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Comment on 'KRAS-mutated plasma DNA as predictor of outcome from irinotecan monotherapy in metastatic colorectal cancer'

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Sir,

We read with great interest the article 'KRAS-mutated plasma DNA as predictor of outcome from irinotecan monotherapy in metastatic colorectal cancer' published by (Spindler *et al*, 2013) in the December 2013 issue of the *British Journal of Cancer*. It is now well established that only patients with wild-type *KRAS* metastatic colorectal cancer benefit from treatment with an anti-epidermal growth factor receptor (EGFR) monoclonal antibody and that patients with *KRAS* mutant metastatic colorectal cancer do not (Karapetis *et al*, 2008; Douillard *et al*, 2010). Up until now, DNA from archival tumour tissue is used to determine *KRAS* mutations in clinical practice. Increased recent data indicate that circulating tumour DNA in plasma, could be a new way to analyse the somatic mutation in tumours and could be a potential biomarker to ensure optimal treatment (Murtaza *et al*, 2013). Spindler *et al* (2013) aimed to investigate the clinical implication of *KRAS* and *BRAF* mutations in both archival tumour tissue and plasma cell-free DNA in 211 metastatic colorectal cancer patients treated with second-line irinotecan monotherapy. Authors observed that plasma *KRAS* mutations, but not tumour *KRAS* mutations, were associated with worse disease control rate, progression-free survival and overall survival. However, contrary to what is mentioned in the title, the predictive impact of the plasma *KRAS* and *BRAF* mutations for the irinotecan response treatment cannot be evaluated in this study because there is no control arm (patients receiving other therapies or no therapy).

In this study, *KRAS* mutations have been detected less frequently in plasma (31%) as compared in tumour (45%) (16 patients with a wild-type *KRAS* plasma had a mutation in the tumour). Tumour *KRAS* mutations were analysed in formalin-fixed paraffin-embedded tissue obtained at diagnosis, whereas plasma *KRAS* mutations were analysed in pretreatment blood samples before the beginning of second-line irinotecan monotherapy. The description of patients receiving an anti-EGFR in first-line therapy would be an interesting information, as acquired *KRAS* mutations can be induced by these therapies (Misale *et al*, 2012). The presence of a minority subclone harbouring *KRAS* mutations within tumours might explain the secondary resistance to anti-EGFR therapy (Tougeron *et al*, 2013) and the emergence of plasma *KRAS* mutations (Diaz *et al*, 2012).

Furthermore, the discordance for the *KRAS* mutation detection rate between tumour and plasma could be explained by a lack of sensitivity for the plasma *KRAS* mutations detection or by the absence of circulating tumour DNA for some patients. The amplification refractory mutation system-quantitative PCR (ARMS-qPCR) methodology, used in this study, has a sensitivity around 0.1% (Fox *et al*, 1998; Nordgård *et al*, 2012). Some studies have suggested that ARMS has an insufficient sensitivity to detect low levels of *KRAS* mutation (Nordgård *et al*, 2012). Indeed, the level of circulating tumour DNA in plasma can be very low and may represent only a small fraction of the total circulating DNA (<0.01%) (Diehl *et al*, 2008; Taly *et al*, 2013). Techniques with very high sensitivity for circulating tumour DNA detection have been recently developed (Taly *et al*, 2012), such as microdroplet technology, which can detect one mutant *KRAS* gene among 200 000 wild-type *KRAS* genes in the plasma (Pekin *et al*, 2011). Thus, we think that the results of the study by Spindler *et al* (2013) should be interpreted with caution because the poor prognosis of patients with plasma *KRAS* mutation could only reflect the poor prognosis of patients with a high level of circulating tumour DNA, as suggested by some others studies (Lefebure *et al*, 2010; Spindler *et al*, 2012). In contrast, the better prognosis could only reflect the low level of circulating tumour DNA that is not detectable by the ARMS assay for the *KRAS* mutation testing.

