Intra-patient heterogeneity of BRAF mutation status: fact or fiction?

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Sir,

We read with great interest the recent publication by Heinzerling et al (2013), demonstrating intra-patient heterogeneity of BRAF mutation status between tumours in 10 of 53 (18.9%) patients. However, we have great concern that the results of the study may reflect the (less than 100%) sensitivity of the results available in the near future (Von Hoff et al, 2013). As these authors propose, the effects of BRAF status on clinical outcome may be a consequence of the direct elimination of cancer cells and interruption of the cancer cell-stroma interactions. Certainly, additional preclinical and translational clinical studies are needed to determine the precise mechanism of action of this, otherwise, clinically effective regimen.

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This is a letter to the editor.
Clinical responses observed in patients treated with BRAF inhibitors do not support the suggestion of intra-patient BRAF heterogeneity as all metastases have a uniform initial metabolic response to BRAF inhibition assessed using FDG-PET imaging (McArthur et al, 2012), and all resistant lesions resected from patients still contain mutant BRAF (McArthur et al, 2011; Poulikakos et al, 2011; Van Allen et al, 2013).

Further clinical studies are required to examine the issue of intra-patient discordance of BRAF. Carefully assigning primary melanomas as culprit lesions, and using accurate BRAF testing methods with adequate tumour cell content would be the requirements to underpin the data.

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CONFLICT OF INTEREST

AMM has received honoraria from Roche and travel support from Roche and GlaxoSmithKline (GSK). JSW declares no conflict of interest. GVL has been a consultant for Roche, Bristol-Myers Squibb, GSK and Novartis, and has received honoraria and travel support from Roche. RAS has been a consultant for Roche and GSK, and has received honoraria from Abbott Molecular.

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**LETTERS TO THE EDITOR**

**Reply: Intra-patient heterogeneity of BRAF mutation status: fact or fiction?**

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We thank Menzies et al (2014b) for their interest in our work and their detailed and informative remarks that extend what we discussed in our paper. They are concerned that our findings of an unexpected high percentage of heterogeneity reflect methodological problems of mutation detection rather than tumour biology. In contrast, our main worry is that acknowledged and widely used diagnostic techniques could exclude a significant percentage of patients from BRAF inhibitor therapy despite the presence of mutated metastases. Indeed, our study was initiated because we could not believe in the intrapatient heterogeneity even though we like other groups (Houben et al, 2004) were occasionally getting divergent results when retesting new metastases from patients. We will try to explain in our reply why we do not believe that there are ‘easy’ explanations such as lack of sensitivity, low tumour content in samples studied and higher sensitivity of immunohistochemical analyses compared with direct mutation detection.

We are aware that our findings could be due to sensitivity of our testing methods. The suggested approach of immunohistochemistry (IHC), however, will not suffice to detect BRAF mutations. Indeed a substantial patient population will be missed as we and others have shown that rare BRAF mutations are not (V600K, V600D, L597S, V600DKE601del, V600R) or not always detected by IHC (Skorokhod et al, 2012; Heinzlerling et al, 2013). Similarly, the COBAS test does not reliably detect rare mutations (Heinzlerling et al, 2013). Rare mutations have been described in up to 20% of BRAF-mutated patients by your group and others (Beadling et al, 2011; Long et al, 2011; Dahlman et al, 2012) and it is crucial to detect them as these patients respond to therapy with BRAF inhibitor (Chapman et al, 2011; Klein et al, 2013). Thus, even though possibly the intrapatient heterogeneity might be lower in the published IHC study by Menzies et al (2014a) using IHC as only detection technique would exclude patients with actionable mutations from effective treatment with a BRAF inhibitor. Furthermore, discordance rates of course also depend on the number of samples tested. And even the study with lowest rates of heterogeneity only using paired samples of primary tumour and one metastatic lesion found heterogeneity in some patients with concordant results in 90.9% (Boursault et al, 2013). It is likely that the rate of heterogeneity is higher when testing more samples per patient (up to 13 in our studies) and as shown by Colombino depends on the number of samples studied.

In summary, we do not believe that our findings are an artefact of our testing method. As we have shown, the intrapatient heterogeneity, is a common finding in melanoma patients with BRAF mutation, even though not all have a clinical response to BRAF inhibition.