Reviewer’s report

Title: The prospective application of a hypoxic radiosensitizer, doranidazole (PR-350) to rat intracranial glioblastoma with blood brain barrier disruption

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Reviewer: Michael Horsman

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The presence of hypoxia in glioblastoma may be one of the major reasons why this tumor type is resistant to radiation, thus investigating potential radiosensitizers like doranidazole to overcome this hypoxia-induced radiation resistance is certainly valid. It is also important to show that the drug can actually cross the BBB and get to the hypoxic cells in brain tumors. This is the basis of the current study, so in that context it is totally valid. Appropriate experiments have been well performed and relatively good data obtained. However, there are a number of issues that need to be addressed.

Minor Essential Revisions

1. Page 3, lines 1-3: Is hypoxia the only reason that glioblastomas are resistant to radiation? This is a bold statement to make in the abstract and the authors must include references in the Introduction that categorically demonstrate that hypoxia in glioblastomas actually reduces the effect of radiation.

2. Page 5, lines 7-16: I can understand that the biological distribution of radiosensitizing reagents in the brain will modulate the effects of hypoxic radiosensitizers. But, how exactly are they modified by hypoxia? If hypoxia exists then radiosensitizers enhance radiation response, but if there is no hypoxia then there is no effect; it is an all or none effect – there is nothing in between, so “modulate” seems inappropriate!

3. Page 15, paragraph 1: It is stated that doranidazole was equally toxic under normoxia and hypoxia, but why? Nitroimidazole compounds are known to be toxic under hypoxia, but generally show little toxicity under normoxia. For completeness it would be a good idea to actually include the levels of cell killing for all conditions; controls under normoxia and hypoxia, and doranidazole under both conditions.

4. Page 22, Competing interests: The authors declare no competing interests, but how can this be? Doranidazole was obtained from POLA PHARMA INC, so surely they own the rights to this drug. That would mean that any person employed by the company, such as N. Kubota, has a conflict of interest!

5. Figure 1: This figure should be deleted; it has been published before in other publications and including the structure of doranidazole here serves no purpose.

6. Figure 2: The difference between the hypoxia and normoxia curves is such that the OER is around 2.0 or just below. This is somewhat lower than one would
expect for this cell line, so some comments on this should be made in the manuscript. Is the lower OER due to the fact that the cells were plated in plastic dishes; ordinary plastic dishes are known to contain oxygen which can leak-out during hypoxia and raise the actual oxygen level. Of course, maybe you used special plastic such as permonox, which is oxygen deficient. This does not mean the study is wrong, but if there is more oxygen than expected under the hypoxic conditions then it could decrease the effectiveness of doranidazole (under complete hypoxia the SER may have been greater than 1.5).

7. Figure 3: For most of the data shown in the various figures the authors include quantitative data, often supplemented with representative examples, which is good. However, for figure 3 only representative examples are shown. The data of figure 3 should also be quantified; having actual values with errors will prove that the effects on the BBB are real and not just due to chance in one tumor. Such data would clearly strengthen the author’s arguments and conclusions. Also it states in the legend that views are from the dorsal surfaces, but the figures seems to show both sides of the brain not just the dorsal side.

8. Figure 4: Again quantifying the doranidazole radioactivity data was an excellent idea. But, why not do the same for the pimonidazole data? In the text on page 17 it is stated that pimonidazole was given 60 minutes prior to doranidazole, so if you have the data for doranidazole you must also have it for pimonidazole. Also, on the histology figures T and N are indicated. I assume these represent tumor and normal tissue, respectively, but this should be explained in the legend.

Level of interest: An article of importance in its field

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.