Reviewer's report

Title: The interaction between pemetrexed, gemcitabine and irradiation: in vitro study to the cell line and schedule dependency.

Version: 1 Date: 26 December 2009

Reviewer: Tina Li

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In this manuscript, the authors determined the effect of different drug delivery schedules of pemetrexed, gemcitabine and radiation combination in two selected human cell lines of NSCLC and SCCHN. The research question of determining the optimal schedule of chemoradiation using preclinical models addresses the unmet clinical need and is clinically relevant to guide clinical trial design. The strengths of the manuscript are: 1) the extensive analyses of in vitro cytotoxicity data; and 2) the manuscript was well written. However, there are several important deficiencies/limitations that do not support the conclusions of the authors and have diminished the enthusiasm for the manuscript.

1. The selection of one NSCLC and one SCCHN for in vitro study only does not represent the heterogeneity and complexity of the human cancers. As the authors stated on page 10 line 5, “The cytotoxicity of pemetrexed was greatly dependent on the cell line used, …”.

2. Since the clinical doses of pemetrexed and gemcitabline were derived from “maximum tolerated dose” in phase I studies rather than the IC50 from in vitro cytotoxicity study, there is a serious concern for the clinical relevance of the results as the antagonistic effect (C.I. value >1.0) was observed at higher concentrations of chemoradiation in Figures 1 and 3.

3. The cell cycle effect of chemotherapy with or without radiation was only assessed by flow cytometry in the manuscript, which could be subjective depending on how the data were analyzed. Confirmation with western blot analysis is recommended for the cell cycle effect of different schedules of chemotherapy and radiation.

4. There were no evaluation of molecular and cellular mechanisms of potential synergism of pemetrexed, oxaliplatin and radiation in the manuscript. Evaluation of induction of apoptosis, and modulation of expression of drug targets and signal transduction molecules are recommended.

5. The authors did not mention some of the important, relevant references. For instances, Dr. AA Adjei has published several manuscripts evaluating the effect of different sequences and schedules of gemcitabine and pemetrexed in solid tumors including NSCLC.

a. Adjei AA, Erlichman E, Sloan JA, et al A phase I and pharmacologic study of


**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 competing interests.