Author's response to reviews

Title: Changes in [18F]FLT and [18F]FDG positron emission tomography following treatment with belinostat in human ovary cancer xenografts in mice

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Author's response to reviews: see over
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Author’s response to reviews: See over
Object: MS: 1477436293799445 Changes in [18F]FLT and [18F]FDG positron emission tomography following treatment with belinostat in human ovary cancer xenografts in mice

Thank you very much for consideration of our manuscript for publication in your journal. We have revised the above manuscript according to your reviewer’s comments.

Reviewer’s report:

Minor Compulsory Revisions

1. There does not appear to be any specific reference to and discussion of in vitro experiments describing the mode of action of belinostat. Does it actually inhibit proliferation in cells? This is normally done before the effect of an experimental agent on a specific process (in this case proliferation) is studied in a xenograft model. This oversight makes data interpretation difficult as it is not possible to know if the agent has limited efficacy in the xenograft or does not actually inhibit cell proliferation. Authors should describe effect of belinostat on cell proliferation in cultured ovarian cells in the introduction. Specifically: Is the main anti-cancer effect of belinostat through decreased proliferation or through a different process?

- We agree that in vitro experiments should be mentioned and this has now been included in the introduction section.

2. Authors should also refer to other papers that study effect of anti-cancer agents on FLT uptake in ovarian cancer xenografts so that the belinostat FLT data can be put into perspective (e.g. Perumal et al, Mol Imaging Biol, 2012; Aide et al, JNM, 2010; Jensen et al PLoSI, 2010). This will help readers to determine whether the limited change in FLT uptake is a result of the experimental agent, FLT as a biomarker, or the tumour type and put the belinostat data into perspective.

- We agree that the data could be put more in perspective and have included a section in the discussion with references to other papers studying FLT uptake in ovarian cancer xenografts.

3. Authors do not discuss why SUVmean produced a significant difference between treatment groups for FDG uptake, yet SUVmax does not. Discuss.

- We have now included a discussion about the difference between SUVmean and SUVmax FDG uptake after initiation of belinostat treatment in the discussion section.

4. There is no description of how SUVmax and SUVmean were calculated (or a reference to how this was done in a previous paper).

- We have now included a description of how SUVmean and SUVmax were calculated.

5. The use of gene expression to study expression of proliferation markers may not translate into changes in protein levels. For this reason the gold standard biomarker for cell proliferation is analysis of Ki67 by immunohistochemistry and not by gene expression.
Can authors confirm that Ki67 gene expression correlates closely with Ki67 expression by immunohistochemistry?

Does the apparent increase in TK1 expression by gene expression (Fig 6) actually result in upregulation of TK1 protein?

- We chose to study the gene expression levels of the molecular markers because with the RT-qPCR method it is possible to quantify the amount of target whereas accurate quantification is often difficult in immunohistochemical analysis.

On the other hand we are perfectly aware of the problems associated with measurements of Ki67 gene expression instead of Ki67 protein measurements by immunohistochemistry and regrettably we cannot confirm the correlation between Ki67 gene expression measurements and protein expression in the tumors acquired in the present study. Actually Ki67 protein measurements were included in the experimental protocol for this study; however, something went unfortunately wrong during the tissue preparations for immunohistochemistry analysis. However, in other studies a positive correlation between Ki67 protein and gene expression has been observed (Tan et al, Mod Pathol, 2005; Yamamoto et al, Breast Cancer, 2012).

We did not measure if there was a connection between TK1 gene expression and up-regulation of TK1 protein.

We agree that the lack of protein measurements of both Ki67 and TK1 is a limitation of this study which we therefore clearly have stated as a limitation in the discussion section.

6. On p1 of discussion ‘This could be an explanation to the increase in TK1’. ‘to’ should be replaced by ‘for’

- “to” has been replaced with “for”

7. On p2 of discussion authors mention ‘lover in the treatment’ Should this be ‘lower’?

- This was a mistake, lover has now been replaced with lower

8. Is reference 3 a book or is it incomplete (no journal title etc)?

- Reference 3 was incomplete, it was a mistake and this has now been corrected. We also became aware of that several other references were incomplete and this has been corrected as well.

**Minor Discretionary Revisions**

9. Authors should clearly state limitations of work.

- We have now included a paragraph in the discussion section describing the limitations of work
10. In the introduction the authors state that ‘new anti-cancer biomarkers for assessing early treatment effect are lacking’. This is not strictly true. There are a number of biomarkers being developed for this role. The key point is that most of these biomarkers have not yet been validated or qualified. FLT is an exception, as it has been validated vs the proliferation marker Ki67 in some tumour types (e.g. breast cancer). This point needs to be corrected.

- We agree that the wording of the first paragraph could be confusing and have therefore reworded the paragraph.

11. Materials & Methods (in section headed microPET and microCT imaging) ‘MBq’ missing before 18F-FDG in sentence 1

- MBq has been added before 18F-FDG in sentence 1

12. On P2 of discussion authors mention the ‘diluting effect of non-responders’. Could this be worded better.

- We agree that the sentence could be worded better and have rewritten the sentence

13. On p2 of discussion authors talk about ‘secondly in clinical practise, treatment modifications in non-responding patients during a treatment course may be undertaken. The gene expression of GLUT1 paralleled the uptake of 18F-FDG.’

This is a very strange (and unexpected) change of topic mid-paragraph. Are the authors talking theoretically, or referring to data in Fig 6 or an unreferenced paper from the literature? This paragraph needs to corrected so that it makes more sense.

- We agree that the change of topic did not make much sense and we have made a paragraph shift so it hopefully has become more readable. Furthermore we have changed the order in which the paragraphs appear.