In conclusion, this promising work published by Spindler *et al* (2013) highlights the impact of circulating tumour DNA on the treatment response of metastatic colorectal cancer. Moreover, it strengthens the need for harmonising detection methods for *KRAS* mutations and to develop highly sensitive techniques for plasma testing. Thus, correlation of *KRAS* mutation in primary tumours, metastases and plasma during metastatic colorectal therapies still needs to be studied.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Response to comment on 'KRAS-mutated plasma DNA as predictor of outcome from irinotecan monotherapy in metastatic colorectal cancer'

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Sir,

We were pleased to read the comments (Tougeron *et al*, 2014)on our recently published data on KRAS mutation detection in plasma, which underline the strong interest these aspects attract.

Cell-free DNA, and tumour mutation detection and quantification in plasma can be discussed from three different but interacting aspects—that is, methodological, biological and clinical. In the comments from our peers, there is a strong biological and methodological focus, whereas in our report of the data, we have chosen to focus on the clinical observations. With the current threshold for mutation detection in our cohort, we found a strong association to outcome in terms of both responses, PFS and OS. However, contrary to the criticism above, we have not definitively concluded that our observations are predictive (hence, the title predictive of 'outcome'), as (as also stated in our concluding remarks) randomized trials are clearly needed to clarify this.

In general, we agree with most of the comments above, all of that are highly relevant from a biological and methodological view.

Of note, the method used in our lab has been developed and refined to a detection level far beyond the reported 0.1% referred to by the authors. The detection sensitivity varied between 0.03 and 0.0005% depending on the type of mutation detected, 12asp (1/200000, 0.0005%), 12Cys (1/200000, 0.0005%), 12ser (1/7000, 0.014%), 13asp (1/3000, 0.033%), 12ala (1/100000, 0.0010%), 12val (1/200000, 0.0005%), 12arg (1/200000, 0.0005%), respectively. However, as also stated above that regardless of the assay, the sensitivity may be determined by the concentration of DNA because of the generally low amount in plasma. Whereas a reliable method with a high sensitivity is needed for mutation detection, it can also be argued that there is a clinical as well as a subclinical detection level. In other words, the importance of the mutations depends on a certain threshold, and that detection of subclinical levels may be less relevant from a clinical point of view.

It is criticised that the detectable pKRAS is merely a surrogate for a high ctDNA level. However, KRAS was detected even in patients with low cfDNA levels (data not presented). It is correct that we and others have reported that a high level of total cfDNA implies a poor prognosis in itself (Spindler *et al*, 2012, 2013a, 2013b, 2013c, 2013d; Hansen *et al*, 2014). Clearly, we have also observed a strong correlation between total cell-free DNA levels and quantitative mutated alleles. In the present report, we did not present the quantitative data of total cfDNA levels (data are submitted for publication elsewhere), but in brief, previously combined analysis suggests that the combination of both parameters has a strong clinical impact, indicating that the presence of KRAS does not merely reflect a high level of cfDNA. Furthermore, the potential utility of plasma KRAS detection with the present method should not be disregarded on the basis of biological assumptions, but rather validated in larger cohorts. For clinical purposes, a simple detection of mutations in a sample is feasible compared with the broad quantitative range that cfDNA measurement provides and makes a clinical application difficult.

The authors comment on the role of mutations for 'acquired resistance' to EGFR inhibition. We and others have indeed presented data that suggest that mutations appear at the time of progression (Diaz *et al*, 2012; Misale *et al*, 2012; Spindler *et al*, 2012; Tougeron *et al*, 2013). Clearly, optimal methods need to be applied with the perspective of gaining further knowledge of tumour biology, heterogeneity and to clarify whether mutations are early events at low concentrations or *de novo* mutations actually do appear along with the development of resistance to a certain therapy.

In conclusion, we are pleased to be able to contribute to the discussion and call for international cooperation to gain further knowledge of methodological, biological and clinical aspects within this interesting field.

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