Author's response to reviews

Title: Limited importance of dominant-negative effect of TP53 missense mutations

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Author's response to reviews: see over
Dear Editor,

Please consider revision of article entitled: Limited importance of the dominant-negative effect of TP53 missense mutations by Stoczynska-Fidelus E, et al. All suggestions of reviewers were addressed and all changes were highlighted. Following paragraph was added in response to Professor Eytan Ruppin proposal to emphasize biological differences between cells in vivo and cell lines:

The data presented above show that although surgical specimens are analysed less precisely than cell lines, single heterozygous mutations are still observed more frequently *in vivo* than *in vitro*. The G16, MOLT13-boost and PF-382 cell lines consist of cells that were selected from subpopulations arising *in vivo* (in the case of the MOLT13-boost minor subpopulation), whereas H-318 cells probably acquired the 17p LOH *in vitro* (adaptation to *in vitro* conditions and further stages of tumorigenesis). Thus, it may be presumed that it is possible to detect many biological differences between cells observed *in vivo* and *in vitro* in terms of TP53 status. For example, artificial selection forces acting *in vitro* may change the TP53 status. Alternatively, rapid selection and generation of subpopulations of the more advanced neoplastic cells may be observed under such conditions. The latter interpretation would favour *in vitro* conditions as selecting the most effective impairment of TP53, i.e., the selection of cells at more advanced stages of carcinogenesis. Nonetheless, an effective DNE would not be easily replaced by elimination or impairment of the second allele under artificial or *in vivo* selection pressure. Moreover, it is very unlikely that a mechanism that is sufficiently effective *in vivo* would become utterly suppressed *in vitro*. In general, *in vitro* selection and generation of cells presenting hemi/homozygous mutations undermines the importance of dominant-negative inactivation of TP53. Still, we cannot exclude that *in vitro* cell culture does not optimally recapitulate all aspects of tumorigenesis involving TP53.

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Reference was added: Our approach goes in accordance to Olivier’s group suggestion to consider the percentage of cases showing LOH in the estimation of DNE importance [15].
Source of the claim was added: It has been confirmed in many cell line analyses that the first examination is not sufficiently thorough, whereas surgery/biopsy sample analyses are rarely repeated [26-28].

Mutation was defined as intronic: In the first paper, the lack of a mutation was suggested [26], however, an additional analysis revealed a homozygous splice-site mutation in intron 4 of TP53 [27].

Sentence was rephrased as well as the whole text.
From: Partially, it could be expected based on the assumption that the percentage...
To: In part, it could be expected that the percentage of single heterozygous mutations correlates with the magnitude of DNE and the importance of specific dominant-negative mutations defined by Dearth et al.

In figure 1 p values were specified.

In addition, accordingly to current nomenclature glioblastoma multiforme was rephrased to glioblastoma.

Article was corrected by professional Language Editing Service.
We hope that our article will be accepted for publication in BMC Cancer.

Sincerely,

PhD Piotr Rieske