Reviewer's report

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

**Version:** 1  **Date:** 25 January 2013

**Reviewer:** Ian Fleming

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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?

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 PLoS1, 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.

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

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

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?

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

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

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

Minor Discretionary Revisions

9. Authors should clearly state 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.

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

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

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.

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