Peer Review File

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Review #1

Comment 1: *The size of radiation doses used (3x 8 Gy on consecutive days to essentially the whole lung cavity). Such a dose is likely to be lethal for the mice at a later time and certainly is significantly larger than could be given clinically. The authors need to justify their choice of such a large dose and address the issue of whether the effects they observe would occur to the same extent at lower doses, more relevant to the clinic?*

Reply 1: Clinically, stereotactic body radiotherapy with doses between 50 and 56 Gy, administered over 5-9 fractions achieved acceptable tumor control without severe complications for primary lung cancer and oligometastasis (Aoki, Hatayama et al. 2016). In the current study, we aimed to induce the acute radiation-induced lung injury (RILI) in mice. It had been reported that RILI can be induced with a single dose of 20 Gy (Zheng, Zhu et al. 2020) or 6 Gy × 5 (Chung, McKay-Corkum et al. 2016) to the full thorax. The mice were alive for at least 12 weeks with these doses administrated. The regimen of 8 Gy × 3 in our current study was based on the published literatures (Dewan, Galloway et al. 2009, Wang, Luo et al. 2019).

We maintained the irradiated mice for 28 days and no death occurred during this period. However, it might result in the death of mice in long-term as the reviewer pointed out. The death happened after 16 weeks following irradiation is probably related to chronic RILI (Paun, Bergeron et al. 2017). We did not address this issue in the current study.

Changes in the text: Based on the reviewer's suggestion we have modified our description in the section of Radiation schedule, Page 7, line 141-144 and added some detailed description in Results section, Page 12, line 245-247 and Discussion section, Page 19, line 404-406.

Comment 2*: It is recognized that PD-L1 can also influence the effects of radiation on the heart. Since the heart was in the radiation field the authors need to consider the*

potential effects of cardiac damage on their observations.

Reply 2: Radiation-induced cardiac toxicity (RICT) is a recognized late sequela of thoracic radiotherapy (Simone 2017). Cardiovascular Toxicities associated with PD-1/PD-L1 checkpoint inhibitors are infrequent in clinical practice (Ball, Ghosh et al. 2019). In the current study, we tried to address the acute toxicities associated with thoracic radiotherapy, such as acute pulmonary toxicity. In the experimental mouse models, acute RILI generally happened within 1 month after irradiation based on the literature (Liu, Kong et al. 2018).

To address the RICT pointed by the reviewer, we examined the histology of the murine hearts on days 7 and 14 following irradiation. These data were provided in our current revised manuscript.

Changes in the text: Methods section, Page 8, line 164-166; Results section, Page 12, line 260-262; Discussion section, Page 19, line 401-406 and supplementary Figure S1

Comment 3: *In the RNA studies did the authors examine whether expression of their control (GAPDH) was affected by the radiation or anti-PDL1 treatment.*

Reply 3: In irradiated mice, there was no significant change in the expression of GADPH between the anti-PD-1 antibody and isotype-treated groups.

Changes in the text: We added data about the levels of GADPH in supplementary Figure S2 in our current revised manuscript.

Comment 4: *It seems possible (likely) that the expression of cellular surface markers could be affected by the digestion procedure used to prepared a cell suspension from the right lung. Did the investigators consider this issue? There is no discussion of this possibility in the text.*

Reply 4: The digestion methods and procedures are widely used for isolation and characterizing cell phenotypes of immune cells (Qu, Edwards et al. 2004). No change of cellular surface markers was reported (Autengruber, Gereke et al. 2012). In our current study, we used a digestion procedure as previously reported with minor modifications (Jin, Lagoudas et al. 2019).

Changes in the text: We added detailed description in our current revised manuscript in Methods section, Page 8, line 170-174.

Review #2

Comment 1: *In the Methods section on flow cytometry the authors would ideally provide refs for the various markers they used for the different cell populations examined.*

Reply 1: Done as suggested.

Changes in the text: Methods section, Page 9, line 179-186

Comment 2: *In the Methods section on MACS it is not entirely clear what was done. In particular were the gamma/delta T cells put into the plates precoated with anti-PDL1.*

Reply 2: The detailed information was provided in our current revised manuscript.

Changes in the text: Methods section, Page 11, line 223-230

Comment 3: *It would be useful to underline the headings in the Methods section.*

Reply 3: Done as suggested.

Changes in the text: Methods section.

