Reviewer’s report

Title: Benzofuroxan derivatives N-Br and N-I induce intrinsic apoptosis in melanoma cells by regulating AKT/BIM signaling and display anti metastatic activity in vivo.

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Reviewer: Ana Gamero

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This study identified 2 benzofuroxan derivatives out of 23 that are analogues of the antibacterial drug nifuroxazide. Compounds #21 and 23 (N-I and N-Br, respectively) were tested for their antitumor effects given that this drug increases ROS generation. Initially, all 23 derivatives made were screened in vitro using a panel of human and mouse cell lines of different types and then further evaluated in vivo using a clone of the murine B16-F10 metastatic cell line. The remaining studies were performed with B16-F10-Nex2 cells using N-I and N-Br, which reduced metastatic potential, increased ROS production, decreased mitochondrial membrane potential and induced cell death. The authors proposed a molecular mechanism in which these 2 compounds increased ROS production thereby attenuating the PI3K/AKT pathways and inducing the pro-apoptotic Bim protein resulting in reduced tumor burden.

Major Comments:

1. The emphasis of this paper is on metastatic melanoma as stated in the abstract and the rest of the manuscript. Yet the authors only used one mouse melanoma cell line to draw several conclusions. To confirm the antitumor effects of their 2 benzofuroxan derivatives, additional melanoma lines (preferably of human origin) should be evaluated as well. Furthermore, melan-a cells were mentioned in the methods section but not thereafter. Were they tested and if so how did they respond to the compounds?

2. The toxicity of their 23 compounds were evaluated also in non-tumorigenic cell lines (Fibro T75 and GM637) and the IC50 was found to be within the range of tumor cell lines (10-30uM) that responded to their 2 selected drugs. No comment on toxicity was made in the discussion, however.

3. There is no explanation as to why a clone from B16-F10 (B16F10-Nex2) had to be generated. The parental line is well documented to be highly metastatic.

4. Figures 1B and 1C are two animal studies performed in two different strains of mice (B6 and Scid-NOD). Yet the corresponding tumor images presented for each figure panel are identical.

5. A subcutaneous tumor model was described in the materials and methods section but no data were presented or mentioned. Did the compounds reduce tumor size?
Minor Comments:
1. A few grammatical errors/typos were found throughout (i.e. ....Line 142; medium were add)
2. Figure 5 has a title but is missing a legend.
3. Line 116; the proper title should read “Cell lines” and not Cell lineages.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.