Reviewer's report

Title: Sensitization of human cancer cells to gemcitabine by the Chk1 inhibitor MK-8776: cell cycle perturbation and impact of administration schedule in vitro and in vivo

Version: 2
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Reviewer: Meredith Morgan

Reviewer's report:

This manuscript by R. Montano et al. provides a detailed examination of the optimal schedules of administration of Chk1 inhibitors with gemcitabine and the underlying mechanisms which can be leveraged by adjusting administration schedules. The authors carefully examine the influences of Chk1 inhibitor and gemcitabine on the cell cycle, homology-directed repair, and DNA damage. In addition, the suggested optimal scheduling of Chk1 inhibitor with gemcitabine was confirmed in 2 independent animal tumor models. This work is important in that it provides evidence supporting the superiority of delayed administration of Chk1 inhibitors (relative to gemcitabine) which is generally in contrast to how these agents are given clinically. The authors should consider a few comments (below) to improve the manuscript.

Major compulsory revisions

none

Minor essential revision

1. Page 10, para 2: The statement ‘inhibition of Chk1 preventing its normal feedback inhibition of ATR such that ATR is activated and phosphorylates Chk1’ is not entirely consistent with the model described in ref. 14. This model states that Chk1 inhibition results in feedback inhibition of PP2A (the phosphatase for Chk1) which ultimately results in accumulation of pChk1 (S317 and 345). An alternative (but not mutually exclusive) explanation for the increase in pChk1 S345 observed in response to Chk1 inhibition is that this is a consequence of increased DNA damage which leads to activation of ATR-mediated Chk1 phosphorylation (Parsels et al., Clin Cancer Res 2011). The latter is consistent with the effects of MK8776 on pDNA-PK and gH2AX observed in Fig. 2A.

2. Figure 5: Please state how many tumors/animals per treatment group.

Discretionary revision

3. The interpretation of the Rad51 focus formation data in Figure 3 is that in the presence of Chk1 inhibitor that Rad51 foci dissociate. The authors discuss mechanisms whereby Chk1 can promote recruitment of Rad51 to DSBs (e.g. Chk1-dependent BRCA2 and Rad51 phosphorylation) however these are mechanisms that when inhibited by a Chk1 inhibitor would be expected to prevent assembly of Rad51 foci not promote dissociation. It would also be of
interest to discuss mechanistically how inhibition of Chk1 might promote disassembly of Rad51 foci.

**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:**

none