Neuro-Oncology

Possible mechanisms and biomarkers of resistance to vismodegib in SHH medulloblastoma

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To
Dr. Kenneth Aldape
Editor-In-Chief
Neuro-Oncology

RE: N-O-D-21-00593-R1

Porto Alegre, December 30, 2021

Dear Dr. Aldape,

Please find attached a revised version of our **Letter to the Editor**, manuscript number **N-O-D-21-00593-R1,** entitled **" Possible mechanisms and biomarkers of resistance to vismodegib in SHH medulloblastoma "**, by **Rafael Roesler, Caroline Brunetto de Farias, André T. Brunetto, Lauro Gregianin, Mariane Jaeger, Carolina Nör, and Amanda Thomaz**, submitted for consideration at ***Neuro-Oncology***.

We are glad that the Reviewer feels very positive about our letter and recommends that it could be accepted after revision. We have extensively revised the letter to address the comments made by the Reviewer.

Note, however, that ***Neuro-Oncology*** allows a maximum of 600 words and 6 references for Letters to the Editor, so we were limited in how much we could extend our discussion and we could include only one additional reference.

Specifically, two new paragraphs addressing the comments made by the Reviewer read as follows:

"In fact, current research has unraveled resistance mechanisms not addressed by the authors in their discussion. Intratumor heterogeneity must be taken into account. Untreated MB tumors contain specific cell subpopulations displaying a spectrum of expression of neural progenitor and stem cell markers. Specific subsets of these cells, showing persistent SHH activation, stem cell features, and expressing transcription factors HES1 and Myod1, as well as SHH pathway markers, are the ones that remain proliferative and keep mediating MB tumor growth after vismodegib treatment. Importantly, MB tumors may change their mutational landscape in between primary diagnosis and relapse, which indicates relapse samples should be evaluated for resistance markers. [2]

Several molecular mechanisms, in addition to mutations of SMO, are likely to mediate resistance to vismodegib in MB. These include amplification of the downstream SHH signaling molecule zinc finger protein GLI2 or of the SHH target gene CCND1, amplification of MYC/MYCN, TP53 mutation, and upregulation of the RAS/mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways. Enhancement of PI3K activity in SHH MB may involve loss of the PI3K negative regulator phosphatase and tensin

homologue (PTEN) and enhanced signaling by insulin-like growth factor 2 (IGF2) and its receptor IGFR. Combining vismodegib with SHH inhibitors that target pathway components downstream of SMO, or with PI3K inhibitors, may provide promising avenues for reducing resistance to vismodegib in SHH MB tumors. [3"].

We hope that you will find our revised letter acceptable for publication in ***Neuro-Oncology***.

This manuscript, in whole or in part, has not been published previously or submitted concurrently to any other journal. All co-authors have read the manuscript and submission of this article for publication was approved by all authors as well as by the responsible institutional authorities.

All the information in the manuscript is in agreement with institutional research ethics guidelines.

The authors have no conflict of interest related to the contents of this manuscript to disclose.

Looking forward to hear from you,

Yours sincerely,

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Institute for Basic Health Sciences

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RESPONSE TO REVIEWS

REVIEWER 1:

1. Reviewer's comment: *The authors submit a letter to the editor as a comment on the article "MEVITEM - A Phase I/II of Vismodegib and temozolomide vs temozolomide in patients with recurrent/refractory medulloblastoma with Sonic Hedgehog pathway activation", published by by Frappaz et al., (Neuro Oncol. 2021; noab087. doi: 10.1093/neuonc/noab087). They thereby pick up the point that the authors of the original article attributed the low efficacy of their approach to primary and secondary resistance against Vismodegib and further elaborate on that hypothesis.*

In addition to the known resistance mechanisms also named by the authors of the original article, namely mutations of PTCH1 and hot spot mutations of SMO, they intend to add additional possible resistance mechanisms in their letter, namely

intratumor heterogeneity and neurotrophin signaling, mediated by the receptor-encoding genes NTRK1, NTRK3 and CD271/p75 (p75NTR). The authors claim that a better understanding of these mechanisms could help in the identification of novel combination therapy strategies to overcome resistance to Vismodegib in patients with SHH medulloblastoma.

