Peer Review File

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Replies to Reviewer Comments

Reviewer A

Comment 1: This article was unfortunately similar in structure with other review article named “radiation and immunotherapy in high-grade gliomas where do we stand?” PMID “28906259”. Those review article was well structured, so the readers could comprehensively get information about radiation and immunotherapy in high-grade gliomas. However, this articles include so large study results that is not easy to follow.

Reply 1: Thank you for feedback. We have read the article referenced above and have found it very helpful. We have structured our paper by broad categories of vaccine therapies, immune checkpoint inhibitors, and adoptive cell transfer, rather than by the categories of passive, active, and gene therapy. We feel that our current structure allows us to discuss the trials in a chronological and historical context, thereby improving the understandability of these complex trials. We agree that it is helpful to clarify for the reader which therapies are active, passive, and gene mediated, so we have included this in the section titles with additional explanation in the text.

Changes in the text: We have modified our text as advised. See titles and subtitles as follows: page 7, line 111; page 10, line 187; page 12, line 213; page 15, line 277; page 17, line 314; page 22, line 426. We have also added explanation in the text on page 6-7, line 105-108; page 17, line 321-323; page 22, line 427-428.

Comment 2: There was no figure in your paper. I think some concept could be expressed with figures such as microglia, PD-1, and other kinds of vaccines. Some results of land-mark trial should be shown with figures. Please add the figures.

Reply 2: We have included a figure to show immune reactions in glioblastoma and immunotherapeutic targets, re-printed from Binder et al. (OncoImmunology 2015) without changes under the terms of the Creative Commons Attribution License.

Changes in the text: Page 37

Comment 3: In radiation oncologist’s perspective, there were lack of information. There were some points that could be described about radiation oncology. Radiation dose (conventional vs. hypofractionated), Radiation field (the subventricular zone), radiation induced lymphopenia and so on. Please add some point of view that radiation oncologists get some information.

Reply 3: Thank you for bringing this to our attention. We have included information on radiation-induced lymphopenia and treatment fields in the section on Radiation and Immunotherapy. We did not include any additional explanation regarding the subventricular zone because, to our knowledge, there is no immunologic correlation. We briefly mention the difference between conventional fractionation and fractionated stereotactic radiosurgery on page 29-30, lines 584-588. Please let us know if this requires further clarification.

Changes in the text: Page 30-31, lines 606-608

Comment 4: Please re-organize your article. Active immunotherapy, Passive immunotherapy, gene therapy could be your options to re-organize.

Reply 4: Please see Reply 1 above.

Changes in the text: Please see Reply 1 above.

Reviewer B

Comment 1: I suggest adding a section to review the different mechanisms by which radiation could improve immunogenicity of tumors. The authors should elaborate on the mechanisms such as generation of neoantigens due to radiation induced DNA damage, upregulation of MHC I and antigen presentation post-radiation, activation of STING pathway and IFNg production, DAMP-induced microglia activation, effect of radiation on TGFb and immunosuppressive microenvironment, upregulation of NKG2D ligand and sensitization to NK mediated cytotoxicity to name a few.

Reply 1: Thank you for your thoughtful comments. We agree that further focus on the combination of immunotherapy and radiation is warranted. We chose to focus on clinical trials more so than mechanisms and preclinical studies, but, as suggested, we have included more information on the mechanisms by which radiation may enhance immunogenicity to provide background to the reader. We attempted to focus on the most salient mechanisms.

Changes in the text: Page 27-28, lines 536-548

Comment 2: Line 519-546 (Sequencing and timing of therapies)-Is there an advantage of using immunotherapy before radiation? Are there any studies to determine this?

Reply 2: There are no studies that compare immunotherapy before versus after radiation, and in general there are very few studies (only two to our knowledge) that have delivered immunotherapy prior to radiation.

Changes in the text: The need for additional studies of immunotherapy given prior to radiation is now mentioned on page 29, line 582-583

Comment 3: Discuss the need to optimize identification of early biomarkers to predict efficacy of radiation combined with immunotherapy.

Reply 3: Ongoing studies to identify biomarkers are mentioned in the section on immune checkpoint inhibition (page 20, line 395-409), and we have added text in the conclusions regarding the need to identify biomarkers to predict the efficacy of radiation combined with immunotherapy

Changes in the text: Page 34-35, line 690-692

Comment 4: Please include references for Line 94, 199.

Reply 4: References included.

Changes in the text: Page 7, line 115; page 12, line 220

Reviewer C

Comment 1: On Page 4 line 62- 64, authors briefly describe lymphatic drainage in the CNS. They should refer to more recent articles by Jonathan Kipnis (Nature Neuroscience 2018) and Kari Alitalo (Journal of Exp Med 2015).

Reply 1: Thank you for bringing these articles to our attention. They are now referenced.

Changes in the text: Page 5, line 82

Comment 2: On Page 5 line 81, authors should specify what the co-stimulatory agents are and include the reference.

Reply 2: Thank you for this comment. This sentence is meant to act as a generalization of the mechanism of therapeutic vaccination with more specific details, such as particular co- stimulatory agents, mentioned in the text below. Poly-ICLC was mentioned in the subsequent section along with GM-CSF (see Comment 3), and additional adjuvants are mentioned in the discussion of particular studies in the tables.

Changes in the text: See Comment 3.

Comment 3: On Page 6 line 94, the authors should mention poly I:C in addition to GM-CSF.

Reply 3: Poly ICLC has been included.

Changes in the text: Page 7, line 115

Comment 4: The section about Viral Vaccines, author should include few sentences at the end of section – discussing / interpreting the outcome of the oncolytic virus trials. The

three year patient survival rate across the three trials mentioned in manuscript are around 20%. Does this survival statistic suggest that all responders are immunologically equal, and they will respond irrespective of the kind of oncolytic viral therapy used?

Reply 4: It is indeed interesting that these three studies each had a similar rate of durable responders, which may imply a common immunologic feature that potentiates this response. We have included this interpretation in the discussion

Changes in the text: Page 16, line 310-313

Comment 5: On Page 19 line 374, there is a typo- should be CD137 and not DC137.

Reply 5: Thank you. This has been corrected.

Changes in the text: Page 21, line 398