Author's response to reviews

Title: KIT exon 10 variant (c.1621 A>C) single nucleotide polymorphism as predictor of GIST patient outcome.

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

Please find enclosed the revised manuscript “KIT exon 10 variant (c.1621 A>C) single nucleotide polymorphism as predictor of GIST patient outcome” (running title: “KIT exon 10 variant predicts GIST patient outcome”) including the requested corrections for publication as a research article in *BMC Cancer*.

First of all, we would like to thank both reviewers for their useful comments in improving our manuscript.

As requested, we included the full name of the ethics committee that approved the study: the Centre Léon Bérard Clinical Trial Review Committee (please see page 9).

The answers to the specific comments/suggestions/queries are as follows.
Response to Reviewer #1 Comments

Major revision: In figure 1 A and B., the authors compare KITL541 expression and the resulting activation signalling pathways (p-AKT, p-RAF, p-ERK). However, to determine the expression levels of the different mutated KIT proteins and the relative activation pathways, the authors should run all samples on the same SDS-PAGE. In general it is not recommended to compare samples from different gels, nevertheless a positive control on both gel was suitable. In this case the control sample should be not overexposed and an accurate densitometric analysis was due.

Moreover the WB shown in Figure 1A is of very poor quality; can the authors provide better pictures that distinguish from 145kDa and 125 kDa KIT forms?

Response: As requested, we provided better pictures of the Western Blot shown in Figure 1A that distinguishes 145kDa and 125 kDa KIT isoforms. Unfortunately, we could not run all samples on the same SDS-PAGE because we used 12-well gels. Nevertheless, the two gels were loaded at the same time.

Minor revisions: Please check the text for punctuation error (for example page 12 line 261 “..”). Page 15 line 338, please change “brut” with “but”. In legend of figure 2. The authors should change “KIT541” in “KITL541” according to figure 2.

Response: Punctuation, spelling and legend error have been corrected as requested.
Response to Reviewer #2 Comments

Major revision

1. The major concern is about the choice of the cell line. Why authors did not ask for GIST cell line, rendering the results more specific to GIST. I understood that GIST cell lines are not commercially available, but they can be obtained for scientific purposes. The use of different cell line make translation of the results to GIST a bit ambiguous.

Response: Previous studies (Tabone-Eglinger et al., Clinical Cancer Research 2008; Bachet et al., Plos One 2013) have already established NIH3T3 cell lines expressing hemizygous or heterozygous KIT mutation and showed that NIH3T3 cell lines are reliable cellular model to characterize biology of human KIT mutants in GIST, with observations which could be translated to GIST in these models (eg regarding phosphorylation and trafficking pattern). Our research team has been working for a long time on this well characterized cell line. For these reasons, we used NIH3T3 cell lines and translated the results to GIST. However, we plan to confirm these results on GIST cell lines.

2. Ref 2 is out of date. I think there are many updated review to be cited in its place (example are Cioffi and Maki JCO 2015; Angelini, et al., Pharmacogenomics 2013). In any case cite a ref published in the last 5 years. Additionally in the 1990 GIST were still misclassified with leiomyosarcomas.

Response: The Reference 2 has been updated and replaced by more recent references (please see page 21).
3. There is no mention regarding Hardy Weinberg equilibrium. Authors should calculate it, as a situation of non-equilibrium would make results ambiguous.

**Response:** We could not calculate Hardy Weinberg equilibrium. Indeed, the CAST-PCR® technology could not determine the heterozygous or homozygous condition of the variant. Thus, observed frequencies of the alleles is unknown.

4. As the authors stated in the abstract, “Tumor genotype plays a crucial role in clinical management of GIST. Whether inherited genetic polymorphism of KIT influences GIST patient outcome is not known” this aspects should be re-considered and deepened in the discussion, as other SNPs besides the one in kit may have influence, as well epigenetic factors, including methylation, and microRNA (the reviews Sioulas et al., Dig Dis Sci 2013; Ravegnini et al., Int J Mol Sci 2015 may be of interest).

**Response:** We agree with these comments. These aspects were reconsidered (please see page 5) and discussion was expanded on this topic (please see page 18).

**Minor Essential Revisions**

5. Background can be shortened; for example the sentence “About 10-15% of GIST do not harbor any mutations in the KIT or PDGFRA genes. Previously referred to as “wild-type” (WT), these GIST are now known to present with mutations on SDHA/B/C/D (6,7), BRAF (8–10), NF1 (11). These mutations are also associated with specific clinical presentations and clinical behaviour” is out of the aim of the paper and can be avoided.

**Response:** We agree with this proposal, the background was shortened in the revised version (please see page 5-6).
6. The sentence “In the literature, variants have been found associated with soft tissue sarcoma incidence (12) and higher risk of relapse in different malignancies (13)” delete or make it more specific to GIST. For example, besides O’Brien et al., authors should cite: Kwon et a., J Korean Surg Soc 2012; Angelini et al., Pharmacological Research 2013; Kang et al., Asia Pac J Clin Oncol. 2014; Angelini et al., Eur J Hum Genet. 2015;

Response: The sentence was corrected accordingly (please see page 5).

7. In material and methods: “of 109 patients with GIST” change to “of 109 GIST patients”

Response: The sentence was corrected accordingly (please see page 8).

8. In results: The heading “Clinical and biological characteristics of the series” change “of the series” to “of the two GIST patients series”

Response: The sentence was corrected accordingly (please see page 13).

9. Discussion should put less emphasis on the results regarding kit SNPs, unlike mutations (that usually have a drastic/dramatic effects) the influence of a single SNP can be rarely the solely responsible of important effects. Besides this, are other KIT SNPs be involved in cancer susceptibility and/or prognosis? In other words why limiting the work to just one SNP?

Response: As suggested, discussion put less emphasis on the results (please see page 18).

Currently, whether other KIT SNPs are involved in cancer susceptibility and/or prognosis is not known. We focused on this SNP because of its location (transmembrane) and because of the previous results in several malignant diseases including aggressive fibromatosis (Gonçalves et al., J Natl Cancet Inst 2010; Dufresne et al., BMC Cancer 2014).
We feel that these modifications have improved the quality of our manuscript and hope they will give satisfaction to both reviewers as well as to the Editor. We remain of course at your disposition for any further modifications.

Sincerely Yours,

Dr Mehdi BRAHMI