobas® z 480 analyzer

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Background: Targeted therapies directed specifically against somatic mutations in genes involved in signalling pathways have been shown to improve outcome compared with cytotoxic chemotherapies in patients with advanced tumors carrying the respective mutations.

Purpose/Objectives: Identification of such mutations is performed in formalin fixed material from the primary tumor. However, such material is not always available and, even more importantly, cells with metastatic potential must be released and travel via the blood during the course of disease to reach their distant loci. Using maintrac®, a non-dissipative approach avoiding enrichment steps, CETCs can be detected and individually isolated in almost all patients with lung, colon cancer and melanoma and therefore can provide a liquid biopsy to monitor the course of disease. We, here, report on the successful analysis of such isolated cells for gene mutations in tumor driver genes EGFR, KRAS and BRAF.

Materials and Methods: Blood from patients with non small cell lung cancer, colon cancer and malignant melanoma was analyzed for cells positive for epithelial antigen (EpCAM) using the maintrac® approach, which avoids cell selection, and an image anlysis system or laser scanning cytometry for detection. Between 8-20 EpCAM positive cells from each patient were isolated individually using a semiautomated capillary approach and deposited one by one into micro cups. The DNA was subsequently amplified by whole genome amplification and assayed using either the cobas® EGFR mutation test, the cobas® KRAS mutation test or the cobas® BRAF V600 mutation test.

Results: DNA could be amplified from all individually isolated cells. An EGFR mutation was detected in 12% of isolated tumor cells from a patient with non-small cell lung cancer, the KRAS mutation was detectable in 28% of cells from a patient with colon cancer and the BRAF mutation in 100% of cells from a BRAF mutated cell line and in 50% of evaluable cells from a patient with melanoma.

Conclusions: Individually isolating epithelial tumor cells from the peripheral blood from patients with non-small cell lung cancer, colon cancer and melanoma allows not only detection of driver mutations in circulating tumor cells but also to determine the frequency of mutated cells. The results were confirmed by single cell analysis of a BRAF mutated cell line. This proves that at least part of the CETCs are derived from the tumor. They can, in the future, be used as markers of response to the action of drugs and contribute insight into how resistance may be acquired.