INTRODUCTION
TP53 is the most frequently mutated gene in human cancer. However, in metastatic melanoma mutations of TP53 occur infrequently and p53 fails to function as a tumour suppressor. The altered expression of p53 family members, including p33/p73 isoforms, as well as of the interactions among them could affect normal function of p53. Furthermore, somatic BRAF mutations have been found in 37%-50% of all melanomas, of which almost 90% harbour the activating V600E mutation. Although initial response to BRAF inhibitors is highly effective, the resistant clones frequently develop, and, in treated patients disease progression is observed within 6 to 8 months. To address this, a better understanding of the genetic basis of melanoma initiation and progression is needed.

MATERIAL AND METHODS
The expression profile of p33 and its potential interaction partners - p53 and p73 isoforms was determined in a panel of melanoma cell lines by western blot analysis and quantitative RT-PCR. We have determined the protein levels of p33/p73 isoforms in response to DNA damage treatment (γ-irradiation and etoposide) in cell lines with different TP53 mutational status using western blot analysis. Furthermore, vemurafenib resistant cells are generated and resistance was confirmed by MTT assay. Expression of p33/ p73 isoforms was determined in these cells.

RESULTS AND DISCUSSIONS
Relative expression analysis of metastatic melanoma cell lines revealed that the most expressed p33 isoforms are p33α and Δ133p33α, while Δ40p33α, Δ40p33y and Δ133p33y are least expressed. Also, interestingly, relative expression of full length TAp73 was higher than ΔNp73. Furthermore, the most expressed proteins were p33α, Δ40p33α, Δ133p33y and Δ160p33y. Contrary to gene expression, the most expressed p73 isoform is oncogenic ΔNp73β. γ-irradiation induced accumulation of all p33α isoforms in p53 mutant melanoma cell lines, but not in p53 wild type cells. Levels of p33 beta isoforms remained the same, while gamma isoforms were undetectable. Upon γ-irradiation, accumulation of ΔNp73 isoforms was observed in p53 mutant cells. Treatment with etoposide induced expression of p33α isoform, and both TAp73 and ΔNp73 isoforms in p53 wild type cells. Furthermore, in vemurafenib resistant clones the changes in p33/p73 protein expression were observed.

CONCLUSION
Taken together, these analyses enabled us to detect p33/p73 isoforms in melanoma cell lines and gave us insight into their abundance in melanoma cell lines for further analyses of p53 interacting partners.