Reviewer's report

**Title:** Inhibition of Radiation Induced Migration of Human Head and Neck Squamous Cell Carcinoma by Blocking EGF Receptor Pathways

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**Reviewer:** Deborah Citrin

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The authors present a study evaluating the role of EGFR and downstream signaling pathways in radiation-induced cell migration in head and neck squamous cell carcinoma cell lines. They report on three cell lines in all assays. In the study cells were first treated with EGF or radiation of varying doses which revealed increased migration via a scratch test. Blocking EGFR or several downstream pathways blocked migration after radiation. Proliferation was also assessed and was found to be reduced in inhibitor treated cells when delivered alone and in combination with radiation. The rationale for the study is clear. The techniques employed are not always appropriate and the discussion and conclusion is superficial and poorly developed. The references are appropriate.

**Major compulsory revisions:**
1) General: There are a number of grammatical mistakes throughout the manuscript and the figures. These should be corrected.
2) Tables and Figures: The manuscript describes references tables which are not present in the provided materials. These should be added.
3) Tables and Figures: Induction of these pathways after radiation, and inhibition of the targets of each agent is critical to the rationale of this study and the results the authors report. The Western blot results should be presented in figure form. The description provided is complicated and difficult to understand.
4) Tables and Figures: The authors report that they do not observe phosphorylation of EGFR in their western blots. This is critical to the rational of the study in that they suggest inhibition of EGFR alters migration. The 24 hour time point may not be the most appropriate time at which to assay this receptor and alternative time points should be considered or an alternative explanation of these results should be provided.

**Minor Essential Revisions**
1) Background: The authors reference invasion and migration. They reference Camphausen et al in regards to the Lewis Lung Model. This reference showed that a molecule liberated at the time of radiation affected dormant metastatic cells in the lung, not that radiation induced new metastases proving an abscopal effect. Although this is an excellent reference, this does not relate to migration or invasion.
2) Methods: The energy and dose rate of the irradiation used should be reported.
3) Results: The authors state in the results that “The increase in migration by radiation was significantly reduced through stimulation with EGF as well as through inhibition with AG1478.” This is not what is evident from review of the figure and figure legend and does not make sense based on the hypothesis. Looking at the figures it appears that there is no effect or a small increase in migration after stimulation with EGF. This should be clarified in the text.

4) Results: The authors state that the use of all 3 inhibitors of downstream pathways blocked migration in all three cell lines. Looking at figure 4, it appears that treatment of the BHY cell line with PD98059 increased migration without and with radiation. This should be described in the results and the possible rationale for this effect should be discussed in the discussion.

5) Discussion: The rationale for evaluating proliferation is not well described and the implications of these results are not discussed. It is clear that proliferation would be an important variable to evaluate in terms of the scratch test as increased proliferation could result in filling in of edges and a increase in migration in the setting of decreased proliferation is a more powerful result. This should be discussed.

Discretionary Revisions
1) In the results section the authors report the decrease in MAPK signaling after inhibition of PI3K. It would be appropriate to discuss this in terms of possible off-target effects.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

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

Declaration of competing interests:

I declare no competing interests