Reviewer's report

Title: gammaH2AX and Chk1 phosphorylation as predictive pharmacodynamic biomarkers of Chk1 inhibitor-chemotherapy combination treatments

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Reviewer: Alan Eastman

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Major concerns

1. This manuscript sets out to determine which drugs combine well with a Chk1 inhibitor, and what biomarkers predict this response. A limitation of this study is its general lack of novelty because it has been preempted by many prior studies, albeit with a different Chk1 inhibitor. Many papers have assessed the activity of Chk1i in combination with other drugs; e.g., one recent paper directly compared multiple drugs (Xiao, Mol Cancer Therap, 2013). The use of phospho-Chk1 and gH2AX have also been well studied as response markers (e.g. Parsels, Clin Cancer Res 2011).

2. A major conclusion of this manuscript is that gH2AX would be a good marker to assess response in patients. Yet the results presented here show that cleavage of PARP, caspase 2 or 3 would be equally appropriate as markers. This raises the question of whether the appearance of gH2AX is a cause or consequence of the onset of apoptosis. I suspect it is a cause, but the authors would need to incubate cells with caspase inhibitors before they can make the conclusion that gH2AX is indeed causal (i.e., line 215).

3. The authors should consider the clinical schedule for administration of the drugs they use as continuous incubation for 72 h or 168 h has little relevance to bolus administration of most of the drugs to patients. Furthermore, while they use two different growth / survival assays, the end point in both is the same and neither can be said to assess survival. Both assays only measure viable cells and provide no data as to whether there are any dead cells in a culture. Hence, as used here they can only be said to assess growth inhibition. The comment on line 147 (“fraction of cells killed”) is therefore incorrect, and figure axis saying “survival” are also incorrect.

4. Many of the changes observed in Fig 2 could be attributed to differential cell cycle perturbation caused by the various drugs, yet it is unfortunate that no attempt was made to assess this important parameter.

5. The results in Fig 3 seem inconsistent at times but the authors fail to address or explain this. In particular, in HT29 cells, 62.5 nM V158411 inhibits Chk1 as assessed by p296Chk1, yet no gH2AX is observed in combination with gemcitabine until 1 µM V158411. In contrast, gH2AX is observed at 62.5 nM in the equivalent Colo25 experiment. Is there some reason why these results are so
different between cell lines? Equally confusing is the observation that the addition of V158411 has no additional impact in gemcitabine treatment in both cell lines despite the fact it is supposed to be collapsing replication forks (Fig 3B).

6. The experiments to demonstrate lack of response in p53 wildtype cells are inadequate because the cell lines used are not isogenic. As a minimum this limitation should be noted. However, there is also a problem in understanding why p53 might protect cells. Consider the text on lines 310 – 320. Gemcitabine stalls cells in S phase, and when Chk1 is inhibited, the replication forks collapse. It is not correct that these cells progress through S phase and enter a lethal mitosis, which p53 might protect from, as the absence of dNTPs in cells on gemcitabine means that 7. the cells remain in S phase. There is no rationale to suppose that p53 would impact the collapse of replication forks.

Minor revisions requested:
8. Lines 49/50 should clarify that the Mre11 complex signals to ATM while 9-1-1 signals to ATR. On line 47 it should be noted that ATR signals in response to single-strand regions of DNA.

9. Line 64 is misleading as all the agents listed inhibit replication fork progression. Etoposide inhibitors topoisomerase II.

10. The antibody to pCdc2-Y15 can not discriminate phospho-Cdc2 from phospho-CDK1 (despite what suppliers frequently say). Furthermore, changes in this phosphorylation can not be said to be due to mitotic entry.

11. Justification should be given as to why anchorage independent cultures were investigated. Similarly, what was the intent of performing experiments in low oxygen or low serum? A clear reason for each experiment needs to be provided.

12. Line 293. There are already a number of papers that have published the lack of sensitization to 5FU, but the authors might find it interesting to note that several of those same papers have shown sensitization with fluorodeoxyuridine.

13. Line 306. It is correct that the greatest sensitization has been seen with antimetabolites, but the greatest sensitization in this class has ben reported with cytarabine and hydroxyurea.

14. Line 328, gemcitabine is administered to mice at doses below the MTD as it is in humans. The difference in schedule of 3 days vs. weekly is a minimal difference given the difference in species.

**Level of interest:** An article of limited interest

**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 conflicting interests