Comment 4: *There are a significant number of minor English errors that need correcting (highlighted in the attached Ms).*

Reply 4: Our current revised manuscript was edited by a professional English writer. We attach the certificate as "Certificate-AME Editing Services" for your information.

Changes in the text: Positions in the manuscript where we have made modifications are highlighted by red color.

References

1. Aoki, M., Y. Hatayama, H. Kawaguchi, K. Hirose, M. Sato, H. Akimoto, I. Fujioka, S. Ono, E. Tsushima and Y. Takai (2016). "Clinical outcome of stereotactic body radiotherapy for primary and oligometastatic lung tumors: a single institutional study with almost uniform dose with different five treatment schedules." Radiat Oncol **11**: 5.

2. Zheng, L., Q. Zhu, C. Xu, M. Li, H. Li, P. Q. Yi, F. F. Xu, L. Cao and J. Y. Chen (2020). "Glycyrrhizin mitigates radiation-induced acute lung injury by inhibiting the HMGB1/TLR4 signalling pathway." J Cell Mol Med **24**(1): 214-226.

3. Chung, E. J., G. McKay-Corkum, S. Chung, A. White, B. T. Scroggins, J. B. Mitchell, M. J. Mulligan-Kehoe and D. Citrin (2016). "Truncated Plasminogen Activator Inhibitor-1 Protein Protects From Pulmonary Fibrosis Mediated by Irradiation in a Murine Model." Int J Radiat Oncol Biol Phys **94**(5): 1163-1172.
4. Dewan, M. Z., A. E. Galloway, N. Kawashima, J. K. Dewyngaert, J. S. Babb, S. C. Formenti and S. Demaria (2009). "Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody." Clin Cancer Res **15**(17): 5379-5388.
5. Wang, F., Y. Luo, X. Tian, S. Ma, Y. Sun, C. You, Y. Gong and C. Xie (2019). "Impact of Radiotherapy Concurrent with Anti-PD-1 Therapy on the Lung Tissue of Tumor-Bearing Mice." Radiat Res **191**(3): 271-277.
6. Paun, A., M. E. Bergeron and C. K. Haston (2017). "The Th1/Th17 balance dictates the fibrosis response in murine radiation-induced lung disease." Sci Rep **7**(1): 11586.
7. Simone, C. B., 2nd (2017). "Thoracic Radiation Normal Tissue Injury." Semin Radiat Oncol **27**(4): 370-377.
8. Ball, S., R. K. Ghosh, S. Wongsaengsak, D. Bandyopadhyay, G. C. Ghosh, W. S. Aronow, G. C. Fonarow, D. J. Lenihan and D. L. Bhatt (2019). "Cardiovascular Toxicities of Immune Checkpoint Inhibitors: JACC Review Topic of the Week." J Am Coll Cardiol **74**(13): 1714-1727.
9. Liu, D., F. Kong, Y. Yuan, P. Seth, W. Xu, H. Wang, F. Xiao, L. Wang, Q. Zhang, Y. Yang and H. Wang (2018). "Decorin-Modified Umbilical Cord Mesenchymal Stem Cells (MSCs) Attenuate Radiation-Induced Lung Injuries via Regulating Inflammation, Fibrotic Factors, and Immune Responses." Int J Radiat Oncol Biol Phys **101**(4): 945-956.
10. Qu, C., E. W. Edwards, F. Tacke, V. Angeli, J. Llodrá, G. Sanchez-Schmitz, A. Garin, N. S. Haque, W. Peters, N. van Rooijen, C. Sanchez-Torres, J. Bromberg, I. F. Charo, S. Jung, S. A. Lira and G. J. Randolph (2004). "Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes." J Exp Med **200**(10): 1231-1241.
11. Autengruber, A., M. Gereke, G. Hansen, C. Hennig and D. Bruder (2012). "Impact of enzymatic tissue disintegration on the level of surface molecule expression and immune cell function." Eur J Microbiol Immunol (Bp) **2**(2): 112-120.
12. Jin, C., G. K. Lagoudas, C. Zhao, S. Bullman, A. Bhutkar, B. Hu, S. Ameh, D. Sandel, X. S. Liang, S. Mazzilli, M. T. Whary, M. Meyerson, R. Germain, P. C. Blainey, J. G. Fox and T. Jacks (2019). "Commensal Microbiota Promote Lung Cancer Development via γδ T Cells." Cell **176**(5): 998-1013.e1016.