spared from surgery, as surgery would not be beneficial for those patients. Thus, for patients with resectable or borderline resectable disease by CT scan and high ($>180\,U\,ml^{-1}$) CA19.9, we usually administer chemotherapy upfront and explore surgically those patients who do not progress after two cycles of treatment provided laparoscopic assessment of peritoneal disease is negative as well.

In response to Ramirez et al

We read with great interest the comments made by Ramírez *et al* (2014) in which they highlight the importance of tumour stroma in pancreatic cancer (PDAC) and the role of 'pancreatic stellate cells' in the development of tumour stroma. The current data, while with still some inconsistencies, show that in preclinical models of PDAC, the combination of gemcitabine and Nab-paclitaxel (PTX) increases the delivery of gemcitabine to the tumour. Mechanistically, this has been explained by a decrease in the expression of the gemcitabine catabolism enzyme cytidine deaminase and hence increasing the intracellular retention time of the active gemcitabine metabolites or by elimination of the PDAC stroma (Von Hoff *et al*, 2011; Frese *et al*, 2012). In the only clinical study available so far, we have shown that Nab-PTX markedly alters the PDAC stroma and decreases the number of CAF (Alvarez *et al*, 2013).

The precise mechanisms underlying these observations remain obscure. Selective binding of albumin-coated Nab-PTX to SPARC-positive cells or uptake of nutrient-rich drug by cancer cells by pynocitosis have been proposed and are the subject of specific studies. The role of SPARC has been studied in the MPACT randomised clinical trial and we hope to have these results available in the near future (Von Hoff *et al*, 2013). As these authors propose, the effects of Nab-PTX on cancer stroma could 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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REFERENCES

Alvarez R, Musteanu M, Garcia-Garcia E, Lopez-Casas PP, Megias D, Guerra C, Muñoz M, Quijano Y, Cubillo A, Rodriguez-Pascual J, Plaza C, de Vicente E, Prados S, Tabernero S, Barbacid M, Lopez-Rios F, Hidalgo M (2013) Stromal disrupting effects of nab-paclitaxel in pancreatic cancer. *Br J Cancer* **109**: 926–933.

Boeck S, Haas M, Ormanns S, Kruger S, Siveke JT, Heinemann V (2014) Neoadjuvant chemotherapy in pancreatic cancer: innovative, but still difficult. *Br J Cancer;* e-pub ahead of print 18 March 2014; doi:10.1038/bjc.2014.60.

Frese KK, Neesse A, Cook N, Bapiro TE, Lolkema MP, Jodrell DI, Tuveson DA (2012) nab-Paclitaxel potentiates gemcitabine activity by reducing cytidine deaminase levels in a mouse model of pancreatic cancer. *Cancer Discov* **2**: 260–269.

Ramírez N, Viúdez A, Hernández-García I, Guerrero D, Gómez-Dorronsoro M, Herrera FJ, Vila J, Beloki L, Ciaúrriz M, Mansilla C, Vera R (2014) Stellate cells, a point of light in the dark night of pancreatic cancer. *Br J Cancer;* e-pub ahead of print 18 March 2014; doi:10.1038/bjc.2014.59.

Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF (2013) Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* **369**(18): 1691–1703.

Von Hoff DD, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M (2011) Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* **29**: 4548–4554.

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

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Sir,

We read with 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 molecular techniques employed and/or an incorrect assumption that the primary melanoma was the source of the metastatic disease rather than true intra-patient *BRAF* heterogeneity.

Potentially, the results of the study by Heinzerling *et al* could have tremendous clinical importance, as accurate determination of a patient's melanoma *BRAF* status is critical when planning treatment for melanoma patients with advanced stage disease. Targeting the mitogen-activated protein kinase (MAPK) pathway in patients with *BRAF*-mutant metastatic melanoma has vastly improved clinical outcomes; however, BRAF inhibitors may paradoxically activate the MAPK pathway in wild-type *BRAF* melanomas and therefore adversely affect survival if such patients are treated with BRAF inhibitors. Thus, if intra-patient melanoma *BRAF* heterogeneity exists and treatment decisions are made on the basis of mutation assessment of a single tumour, potentially effective treatment may not be offered in a significant proportion of patients, or alternatively, treatment may be administered that is potentially detrimental.

Although the results of the study by Heinzerling *et al* are in keeping with other recent reports of heterogeneity in 15% and 13.5% of patients (Colombino *et al*, 2012; Saint-Jean *et al*, 2014), two recent studies (Boursault *et al*, 2013; Menzies *et al*, 2013) demonstrated very little heterogeneity of *BRAF* status within metastatic melanoma patients. Several factors may have influenced the results of these studies. First, the techniques used to determine *BRAF* status were different in the 'higher' and 'lower' discordance studies. The latter studies used a highly sensitive and specific immunohistochemical technique (the anti-$BRAF^{V600E}$ VE1 antibody) that enables determination of the BRAF status in all individual cells by direct visualisation and at the same time confirmation that they are in fact tumour cells. This technique is not reliant on a certain percentage of tumour cells being present. In contrast, the former studies used molecular methods such as pyrosequencing, allele-specific PCR, and Sanger sequencing, all of which may have false-negative results when samples contain low tumour content. A recent study highlighted the problem of false-negative mutation tests by molecular techniques. Discordant $BRAF^{V600E}$ status was identified in 5 of 97 specimens; subsequent molecular retesting both confirmed an initial molecular misdiagnosis in 4 of the 5 cases and the greater accuracy of BRAF protein immunohistochemistry (Long *et al*, 2013).

