Author's response to reviews

Title: Anti-invasive and antiangiogenic effects of MMI-166 on malignant glioma cells

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Version: 3 Date: 3 June 2010

Author's response to reviews: see over
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Title: Anti-invasive and antiangiogenic effects of MMI-166 on malignant glioma cells

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Version: 2 Date: 3 June, 2010

Author’s response to reviews: see over
Reviewer's report
Title: Anti-invasive and antiangiogenic effects of MMI-166 on glioma cells
Version: 1 Date: 26 March 2010
Reviewer: Ian Lorimer

Major Compulsory Revisions:

1. As this is a preclinical evaluation of the potential of MMI-166 to treat glioblastoma, data on invasion, angiogenesis and proliferation/cytotoxicity for all three cell lines U87MG, T98G and ONS12) should be shown.

   We showed data on invasion, angiogenesis and proliferation for all three cell lines (T98G, U87MG, and ONS12).

2. For the VWF staining (Figure 6), there needs to be quantification from multiple sections to ensure that the differences in VWF staining are real and not a sampling artifact.

   We have improved the image quality of VWF staining..

3. The structure of MMI-166 should probably be shown (I am not sure of the journal policy on this).

   We described the chemical structure of MMI-166 in Figure 1.

4. The authors should include a discussion of the limitations of the U87MG model, with particular reference to invasion.

   For the animal model, the data of U87MG was replaced by the data of T98G that we obtained after sending the first manuscript. Furthermore, in vitro experiments, the representative image data of U87MG were also replaced by the data of T98G.
5. There should be an explanation or discussion as to why an inhibitor down-regulates the total protein levels of the MMPs, rather than simply inhibiting their activity.

   We examined the effect of MMI-166 protein expression level of MMP-2 and MMP-9 by using the ELISA again. As a result, there was no difference in the protein expression. Therefore, it turned out that MMI-166 simply inhibiting MMP activity.

6. There should be an explanation/ discussion as to why, in Figure 4, angiogenesis is further affected at 100uM drug, even though Figure 2 indicates that MMP inhibition is complete at 10 uM.

   Although MMP-9 activity was completely controlled by 10µM MMI-166, MMP-2 activity remained by 10 µM MMI-166 and was mostly controlled by 100 µM MMI-166 in zymography. Therefore, it is reasonable that angiogenesis is further affected at 100µM.

7. There should be some discussion of what stage this drug is at in clinical development. The drug has been around for ten years and has been assessed preclinically in multiple other cancer types - is there some obstacle to further development?

   We described about the clinical trial of a relative compound of MMI-166 in discussion.

Minor Essential Revisions:

1. Details of the ONS12 cell line should be included (patient tumour classification, tumorigenicity in nude mice if known, available data on markers or genetic aberrations).

   The ONS12 cell line was established from the resected tumour tissue of a 48-year-old female with glioblastoma in our hospital. Tumorigenicity in nude mice and genetic aberrations has not been studied.

2. There should be a reference for the culture insert method used to evaluate
angiogenesis.

The angiogenesis assay used in the present study is a new method that we designed. Therefore, there is no reference literature.

3. It should be clarified that MTT assays assesses changes cell numbers: this can be due to changes in proliferation or cytotoxicity or both (not simply cytotoxicity).

We changed the content of the description of the MTT assay.

4. In Figure 2, there is no explanation for what the different lanes show. Similarly in Figure 3A and 4A, there is no explanation as to what the different panels show in the Figure legend.

We added the explanation for the lanes and the panels.

5. In Figure 5, the data should be shown as a bar graph.

We adopted a bar graph.

6. In Figure 1b, GAPGH should be GAPDH.

Old figure 1 was removed. New Figure 1 shows the chemical structure of MMI-166.

7. On p.20, it was unclear what was meant in the last sentence (“coeval” is not a word).

We changed the expression.

8. There are minor problems with English grammar in the text – perhaps the journal can help with this.

The manuscript was checked again by the company for professional English editing (Editgae™).
Discretionary Revisions:
1. The paper would be greatly strengthened if a second glioblastoma model was used that is more representative of the human disease.

   For the animal model, the data of U87MG was replaced by the data of T98G.

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

**Quality of written English:** Needs some language corrections before being published

**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
Reviewer's report
Title: Anti-invasive and antiangiogenic effects of MMI-166 on glioma cells
Version: 1 Date: 7 April 2010
Reviewer: John F de Groot

Reviewer's report:
This is a straight-forward evaluation of the effects of a new MMP inhibitor, MMI-166, on glioma proliferation, invasion, angiogenesis and tumor growth in an orthotopic glioma model. The effect of MMP inhibitors on glioma invasion and angiogenesis are known and this study confirms that MMI-166 is able to suppress these malignant phenotypes. The study is well written and the objectives, methods and results are clearly explained.

Major Compulsory Revisions:
None

Minor Essential Revisions:
None

Discretionary Revisions:

1. It would be helpful for the authors to highlight the novel findings in their study.

   We emphasized that this is the first report of the effect of a third generation MMP inhibitor to glioma cells, and having performed an angiogenesis assay newly designed.

2. Several MMP inhibitors have failed in phase III clinical trials. It would be helpful if the authors put their findings in the broader context of using MMP inhibitors for the treatment of malignant gliomas.

   We added the description about the clinical trial of MMP inhibitor to glioma in discussion.

3. T98G, in some laboratories, is invasive in orthotopic models. It would be interesting to know if MMI-166 blocks tumor invasion in vivo. U87 is not invasive and thus no anti-invasive effect can be expected in this model.
For the animal model, the data of U87MG was replaced by the data of T98G.

4. MMI-166 is not highly potent - the effective dose for 50% effect is at or above 10 micromolar. Does this agent have off target effects at higher doses?

   In our data, the effect of MMI-166 increase up to 100μM in dose-dependent fashion. Therefore, in the density to at least 100μM, it is thought that MMI-166 doesn’t have the off-target effect.

**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.