Author's response to reviews

Title: Massively parallel sequencing fails to detect minor resistant subclones in tissue samples prior to tyrosine kinase inhibitor therapy

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Version: 2
Date: 11 October 2014

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Dear Editor,

we would like to submit our manuscript entitled “Massively parallel sequencing fails to detect minor resistant subclones in tissue samples prior to tyrosine kinase inhibitor therapy" with the authors Carina Heydt, Niklas Kumm, Dr. Jana Fassunke, Helen Künstlinger, Michaela Angelika Ihle, Dr. Andreas Scheel, Prof. Dr. Hans-Ulrich Schildhaus, Prof. Dr. Florian Haller, Prof. Dr. Reinhard Büttner, PD Dr. Margarete Odenthal, Prof. Dr. Eva Wardelmann, PD Dr. Sabine Merkelbach-Bruse as a Full Paper to *BMC Cancer*.

The aim of the study was to evaluate whether drug resistance mutations develop during therapy by mutagenesis or are already present in minor subclones prior to therapy. In this study, primary tumours of an FFPE collective of 33 corresponding primary and recurrent GISTs with known mutational status were analysed for the secondary mutations of the recurrences. For this purpose three ultrasensitive massively parallel sequencing approaches on the GS Junior (Roche) and the MiSeq™ (Illumina) were applied.

With the currently available ultrasensitive methods and with the achieved sensitivity of 0.02%, no pre-existing resistant subclones could be detected in 33 analysed primary FFPE GISTs with known secondary resistance mutation and in nine fresh-frozen GISTs prior to therapy. On both sequencing systems the sensitivity was limited by sequencing artefacts. Our findings support the theory that such mutations develop under treatment by “de novo” mutagenesis.

The assessment of the probability of pre-existing resistant subclones is an ongoing challenge and of major importance for therapy decisions. In other tumour entities, pre-existing resistant subclones could be detected with a comparable sensitivity of methods. However, in previous studies pre-existing subclones were determined in blood samples and cell cultures in combination with mathematical modelling.

Our results provide an important rationale for cancer diagnostics and therefore we think that our findings are of interest to you and the audience of *BMC Cancer*.

Thank you for your kind consideration.

Yours sincerely

Carina Heydt