Abstract

- *Introduction*

Malignant melanoma is one of the most aggressive skin tumors with unfavorable prognosis and high mortality, especially in the connection with advanced stages. Regarding the incidence growth, it has recorded an increase of 50 % in the last 10 years. The most important for prognosis of the patient with this malignant disease is early diagnosis and radical surgery without delay. In recent years, the benefit of targeted treatment and immunotherapy has been demonstrated in the patients with locally advanced melanoma after resection of primary tumor and nodal metastases.

- *Aims*

The main goal of our study is to identify patients with a high risk of relapse using biomarkers from fluid biopsy (sections of free DNA circulating in blood) who went through a curative surgical treatment and for whom costly adjuvant therapy would be beneficial. Another goal is to use the above-mentioned biomarkers to detect early recurrence itself and residual disease after excision of the primary tumor site.

- *Methods*

Whole blood samples were collected at regular time intervals from patients with early-stage malignant melanoma undergoing curative surgery. Plasma was first separated from the collected whole blood samples by two-phase centrifugation. Cell-free DNA (cfDNA) was isolated at the MD Anderson Cancer Center in Houston, USA. The presence of the BRAF V600E mutation in patient tissue and plasma samples was determined using droplet digital polymerase chain reaction (ddPCR). The patients subsequently underwent regular follow-up care at the Department of Plastic Surgery and the Dermatovenerology Clinic of the University Hospital in Pilsen. We then processed the individual results statistically. IBM SPSS Statistics software (v.26.0, IBM Corp.) was used for data analysis. We considered values of $P < 0.05$ to be statistically significant.

- *Results*

Only 52 of the eighty patients included in our study had primary tumor samples available for BRAF V600E mutation determination. Regarding RR (recurrence rate), DFI (disease free interval) and OS (overall survival), the effect of the BRAF V600E mutation in the tumor sample was not proven. In a preoperative plasma samples, the presence of a BRAF mutation was found to be associated with a high risk of both recurrence and death probability compared with BRAF V600E WT. In plasma samples taken one hour after surgery in 76 patients, the BRAF V600E

mutation was found in 26.3 % of cases (n = 20). Compared with patients with ctDNA BRAF V600E WT, patients with ctDNA BRAF V600E mutation had a higher overall recurrence rate, RR within 6 months of surgery and RR within 2 years. Patients with BRAF V600E mutation also showed shorter DFI and OS. In the plasma samples carried out on the second postoperative day, a BRAF ctDNA mutation with a higher risk of recurrence is found again. In all other timepoints, there was no longer a significant difference in RR, DFI or OS between BRAF mutant ctDNA and BRAF WT ctDNA.

- *Conclusion*

Our results point to the fact that by determining ctDNA one hour after surgery, it is possible to identify high-risk patients who would benefit significantly from adjuvant therapy. Although this study still needs to be confirmed in a larger sample of patients, ctDNA testing may be considered as a future parameter for the indication of adjuvant therapy in patients with early-stage malignant melanoma.