Reviewer's report

Title: Enhancement of Cetuximab-Induced Radiosensitization by JAK-1 Inhibition

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Reviewer: Michael Spiotto

Reviewer's report:

In this manuscript, Bonner et al. attempt to enhance the radiosensitization of targeting EGFR with cetuximab by adding a JAK inhibitor to prevent STAT-3 activation. This work was motivated by the hypothesis that targeting downstream effectors of EGFR which are also activated by separate signaling pathways that may enhance radiosensitization. Here, the target is STAT-3 which is activated by both EGFR as well as and JAK-STAT pathway. To address this hypothesis, the authors use 4 distinct HNSCC cell lines, one of which has a STAT3 knockdown. The authors show that combined EGFR and JAK1 inhibition prevents STAT3 phosphorylation at the Y705 residue (and possibly at the S727). The authors then show that dual inhibition decreases cell proliferation and increases cell death. Next, the authors contend that dual inhibition increases radiosensitization as measured by cell proliferation and clonogenics. Finally, the authors contend that the dual inhibition causes radiosensitization by inhibiting DSB repair.

While the hypothesis is strong and clinically important, I do not think the data provide sufficient support. Unfortunately, I have major caveats regarding this manuscript.

Major Compulsory Revisions:

1. I am unclear how well radiosensitization is proven by the dual inhibition with JAK and EGFR inhibition. The only evidence of radiosensitization that I see is in Figure 3 at doses of 4 Gy or greater. In Fig 2A, the decreased cell proliferation in RT+Cetux+JAKi appears similar to the decreased cell proliferation of the Cetux+JAKi for all 4 cell lines. Given that radiation alone groups inhibited proliferation, the lack of difference between the Cetux+JAKi and RT+Cetux+JAKi makes me concerned for a lack of radiosensitization. This coincides with no obvious radiosensitization at 2 Gy in Fig 3. Furthermore, no analysis was performed to assess if the effects were additive or synergistic and no other experiments used radiation doses at 4Gy or above where radiosensitization may be occurring.

2. It is unclear if STAT3 inhibition mediates radiosensitization for the following reasons.
   a. The level of inhibition (especially in UM-SCC-1) does not correlate with radiation induced apoptosis. JAK1 inhibition (in the absence of EGF supplementation) was sufficient to inhibit STAT3 phosphorylation yet JAK1 had minimal if any radiosensitizing effects. By contrast, Cetux did not cause JAK1
inhibition in the absence of EGF stimulation but had more radiosensitization than JAK1.

b. When measuring proliferation, the sensitivity of STAT3 KD to radiation+dual inhibition does not appear much different than dual inhibition alone. I would have expected increased sensitivity in this case. As a side note, the STAT3-2.4 doesn’t show much responsiveness to apoptosis due to radiation alone or cetux alone compared to the control NEG4.17 that I would expect.

3. I am not convinced that the increased radiation sensitivity is due solely to increased DNA repair as indicated by the comet assays. Why would it not also increase DNA damage with the dual inhibition? Are any DNA repair processes/proteins inhibited to support this point?

4. Statistical comparisons should also be made for Cetux+JAKi vs. Cetux+JAKi+RT.

5. Dose titration experiments to demonstrate an interaction between Cetux and JAKi on growth/apoptosis would be more convincing.

Minor Essential Revisions:
1. Page 13, paragraph 2: Figure 3 should be Figure 4.

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