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# NRASQ61R immunohistochemistry: a new tool for mutational status screening in challenging melanoma samples

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**To the editor:** We read with interest the recent study by Massi *et al*[1] concerning the evaluation of the new anti-human N-Ras (Q61R) monoclonal antibody (clone SP174) in the mutational screening of melanoma samples. As the recently described BRAFV600E mutation-specific immunohistochemistry, an anti-NRASQ61R mutation-specific antibody may consist of a cost-effective and faster ancillary tool in the mutational screening process that has now become a major requirement for the management of patients with metastatic melanoma. The sensitivity and specificity of 100% reported in this study are very encouraging although they need to be confirmed by additional studies as mentioned by the authors, especially in metastatic samples. In our opinion, some other technical points are worth to be notified.

First, we want to point out the importance of the characteristics of melanoma samples. The authors have only selected primary melanomas with Breslow thickness over 4 mm, although many melanomas have a far thinner Breslow thickness. No data is provided concerning the percentage of tumoral cells in the samples or the modalities of DNA extraction from formalin-fixed and paraffin-embedded tissues used for molecular genetic analysis. Notably, the use of macro- or micro-dissection based on histopathological examination of hematoxylin and eosin-stained sections is not mentioned. The percentage of tumoral cells in the samples and the mutated allele proportion in the extracted DNA can highly influence the results of molecular analysis and the ability of the genotyping method to detect a mutation. In our opinion, these parameters have to be taken into account when interpreting a molecular mutational status result.[2] If we keep in mind the thicknesses over 4 mm of the melanomas analyzed in this study, we can postulate that all samples contained a great proportion of tumoral cells. Nevertheless, we cannot rule out the hypothesis that a high amount of inflammatory reactive cells in some samples can decrease the relative amount of tumor in the extracted DNA. Another major interest of mutation-specific immunohistochemistry is the ability to stain a minority of tumoral cells within a sample containing a majority of nontumoral cells (ie, thin primary melanoma or lymph node micrometastasis).[3] These data are relevant for diagnosis and could explain that one sample in the study by Massi *et al* that was initially considered as *NRAS* wild type by molecular analysis was indeed positive for anti-NRASQ61R immunolabeling and finally found to be $NRAS^{Q61R}$ mutated after molecular reanalysis.

Second, the authors analyzed 97 samples as the last sample of their series was excluded because of amplification failure preventing molecular analysis. In our experience, another advantage of mutation-specific antibodies is the opportunity of bringing out a mutated protein in samples that are not conclusive using genotyping methods (about 2–3% of samples in our experience, unpublished data). The immunohistochemical analysis of this remaining sample could have been of interest, despite the fact that, in case of negativity, an *NRAS* mutation could not have been definitively ruled out.

Third, the authors defined the cutoff for positivity at 60% or more of viable tumoral cells with moderate to strong immunelabeling intensity. Weaker labeling and/or single interspersed immunostained cells were considered as negative and nonspecific. This fact points out the real difficulty in identifying melanoma cells *versus* histiocytic/macrophagic cells. We agree with Massi *et al* that the interpretation of isolated NRASQ61R- or BRAFV600E-immunolabeled cells can be difficult, and sometimes not conclusive especially in case of florid reactive inflammatory infiltrate. Nevertheless, it is still not perfectly clear whether

heterogeneous staining pattern results from technical artifacts or real intra-tumor heterogeneity as mentioned in a study dealing with anti-BRAFV600E immunohistochemistry.[4] In the study of Massi *et al*, intratumoral heterogeneity is illustrated in their Figure 4 by a unique case with only 25% of NRASQ61R-immunolabeled tumoral cells.

Finally, this study is, as far as we know, the first to demonstrate a case of double *BRAF*- and *NRAS*-mutated melanoma with a real co-expression in a single cell of both BRAFV600E and NRASQ61R proteins. Until now, this co-expression was supposed to be lethal for cells.[5,6] Even if these co-mutations are rare, they must be taken under consideration in the strategy for determining the *BRAF* and *NRAS* mutational status. The co-determination of both *BRAF* and *NRAS* mutational status is necessary and an *NRAS*-mutated status does not exclude a BRAF-mutant targetable protein in the same tumor.

To conclude, the confrontation of both immunohistochemical and molecular data could be an efficient strategy because, as illustrated by the discordant cases reported by Massi *et al*, the use of both methods is a more secure approach to avoid false results and inappropriate clinical management of melanoma patients.

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## Disclosure/conflict of interest

The authors declare no conflict of interest.

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