The point of the authors is well taken; they should however, in such a letter to the editor, extend their view and not only comment on possible resistance mechanisms that are associated to their own research, but should also comment on other primary or secondary resistance mechanisms as downstream mutations in the SHH-pathway, e.g. in Gli, MYC/MYCN amplification, p53 mutation, and changes in the PI3K/mTOR and RAS/MAPK signaling pathways that can also modulate resistance. In addition, they should more clearly explain their comment on intratumoral heterogeneity in medulloblastoma. Medulloblastoma is subgroup-homogenous in most cases, but may still bear distinct driver mutations within the same subtype, and specifically may develop a mutational history in between primary diagnosis and relapse which mandates a target gene evaluation in relapse tissue, if possible.

If these additional points are accounted for, the letter can be accepted for publication.

Response: We thank the Reviewer for the thoughtful comments that have contributed to improve our letter. We have extended the Letter to include the topics mentioned by the Reviewer. Note, however, that Neuro-Oncology allows a maximum of 600 words and 6 references for Letters to the Editor, so we were limited in how much we could extend our discussion and we could include only one additional reference.

Two new paragraphs addressing the comments made by the Reviewer read as follows:

"In fact, current research has unraveled resistance mechanisms not addressed by the authors in their discussion. Intratumor heterogeneity must be taken into account. Untreated MB tumors contain specific cell subpopulations displaying a spectrum of expression of neural progenitor and stem cell markers. Specific subsets of these cells, showing persistent SHH activation, stem cell features, and expressing transcription factors HES1 and Myod1, as well as SHH pathway markers, are the ones that remain proliferative and keep mediating MB tumor growth after vismodegib treatment. Importantly, MB tumors may change their mutational landscape in between primary diagnosis and relapse, which indicates relapse samples should be evaluated for resistance markers. [2]

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increasing our understanding of molecular mechanisms determining resistance to therapy in MB.

In fact, current research has unraveled resistance mechanisms not addressed by the authors in their discussion. Intratumor heterogeneity must be taken into account. Untreated MB tumors contain specific cell subpopulations displaying a spectrum of expression of neural progenitor and stem cell markers. Specific subsets of these cells, showing persistent SHH activation, stem cell features, and expressing transcription factors HES1 and Myod1, as well as SHH pathway markers, are the ones that remain proliferative and keep mediating MB tumor growth after vismodegib treatment. Importantly, MB tumors may change their mutational landscape in between primary diagnosis and relapse, which indicates relapse samples should be evaluated for resistance markers. [2]

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Over the past few years, we have put forward neurotrophin signaling as a possible mechanism of resistance to molecularly targeted therapies in cancer, and we have also

reported antitumor effects of neutrophin receptor blockade in experimental MB. [4] Moreover, we have recently found that transcription levels of *NTRK1*, the gene encoding TrkA, which is the receptor for neurotrophin family member nerve growth factor (NGF), is increased in sets of SHH MB tumors from patients, compared to normal cerebellum or Group 3 and Group 4 MB tumors. In addition, levels of *NTRK3*, which encodes TrkC, the receptor for neurotrophin 3 (NT-3), are higher in SHH MB compared to all other MB molecular subtypes. [5] Importantly, Liang et al. [6] showed that the expression of CD271/p75 (p75NTR), a neurotrophin receptor that regulates stem and progenitor cells in MB, may be a biomarker identifying cells resistant to vismodegib within SHH MB tumors. Vismodegib failed to affect the growth of experimental SHH MB tumors positive for p75NTR. Together, these findings indicate that neurotrophin signaling might be another mechanism of resistance against vismodegib in SHH MB, and neurotrophin receptors should be investigated as possible biomarkers to identify tumors with primary or acquired resistance to vismodegib. Advancing our understanding of this issue could help in the identification of novel combination therapy strategies to overcome resistance to vismodegib in patients with SHH MB.

Funding

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Authorship statement: All authors contributed to the conception, writing, and revision of this article.

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