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Vemurafenib eliminates BRAF mutated circulating epithelial tumor cells (CETCs) from blood of patients with malignant melanoma

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Background: Almost 50% of melanomas harbor mutations in BRAF, mainly V600E. The mutations are usually identified in the primary tumor. However, the primary tumor is often no longer available and metastases not always reflect the characteristics of the primary tumor. In melanoma it is not clear at what stage epithelial antigen is expressed. CETCs are present in peripheral blood in a significant proportion of patients with malignant melanoma. The aim of the present study was to analyze whether these cells belong to melanoma clone due to the presence of the BRAF gene mutation in these cells. For this reason, the analysis of multiple isolated CETCs from individual patients for BRAF gene mutations was performed.

Materials and Methods: Blood from patients with malignant melanoma was analyzed for cells positive for the EpCAM and Melan-A using the maintrac® approach, avoiding cell selection and using an image analysis system for detection. Subsequently, between 8-20 EpCAM and Melan-A positive cells from each patient were isolated individually using a semi-automated capillary approach and deposited one by one into micro cups. The DNA of individual cells was amplified by whole genome amplification and assayed using the cobas® BRAF V600 mutation test. Furthermore, we performed mutation analysis of cells after magnetic bead enrichment which is known to contain a mixture of CETCs and leukocytes.

Results: DNA could be amplified from all individually isolated cells. In addition, we analyzed the presence of V600 mutation after magnetic bead enrichment. A BRAF mutation was detected in 20 - 75 % of evaluable cells in patients with BRAF mutation in primary tumor. In advanced stage or metastatic patients under Vemurafenib therapy, we were not able to find mutated CETCs.

Conclusions: Individually isolated CETCs from the peripheral blood from patients with melanoma allow not only to detect mutations but also to determine the frequency of mutated cells. This proves that at least part of the CETCs is originated from the primary tumor. Furthermore, detection of BRAF mutation in CETCs may be crucial for a successful molecular-targeted therapy.