Author's response to reviews

Title: Effect of dabrafenib on melanoma cell lines harboring the BRAFV600D/R mutations

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

Please find enclosed the revised version of the manuscript by Gentilcore et al., entitled “Effect of dabrafenib on melanoma cell lines harbouring the \( {BRAF}^{V600D/R} \) mutations” (MS# 1889377177017855). Revision of the manuscript was based on the remarks of Reviewers. Changes have been highlighted (in yellow) into the paper and here listed point-by-point [page numbers are referred to this revised version of the manuscript].

Reviewer Michael Davies

Major Compulsory Revisions

1. In BACKGROUND (page 3, paragraph 3), it has been indicated that vemurafenib/PLX4032 “has been demonstrated to also inhibit proliferation of melanoma cell lines expressing other codon 600 \( {BRAF} \) mutations: \( V600D, V600K, \) and \( V600R \)”, also including the two References suggested by the Reviewer [Reference numbers have been modified accordingly]. Moreover, a new sentence (page 3, paragraph 4) has been added in order to better clarify the reasons why we carried out the present study and the goal we would like to achieve: “To investigate whether dabrafenib may exert inhibiting effects on a wider range of \( {BRAF} \) mutants (similarly to those previously reported with vemurafenib/PLX4720 [4, 6]), melanoma cell lines carrying the two remaining most prevalent \( {BRAF} \) mutations (\( V600D \) and \( V600R \)) were here treated with dabrafenib and cellular proliferation was then assessed”.

Minor Essential Revisions

1. In Figure 1B, error bars reflecting the standard deviations have been reported.

2. Figure 1C has been modified by showing the GAPDH expression levels as total protein loading controls. In METHODS (page 5, paragraph 1), it has been also indicated that “the rate of phosphorylated ERK was estimated on equal amounts of total protein for cell lysates; GAPDH was used as an internal control for total protein expression levels”.

In METHODS (page 5, paragraph 1), it has been specified that the treatment with the \( {BRAF} \) inhibitor was for 72 hrs. In Legend of Figure 1C, it has been further indicated that the effects on \( \text{ERK}_{1,2} \) phosphorylation were evaluated “under the same experimental conditions (concentrations of dabrafenib and time of treatment) as above”.

3. Manuscript has been revised for the English language.

4. In RESULTS AND DISCUSSION (page 4, beginning of paragraph 3), it has been specified that “Although experiments were carried out on a single cell line of each of the genotypes of interest, our findings strongly suggest that dabrafenib…”.

Discretionary Revisions
1. We think that the assessment of the effects of dabrafanib on an additional cell line carrying the \( \text{BRAF}^{V600E} \) mutation is unnecessary since the purpose of this brief study was to evaluate the activity of dabrafanib on cells with \( \text{BRAF}^{V600D} \) and \( \text{BRAF}^{V600R} \) mutations, also because the inhibitory effect on \( \text{BRAF}^{V600E} \) (as well as on \( \text{BRAF}^{V600K} \)) mutants has been previously defined.

**Reviewer Richard K Kefford**

- The **ABSTRACT** has been rewritten in order to emphasize the results of the study.
- Manuscript has been revised for the English language.
- The statement that vemurafenib/PLX4032 specifically acts on \( \text{BRAF}^{V600E} \) mutants has been modified, indicating that this compound “has been demonstrated to also inhibit proliferation of melanoma cell lines expressing other codon 600 BRAF mutations: V600D, V600K, and V600R” (see answer to the other Reviewer).
- Reference 6 in the previous version of the manuscript (actually, Ref. 9) has been replaced with that suggested by Reviewer.
- In **BACKGROUND** (page 3, paragraph 2), frequencies of the rarer \( \text{BRAF} \) mutations have been provided, also including the Reference suggested by the Reviewer [Reference numbers have been modified accordingly].

Reference 4 (corresponding to Ref. 3 into the previous version of the manuscript) has been updated.
A missed author, Antonio Cossu, has been added.

Hoping to have addressed all issues and looking forward to receiving good news from You.

Yours Sincerely,