Reviewer's report

**Title:** Enhanced cytotoxic effect of radiation and temozolomide in malignant glioma cells: Targeting PI3K-AKT-mTOR signaling, HSP90 and Histone deacetylases

**Version:** 2  **Date:** 10 October 2013

**Reviewer:** Peter Sminia

**Reviewer's report:**

General comments

The revised manuscript by Choi et al. has been improved compared with the original version. More details are added to the Materials and Methods chapter, and the authors considered my comments and suggestions regarding the reproducibility of the results, analysis and quantification of the experimental data. Thanks to the authors for the detailed answers to my questions. I have only one remaining question and one remark as well.

This reviewers' original comment:

Figures 5A&B: How exactly was cell migration scored? Because the test took 24 hours, the effect of (drug induced inhibition of ) cell proliferation might result in a higher reduction of cell migration in the test groups where drugs were present. How about the effects of the drugs alone, without TMZ and or IR?

Authors answer:

“The distances between the edges of the wound were measured by using Image J software. The sixty measurements were taken for each experimental condition. The degree of mobility is expressed as percent of wound closure as compared with the zero time point. Migration rates were calculated using the following equation: \((\text{initial distance-final distance }/\text{initial distance}) \times 100\). We added this paragraph at p8-9. PI103 or 17-DMAG or LBH589 alone also increased migration and invasion of U251 cells.

Remaining question:

Is it correct that all those drugs stimulated cell migration and invasion? If true, this is of concern; could you please show me the data?

This reviewers' original comment:

Figure 5C: Addition of TMZ has probably no extra effect on all combinations, and also no radiosensitizing effect. Quantitative data are required to draw a definite conclusion.
Authors' answer:
We added the bar graph showing quantitative data to clarify this point.

Reviewers' remark:
Please notice that X-axis legend /data does not match with the histogram columns. Correction is required.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
'I declare that I have no competing interests'