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Prof Lian-Sheng Ma, Company Editor-in-Chief,
World Journal of Gastroenterology.

17th of February, 2022

Dear Prof Lian-Sheng Ma,

Re; Manuscript NO: 73485

The impact of radiotherapy on the immune landscape in oesophageal adenocarcinoma

Thank you sincerely for your review of the above manuscript, and for giving the authors an opportunity to submit a revised paper. The following are responses to specific comments from each reviewer:

In the Figure 2, these experiments should be performed in the OAC xenograft model instead of cell lines because the anticancer effect of ICI is induced in the situation of the presence of CD8+ T cells. What is the scientific rationale in the experimental design?

If the experimental design in Figure 2 has a scientific rationale, what mechanisms might explain the induction of anti-cancer efficacy of the combined RT and ICI in an experimental system without CD8+ T cell? Some minor concerns: λ Can you provide the original dot plots of Flow cytometry in Figure 1?

Many thanks for your excellent observation. The premise of this set of experiments was to demonstrate the effect of radiation and immune checkpoint inhibition directly on the tumour cell viability. While we appreciate and do understand your observation vis a vis mouse models, this seminal study presents novel findings and is an important translational discovery. Certainly, there will be strength in using a mouse model in further studies to elucidate mechanistic insights such as resistance and more in depth questions to complement these findings.
With respect to the effect on CD8+ T cells, immune checkpoints and their ligands are expressed on tumour cells which can have direct effects on the tumour cells themselves in the absence of immune cells and this is found to be the case in this experiment that there is a synergistic decrease in viability combining both treatments.

We have now included the gating strategy and representative dot plots as requested by the reviewer, please see below for your convenience, attached as a PDF.

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The authors propose an interesting study exploring the role of hypofractionation and immunotherapy in oesophageal adenocarcinoma. The results of this study provide a preclinical basis for randomised trials using hypofractionated treatment schedules and/or adding immunotherapy to conventional radiochemotherapy. The only useful addition, for future clinical use of the study results, is to discuss the possible patient toxicity scenarios of a hypofractionated approach + concomitant immunotherapy.

Thank you for your valuable suggestion, we agree that the immune related adverse events of combination radiotherapy and immunotherapies is of critical importance particularly regarding the translational potential of this study. The literature to date is concentrated primarily on evaluation of adverse events and of radiotherapy or immunotherapy in isolation. With improvements in targeted radiation delivery modalities, and technological advances, hypofractionated radiotherapy is now utilised without evidence of increased toxicities in a number of malignancies [34, 35]. The HRT regimen described here was safe and tolerable in patients unable to receive CRT, and delivered promising survival outcomes [36]. Furthermore, there is data in lung that conventional radiation dosing and immunotherapy is safe and feasible with no increases in adverse events [37-39].
The ATTRACTION 3 trial demonstrated a 50% reduction in serious adverse events in patients treated with nivolumab versus conventional chemotherapy in Esophageal Squamous Cell carcinoma [40]. In a study evaluating hypofractionation and immunotherapy in renal cell cancer, melanoma and lung, the most common grade 3 AEs were fatigue and pneumonitis. They found that toxicity did not correlate with H-RT site, dose, fraction number, tumor type, or ICI and H-RT sequencing. Hypofracitonated Radiotherapy of lung lesions was more likely to achieve CR than other sites. This study found that combining body Hypofractionated Radiotherapy with ICIs is safe and promising, however, prospective validation is warranted [41].

34. Rodin, D., et al., *Hypofractionated radiotherapy in the real-world setting: An international ESTRO-GIRO survey.* Radiother Oncol, 2021. **157**: p. 32-39.
35. Vapiwala, N., et al., *A Pooled Toxicity Analysis of Moderately Hypofractionated Proton Beam Therapy and Intensity Modulated Radiation Therapy in Early-Stage Prostate Cancer Patients.* Int J Radiat Oncol Biol Phys, 2021. **110**(4): p. 1082-1089.
36. Jones, C.M., et al., *Hypofractionated Radiotherapy in Oesophageal Cancer for Patients Unfit for Systemic Therapy: A Retrospective Single-Centre Analysis.* Clin Oncol (R Coll Radiol), 2019. **31**(6): p. 356-364.
37. Spaas, M. and Y. Lievens, *Is the Combination of Immunotherapy and Radiotherapy in Non-small Cell Lung Cancer a Feasible and Effective Approach?* Front Med (Lausanne), 2019. **6**: p. 244.

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38. Meng, L., et al., *The Combination of Radiotherapy With Immunotherapy and Potential Predictive Biomarkers for Treatment of Non-Small Cell Lung Cancer Patients.* Front Immunol, 2021. **12**: p. 723609.
39. Fitzgerald, K. and C.B. Simone, 2nd, *Combining Immunotherapy with Radiation Therapy in Non-Small Cell Lung Cancer.* Thorac Surg Clin, 2020. **30**(2): p. 221-239.
40. Kato, K., et al., *Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial.* Lancet Oncol, 2019. **20**(11): p. 1506-1517.
41. Mohamad, O., et al., *Safety and efficacy of concurrent immune checkpoint inhibitors and hypofractionated body radiotherapy.* Oncoimmunology, 2018. **7**(7): p. e1440168.

We hope that this is to your satisfaction and look forward to a favourable outcome. Please do not hesitate to contact me at any stage.

Kind regards,

Noel E Donlon & co-authors.

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