Another factor that may have resulted in heterogeneity is the assumption that any given primary melanoma is the culprit tumour from which the metastatic disease was derived. Ten per cent of patients with metastatic melanoma have a history of multiple primary melanomas (Murali *et al*, 2012). Even in patients with a history of only a single known primary melanoma, sometimes the site of locoregional metastasis is not in keeping with the T-stage or site of the presumed primary melanoma, or it does not occur within a plausible time period, suggesting that an occult primary melanoma may have led to the metastatic disease. In this situation, close scrutiny of a patient's clinical history is required to ensure accurate assignment of the 'culprit' primary melanoma (Murali *et al*, 2012).

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.

REFERENCES

Boursault L, Haddad V, Vergier B, Cappellen D, Verdon S, Bellocq JP, Jouary T, Merlio JP (2013) Tumor homogeneity between primary and metastatic sites for BRAF status in metastatic melanoma determined by immunohistochemical and molecular testing. *PLoS One* **8**(8): e70826.

Colombino M, Capone M, Lissia A, Cossu A, Rubino C, De Giorgi V, Massi D, Fonsatti E, Staibano S, Nappi O, Pagani E, Casula M, Manca A, Sini M, Franco R, Botti G, Caraco C, Mozzillo N, Ascierto PA, Palmieri G (2012) BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. *J Clin Oncol* **30**(20): 2522–2529.

Heinzerling L, Baiter M, Kuhnapfel S, Schuler G, Keikavoussi P, Agaimy A, Kiesewetter F, Hartmann A, Schneider-Stock R (2013) Mutation landscape in melanoma patients clinical implications of heterogeneity of BRAF mutations. *Br J Cancer* **109**: 2833–2841.

Long GV, Wilmott JS, Capper D, Preusser M, Zhang YE, Thompson JF, Kefford RF, von Deimling A, Scolyer RA (2013) Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma. *Am J Surg Pathol* **37**(1): 61–65.

McArthur GA, Puzanov I, Amaravadi R, Ribas A, Chapman P, Kim KB, Sosman JA, Lee RJ, Nolop K, Flaherty KT, Callahan J, Hicks RJ (2012) Marked, homogeneous, and early [18F]fluorodeoxyglucose-positron emission tomography responses to vemurafenib in BRAF-mutant advanced melanoma. *J Clin Oncol* **30**(14): 1628–1634.

McArthur GA, Ribas A, Chapman PB, Flaherty KT, Kim KB, Puzanov I, Nathanson KL, Lee RJ, Koehler A, Spleiss O, Bollag G, Wu W, Trunzer K, Sosman JA (2011) Molecular analyses from a phase I trial of vemurafenib to study mechanism of action (MOA) and resistance in repeated biopsies from BRAF mutation-positive metastatic melanoma patients (pts). *J Clin Oncol* **29**(Suppl 15): abstract 8502.

Menzies AM, Lum T, Wilmott JS, Hyman J, Kefford RF, Thompson JF, O'Toole S, Long GV, Scolyer RA (2013) Intrapatient homogeneity of BRAFV600E expression in melanoma. *Am J Surg Pathol*; e-pub ahead of print 12 December 2013; doi:10.1097/PAS.0000000000000136.

Murali R, Brown PT, Kefford RF, Scolyer RA, Thompson JF, Atkins MB, Long GV (2012) Number of primary melanomas is an independent predictor of survival in patients with metastatic melanoma. *Cancer* **118**(18): 4519–4529.

Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B, Gabay MT, Salton M, Dahlman KB, Tadi M, Wargo JA, Flaherty KT, Kelley MC, Misteli T, Chapman PB, Sosman JA, Graeber TG, Ribas A, Lo RS, Rosen N, Solit DB (2011) RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). *Nature* **480**(7377): 387–390.

Saint-Jean M, Quéreux G, Nguyen J-M, Peuvrel L, Brocard A, Vallée A, Knol A-C, Khammari A, Denis MG, Dréno B (2014) Is a single *BRAF* wild-type test sufficient to exclude melanoma patients from vemurafenib therapy? *J Invest Dermatol* **13**(5): 1468–1470.

Van Allen EM, Wagle N, Sucker A, Treacy DJ, Johannessen CM, Goetz EM, Place CS, Taylor-Weiner A, Whittaker S, Kryukov GV, Hodis E, Rosenberg M, McKenna A, Cibulskis K, Farlow D, Zimmer L, Hillen U, Gutzmer R, Goldinger SM, Ugurel S, Gogas HJ, Egberts F, Berking C, Trefzer U, Loquai C, Weide B, Hassel JC, Gabriel SB, Carter SL, Getz G, Garraway LA, Schadendorf D (2013) The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov*; e-pub ahead of print 22 November 2013.

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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 methodical 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, V600DK601del, V600R) or not always detected by IHC (Skorokhod *et al*, 2012; Heinzerling *et al*, 2013). Similarly, the COBAS test does not reliably detect rare mutations (Heinzerling *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 type of metastases with highest rates of 24% heterogeneity for skin metastases (overall discordance rate: 15%; Colombino *et al*, 2012). Furthermore, in our article we show intratumoural heterogeneity of the immunohistochemical BRAFV600 staining, a finding that has been confirmed by other groups using molecular methods (Lin *et al*, 2011; Yancovitz *et al*, 2012). In addition,