Reviewer's report

Title: The radiosensitising effect of gemcitabine and its main metabolite dFdU under low oxygen conditions is in vitro not dependent on functional HIF-1 protein

Version: 2  Date: 22 May 2014

Reviewer: Elisa Giovannetti

Reviewer's report:

The article by Wouters and collaborators describes the radiosensitising effect of gemcitabine in normoxic and hypoxic conditions, using in vitro models with differential activity of HIF-1alpha. Moreover, The Authors describes for the first time the radiosensitising effect of the main metabolite of gemcitabine, dFdU, under both normoxic and hypoxic conditions.

Overall, the manuscript deals with an issue of interest, which has not yet been the focus of preclinical studies and might lead to clinically relevant applications. DfdU has indeed a half-life longer than 14 hours, and can substantially contribute to the radiosensitising effect of gemcitabine in the patients treated with this commonly used anticancer drug.

The study is well designed and executed. The background and the cited literature are up-to-date and properly discussed, and the results are summarized appropriately. The authors discuss in detail the complex role of HIF-1alpha in hypoxia, as well as their novel findings on the potential role of dFdU in the sensitivity of cancer cells to radiotherapy.

There are only a few minor revisions that should be taken in consideration to improve the presentation of this study:

- It is not clear, from the methods and the results section, why the 18-hour exposure time utilized to expose the cells to hypoxic conditions in this study was selected.

- The paragraph on western blot should include more details, such as information on antibodies and experimental conditions

- The authors should report more results about the “Human hypoxia signaling pathways PCR array”. Although significance was lost when the Authors performed the appropriate correction for statistical analysis, I would suggest adding a supplemental table showing at least the 11 genes with more than two-fold up or down-regulation.

- The Tables and figures are instructive and give a comprehensive overview of the studies that are described in this manuscript. However, the Tables 1 and 2 need to be simplified and more than half of all data presented should be moved into supplemental tables.
- The Authors do not provide much insight into how these findings could or should be clinically applied and whether the inclusion of molecular tests for specific biomarkers of hypoxia (different from HIF-1alpha), could help in the screening for patients who should (or not) be treated with gemcitabine and radiotherapy. Although gemcitabine is rarely used in breast cancer I would suggest adding a paragraph on the clinical implications of these studies/results.

- Validation of these studies in in vivo models is eagerly anticipated and should be further discussed by the Authors.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests