Author’s response to reviews

Title: Nonsteroidal sulfamate derivatives as new therapeutic approaches for Neurofibromatosis 2 (NF2)

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Author’s response to reviews:

Dear Dr. Brogi,

Thanks again to you and to the reviewers for their consistently prescient and helpful comments, and for allowing us to revise and resubmit the manuscript. We hope that we have satisfied all of the reviewers’ and the editor’s concerns and that the manuscript can now be accepted by the journal.

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We have taken care to follow their suggestions on the minor revisions they suggested in all but a few instances, as detailed in this response letter.

Technical Comments:
Provide email addresses of all authors in the Title page.
We have included the email addresses of all of the authors.

Our responses to the reviewers are in the Italic font.
Reviewer reports:
Alessandra Gianoncelli (Reviewer 2): I suggest the authors to better clarify the legend of figure 3. Now it is clearer than before but there are still some small ambiguities.
In particular, in the graphs relating to the PF, the concentration curve at 7.5 µM was not reported anywhere although it was present in the legend. The Frax graphs did not show anywhere the concentration curves of both 3 and 4 µM although they were mentioned in the legend.
Finally in the bar graphs shown below there were not complete data and it was not clear why the authors wanted to put only those data; moreover, it was not even clear to which cell line they were referred respectively. Finally, the data related to the 2 µM reported in the second bar graph was not mentioned in the legend and the corresponding concentration curve was not present.

Best regards

Thank you, Dr. Gianoncelli, for pointing out these errors. We have removed PF 7.5 µM, Frax 3 and 4 µM from the legend. We also removed the bar graphs from this figure since they did not add additional information but apparently caused more confusion for readers. We hope that figure 3 is now clear.

Guzmán Alvarez (Reviewer 3): line 21 pg 16, please incorporate which concentration are you saying (as low concentrations?).
also, there are not page numbers, please take into account for the future.
there are not conclusión, please incorporate this section!!!!!!!

Thanks, Dr. Alvarez, for pointing out the omissions. We have now incorporated the concentration (300 nM) into the text (pg12).
Both page numbers and the conclusion section requested (pg15) have been added to this version of the manuscript.

Fig 2 and Fig 3 still been hard to understand, It could be better if the graph in column change for a dose-response graph with individuals points, both compounds, and dmso, an also the authors could use this graph to see the ic50 in those conditions.

We have read this comment very carefully, but, unfortunately, could not comprehend the points the reviewer wishes to make. As we have mentioned in the course of the previous revision, we used the same experimental analyses in this study as in the earlier study of STX agents to provide evidence that these agents are also effective against NF2-related human tumour cells. The format of the analysis and its presentation is the commonly accepted format in such tissue culture-based cancer studies (please see our previous report using these agents in the British J. Cancer: Shen et al., 2015).

About the antibiotic, was not my mistake, was a suggestion for your feature work, if you use antibiotics in cell culture when you are studying the mechanism of action of another drug, you are incorporating drug interactions in your model, then the metabolic behavior of the cells change, because that I said that is not recommended the use of antibiotics.
Thank you for this suggestion, Dr. Alvarez. Sorry for the misunderstanding. Although it is extremely difficult to maintain cultured cells for the duration these experiments require, without antibiotics, we have, through very careful husbandry, accomplished this in the past in our studies of inner ear-derived cell lines, since antibiotics are toxic to many cells (particularly sensory hair cells) of the inner ear (e.g.
Germiller et al. (2004) Molecular characterization of conditionally-immortalized cell lines derived from early mouse embryonic inner ear. Developmental Dynamics 231: 815-827. We will not add antibiotics to the cell cultures in our future experiments.