Reviewer's report

Title: Promoting E2F1-mediated apoptosis in oestrogen receptor-alpha-negative breast cancer cells

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Reviewer: V. Craig Jordan

Reviewer's report:

The authors provide an interesting yet unanticipated result in ER negative breast cancer that has potential for addressing the initiation of apoptosis in ER negative breast cancer. As a result, this is of interest to a broad constituency of the cancer research community. The authors provide unanticipated results, but they have clinical consequences that do not appear to have been noticed by the clinical community, ie, don't give tamoxifen to ER negative patients because this makes things worse. This is an important point to consider when translating the current studies to the clinical arena. I have created points of expansion that the authors should consider to improve the value of their already excellent study.

Major Compulsory Revisions

1. Page 3, 2nd paragraph, the authors describe the effects of tamoxifen but do not mention the fact that tamoxifen as a competitive inhibitor of estrogen action blocks the cell cycle at the G1. There are references to this action of tamoxifen by both Osborne's group and Sutherland's group in the early 1980s.

2. Page 10: I am intrigued why inhibiting MAP-ERK prevents RNA production during 4OHT treatment. Can this be explained a little better, as phosphorylation of the Estrogen Receptor is the target.

3. On page 11 (top) The authors suggest a novel way that the ER self regulates to produce further ER. This as a fact has been known for 15 years and first published by Pink and Jordan in Cancer Research in 1996.

4. Page 11, line four from the bottom, the recurrent use of the word 'resistance' seems inappropriate for 4OHT in MDA-MB-231, from what I can gather from the authors' data, this is 4OHT stimulated growth, and a form of resistance first described by Gottardis and Jordan in Cancer Research 1988 when they first discovered that tamoxifen stimulated growth occurs; so this is a principle.

5. Page 12, three lines from the top, the authors focus much attention on the MAPK cascade so that some of the ER is actually membrane bound?

6. Page 12, five lines down, the discussion about the mechanism of action does not include the fact that fulvestrant binds to the ER and causes rapid ubiquitinylation and destruction of the aberrant receptor complex.

7. Page 12, four lines from the bottom, the ChIP assays are interesting but compared to what? The authors stress that the ER:4OHT complex binds rapidly to the E2F1 promoter; but, how does this work? The 4OHT:ER complex usually
weakly binds to any promoter and the major antiestrogenic action of this complex is that it does not bind a coactivator. Can the authors reconcile this with their data?

8. Page 15, The authors visually quantify the amounts of apoptotic cells, but they stress the role of their combination in enhancing pro-apoptotic genes. In the conclusions, they mention some of these. Because their effects are so profound, can RT-PCR now quantify before and after treatment for pro-apoptotic genes that they select?

9. The authors do everything in the presence of 4OHT for their combination, but what happens without 4OHT? I see this is in figure 4, but more should be made of this synergistic effect.

9. It is clear that 4OHT does something to stimulate the growth of these cells, but the authors should consider adding the article by Sipila et al, EJC, vol. 29A, pp2138-2144, 1993, as this fits very nicely into what they are now finding in culture. This group showed that long term TAM treatment of MDA-MB-231 cells increased their growth and became tetraploid and also grew aggressively into tumors in animals. This would be a useful addition.

10. All of this refers to 4OHT, but what happens with Estradiol and what happens with estradiol after conversion by 4OHT? Does this still all work?

11. In figure 1B, shows Western blots for the 4OHT modulated MDA-MB-231 cells, but 1C shows the relative levels of mRNA compared to MCF-7. To me the Western blot in 1B is an enormous amount of ER not consistent with 3.3 copies of mRNA per million. Can the authors reconcile this?

Minor Essential Revisions
1. The MDA-MD-231 cells should be genotyped to produce fidelity with these studies.

2. Page 3, first sentence of 2nd paragraph, the word 'allosterically' is incorrect as tamoxifen and other SERMs are competitive inhibitors within the ligand binding domain of the ER. Allosteric defines a separate site other than the active site. This must be changed.

3. Page 13, 2nd paragraph, the MDA-MB-231 cells are classified as triple negative breast cancer. They have a mutated fas that is unusual. Important for the authors hypothesis concerning apoptosis, do these cells also have mutated p53?

Discretionary Revisions
1. This is a suggestion to the authors for future studies that they should consider this combination in ER positive breast cancer cells that have acquired resistance to 4OHT. These data would be extremely valuable as this is what is occurring in the clinic and requires a solution.

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