cysteine (SPARC) although they could not find the correlation between the degree of SPARC expression and clinical or pathological responses due to its high affinity to albumin protein of nab-paclitaxel. SPARC strengthens the accumulation of nab-paclitaxel mediated by albumin in the 'tumour's Achilles' heel' (Von Hoff, Annual meeting of the American Society of Clinical Oncology, 2009); however, the mere use of this endogenous transport system would not justify the clinical impact of this combined treatment. From our point of view the key may be in what Omary *et al* (2007) described as 'a star on the rise' in pancreatic disease: the pancreatic stellate cells (PaSCs; α-SMA + Nestin + + Vimentin + +) because of their role as the main manufacturers of profibrotic extracellular matrix (ECM) components of the pancreatic tumour stroma, and which should match with the activated CAFs (SMA + Vimentin + fibroblasts) in Alvarezś paper.

In pancreatic cancer, PaSCs show increased proliferation and migration properties, and so they could be a suitable target for *nab*-paclitaxel because of their ability to interfere with the mitotic activity (Gradishar, 2006). Alvarez *et al* (2013) demonstrate that although CAFs number decreases in patients treated with *nab*-paclitaxel plus gemcitabine in neoadjuvant setting, the average of activated CAFs remains unchanged.

We would like to explain our hypothesis related to what is happening in the stroma: it could be a transient blockade of activated CAFs metabolism, a quiescent status forced for a pharmacologically active substance. Bachem *et al* (2005) show that cancer cells (CCs) induce a desmoplastic reaction in pancreatic adenocarcinoma by stimulating PaSCs in a paracrine way. So it could be an indirect elimination of principal fibrogenic mediators that stimulate proliferation (platelet-derived growth factor) and ECM synthesis (fibroblast growth factor -2 and transforming growth factor -ß1) of activated PaSCs through the abrogation of CCs. In this sense, the hypothetical presence of surface cellular receptors for *nab*-paclitaxel in CCs could be an interesting pathway biomarker for the effectiveness of the drug as authors related. On the other hand, the ablation of physiological PaSCs functioning after *nab*-paclitaxel inclusion, in turn, would break the two-way communication between PaSCs and CCs (Apte *et al*, 2013). In this context, under normal conditions CCs recruit new PaSCs to their vicinity (Vonlaufen *et al*, 2008), whereas *nab*-paclitaxel would temporarily inhibit the main mechanism that rules the desmoplastic reaction.

All these molecular mechanisms would allow to maintain and stabilise the activated CAF's number despite them being dysfunctional, and also would decline its CAF progeny. In this sense, Apte *et al* (2013) and Bachem *et al* (2005) propose that activated PaSCs can transform into a myofibroblast-like phenotype sub-population with the ability of secreting excess amounts of ECM.

In conclusion, due to the dynamic nature of the stromal compartment, it is critically involved in the development and progresion of pancreatic tumours (Heinemann *et al*, 2013). Before using neadjuvant treatment it may be important to know the stroma's cellular activation grade with regard to PaSCs plus their number. In this sense, PaSCs or activated CAFs could give us ample information about tumoral potential of the stroma itself and so could have an important contribution for the patient's prognosis. Also, the fact that activated CAFs do not decrease after *nab*-paclitaxel treatment could not mean that these cells are not one of the main actors of stromal disruption but the primary target of *nab*-paclitaxel.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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*Correspondence: Dr I Hernández-García; E-mail: irene.hernandez.garcia@cfnavarra.es or Dr N Ramírez; E-mail: natalia.ramirez.huerto@cfnavarra.es

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Reply: 'Comments on Stromal disrupting effects of nab-paclitaxel in pancreatic cancer'

R Alvarez[1], M Musteanu[2], E Garcia-Garcia[2], P P Lopez-Casas[2], D Megias[2], C Guerra[2], M Muñoz[2], Y Quijano[1], A Cubillo[1], J Rodriguez-Pascual[1], C Plaza[1], E de Vicente[1], S Prados[1], S Tabernero[1], M Barbacid[2], F Lopez-Rios[1] and M Hidalgo[*,1,2]

[1]*Centro Integral Oncológico Clara Campal (CIOCC), Oña 10, 28050 Madrid, Spain and* [2]*Spanish National Cancer Research Centre (CNIO), Melchor Fernández Almagro 3, 28029 Madrid, Spain*

Sir,

In response to Boeck et al

We read with interest the comments made by Boeck *et al* (2014) about our study. We appreciate their attention to the units used to report CA19.9 levels, which indeed should have been U ml^{-1} and not U dl^{-1} as stated. We certainly agree that a high level of CA19.9 at diagnosis may be an indication of advanced disease and that this should be considered in the selection criteria in preoperative studies. Indeed in our study, with small sample size, one patient with very high CA19.9 level who actually progressed during chemotherapy skewed that average level of CA19.9. This patient was not operated and therefore does not affect the tissue results. As Boeck *et al* mention, levels of CA19.9 should be either a selection criteria or a stratification factor in outcome-oriented preoperative studies that should also include better imaging methods to determine responses and histological, rather than cytological, diagnosis. In our study, however, as the goals were to determine the effects of Nab-paclitaxel in tumour tissue, this criterion was not part of the eligibility criteria. We agree, however, that future controlled studies to confirm our observations should exclude patients with elevated CA19.9 and plan to do so.

In clinical practice, however, one of the goals of preoperative treatment is to identify patients with more advanced or resistant disease who can be

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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*Correspondence: Dr M Hidalgo; E-mail: mhidalgo@cnio.es
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Intra-patient heterogeneity of *BRAF* mutation status: fact or fiction?

A M Menzies[1], J S Wilmott[1], G V Long[1] and R A Scolyer*[,1,2]

[1]*Melanoma Institute Australia and The University of Sydney, Sydney, NSW, Australia and* [2]*Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Missenden Road, Sydney, NSW 2050, Australia*

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).