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## **BIC**

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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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