**Author's response to reviews**

**Title:** Antiprogestin mifepristone inhibits the growth of cancer cells of reproductive and non-reproductive origin regardless of progesterone receptor expression

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**Version:** 5 **Date:** 8 April 2011

**Author's response to reviews:** see over

# COVER LETTER

8 April 2011

**Response to the Reviewers**

**BMC Cancer; MS: 2045093735068547; Tieszen et al.**

*"Antiprogestin mifepristone inhibits the growth of cancer cells of reproductive and non-reproductive origin regardless of progesterone receptor expression"*

Thank you very much to the Associate Editor and Reviewers for the critiques, comments and suggestions to improve the message delivered through this work. Below are the detailed responses to each one of the reviewer's questions. Deleted text from previous version of the manuscript is indicated in red, whereas new text is indicated in blue.

**Referee #1**

Major compulsory revisions:

1. The comment of the reviewer is well taken. There is some literature, yet limited, showing growth inhibition by mifepristone in the absence of nuclear PR. Those papers were mentioned in the article. Our work expands the concept to 10 cancer cell lines of different aggressive behavior, tissues or origin, and hormone responsiveness, ruling out that at pharmacological concentrations mifepristone's growth inhibitory effect should be linked to the presence of nuclear progesterone receptors. The concept of utilizing mifepristone in the clinic without relying in the hormone responsiveness of the cancer tissue is not embodied in the clinical arena. The reliance on the detection of nuclear progesterone receptors before considering the utilization of mifepristone for cancer therapy in patients is a dogma that needs to be eliminated. We are convinced that the results of this work expanding many cancers, helps to do so.

2. We apologize for missing the article by Fjelldal and colleagues. The referee is correct that such work pertains to ours. We missed it likely because we had our manuscript ready for submission early January 2011 and the work of Fjelldal et al. was published late December 2010. We have included and discussed this important work as well as other articles indicated by the reviewer where progesterone and mifepristone have a synergistic cytotoxic interaction in endometrial cancer cells.

3. It is in our experience with cancer cell lines that mifepristone acts as a cytostatic agent in concentrations ranging from 2.5-20 micromolar depending on the cell type. Usually beyond the 20 micromolar threshold mifepristone begins to manifest its lethal effect mostly leading to apoptosis. We made clear this distinction in another work we recently published on the comparison between cytotoxicity of mifepristone and other two antiprogestins named ORG-31710 and CDB-2914 (Ulipristal; Goyeneche et al., Investigational New Drugs, Epub May 22, 2011; Ref #46 of the revised version). We have emphasized this concept throughout the manuscript.

The discussion has been limited as much as scientifically possible, considering that both reviewers suggested adding new subjects to debate.

The alternative mechanisms of mifepristone acting on the membrane progesterone receptors as well as on the newly discovered progesterone receptor membrane component 1 (PGRMC1) were discussed in the last paragraph of the original discussion. Indeed the recent demonstration that at micromolar concentrations, mifepristone acts as agonist on membrane PR expressed in yeast, is promising as an explanatory mechanism of action as discussed. Nonetheless we have expanded this discussion and added other potential mechanisms of action upon request of referee 2.

4. We understand that the variable of doubling time (DT) is not frequently used to correct for potency of a drug in terms of growth inhibition. We were looking for a parameter that better reflects a difference in potency of mifepristone towards cell lines having very different DT. Not to confuse the reader, we have followed the recommendation of the reviewer, and removed such correction which is not relevant to the message this work is intended to provide. Eliminating this correction also shortened the discussion and provided space for discussing other potential mechanisms of action.
5. We have added to the hypothesis that mifepristone was used as a growth inhibitor agent at pharmacologic, micromolar concentrations.

Once again, the correction for DT has been removed, consequently the column 5 of Table 1 has been deleted, and Figure 2 has been removed. Furthermore, section C of Figure 4 indicating the relative lethality of high concentrations of mifepristone towards two different sets of cell lines has been removed from the figure and instead described in the text of the result's section.

**Referee #2**

Discretionary revisions:

1. The referee raises a reasonable question. The presence of phenol red in the culture media has been shown to have hormonal-like activity and to affect the expression of steroid hormone receptors. In a previous work (Goyeneche et al., Clin Cancer Res 2007; Ref # 13, revised version) we studied the effect of mifepristone in both phenol red free and phenol red containing media obtaining similar results. Consequently, at least at the concentrations of mifepristone utilized, phenol red does not have an effect in the

outcome of the experiments. Moreover, in our current work the PR positive control, MCF-7 cells that responded to MF with growth inhibition, did express PR even in the presence of phenol red and unextracted serum.

2. A new paragraph mentioning potential mechanisms as to how mifepristone blocks growth targeting other cellular systems not involving cell cycle regulatory proteins and steroid hormone receptors has been added to the revised discussion.

3. The limitations of the model have been provided in the conclusion section of the discussion.

4. Future directions for this study have now been included at the end of the discussion section.