Author’s response to reviews

Title: Dynamic and unpredictable changes in mutant allele fractions of BRAF and NRAS during visceral progression of cutaneous malignant melanoma

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Version: 2 Date: 04 Jul 2019

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BCAN-D-18-01545R2

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Editor, BMC Cancer

Reply to comments

1. Both reviewers suggested to include additional figures and tables.

Response: The manuscript contained 4 figures, where fig 2 was a composit of 2 and Fig.3 was a composit of 3. When correcting/revising the manuscript the authors did not feel that additional figures can help to better understand our findings/data. Instead, we have reshaped the Fig.3 and 4 to better visualize our message. Also in the Result section p8 L3-14 we have explained in details those figures and referred to individual cases. We think these changes clarify and also justify our messages. Concerning the supplementary Table 1 and 2 which was prepared for Rev1. Our intention was to provide the raw data on what the Figures as well as the new Table 3 and Table 4. were based. Also we have simplified the Table 3 and prepared a novel Table 4. for better understanding, see p8 L15-26.

2. English

We have corrected the English by a native English speaker.
The mutant allele fraction of BRAF and NRAS was determined in this study by high sensitivity (2%) CE-IVD pyrosequencing technology of Qiagen: PyroMarkQ24 using the manufacturer’s software for quantitation. The details of the technology is described in Materials and Methods and also can be seen on the homepage of the manufacturer. Using these KITs we believe we have used a high standard technology which is also used in routine diagnostics. Details now can be found on p6L4-25. This technology was extensively used in the literature of melanoma and colorectal cancer and was compared to other techniques for example:


Critique was the controversial method of MAF determination. First (and also see resp 3.) we have used a CE-IVD test for BRAF and NRAS MAF measurement which is not controversial since it was approved in US as well as in EU for routine determination of BRAF and NRAS mutation testing and measurement. Concerning the normal tissue contamination, we have used a semiquantitative approach which is direct counting the number of tumor cells to normal cells in a macrodissected sample with the highest tumor content, which was performed by an experienced pathologist and lab technician. In this way we have obtained a relatively correct estimate of T/N ratio in the sample. Next the MAF value determined by PyroMarkQ24 testing was corrected with this value to obtain a adjusted value of MAF which approach best the reality in tumor tissue. (MAF of PyroMark multiplied by 100/x% of T proportion) see novel Materials and Methods (p6L4-25). This type of adjustment usually is not done neither in IVD tests nor in NGS therefore a variable T/N ratio can distort the data of MAF. Here we think we have used a more evaluation. On the other hand, the high variability in MAF values in primary or metastases of melanoma is not due to extreme T/N ratios since high T/N or low T/N both were associated with high or low MAFs (see supplementary Tables 1 and 2). Also the high sensitivity of the tests (2% threshold) provided assurance for correctness. Concerning the CNV issue, since a parallel large scale study analyses the CNV changes in primary and metastatic melanoma we dont have those data yet. On the other hand, since we dont have the CNV data we did not use MAF terminology of heterozygozity or homozygozity and clonality and subclonality just the low, medium high categories, where it is known that in case of heterozygosity (one mutant allele) 50% MAF is optimal in case of 100% tumor purity. We have on the other hand found higher than 50% MAF values frequently in primary as well as metastatic tumors which is a sign of possible amplification of the mutant allele or LOH of the wild type allele. Our ongoing studies will answer these questions. (we now discuss this issue in the revised Discussion p9 L11-16).

In summary, we really believe that we made as much as we can to best reply to the critique of the reviewers to improve our manuscript.