Peer Review File

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Reviewer comments

1. This commentary paper should clearly articulate its primary objectives. While the introduction discusses various treatments for LARC, it could benefit from a more explicit statement of the paper's aim why choosing and regarding the use of DNA-PK inhibitors as a radiosensitizer in LARC.

Thank you very much for your supporting comment. We revised manuscript as below, on line 39 page 3 – line 61page 5.

In this context, the development of combination radiotherapy with immunological agents or molecular targeted therapies is also underway, with the objective of enhancing the efficacy of radiotherapy. Phase Ib results of peposertib, a DNA-dependent protein kinase (DNA-PK) inhibitor, as a sensitizer for chemoradiotherapy for LARC were published in the Clinical Cancer Research journal in 2024 (3).

Peposertib has been preclinically demonstrated to inhibit the catalytic activity of DNA-PK with high potency and the double-strand breaks (DSB) repair in human cancer cell lines (4). Furthermore, peposertib inhibits radiation-induced DNA-PK autophosphorylation and DSB repair in tumor xenograft models. Oral administration of peposertib to two xenograft models of human cancer, utilizing a clinically established 6-week fractionated radiation protocol, markedly enhanced the tumor growth inhibition of radiotherapy, leading to complete tumor regression at nontoxic doses. Therefore, peposertib was a strong candidate for combination with radiotherapy based on preclinical data.

2. While the paper mentions ongoing trials and potential applications, it would benefit from a more thorough discussion of future research directions. Highlighting specific areas where further investigation is needed, such as optimizing treatment combinations or identifying predictive biomarkers, would provide valuable insight into the next steps for advancing this field of study.

Thank you very much for your supporting comment. We revised manuscript as below, on line 102 page 8 – line 154 page 13.

The development of drugs that selectively inhibit the backup pathway may potentially induce cell death

in cancers with specific mutations. Furthermore, drugs that target DDR could be combined with radiotherapy or chemotherapy to enhance the treatment of specific cancers. For LARC, the combination of veliparib, a poly (ADP-ribose) polymerase inhibitor (PARPi), with capecitabine and radiotherapy was found to be safe and exhibited a dose-proportional pharmacokinetic profile (10). Although a 29% pCR rate was achieved, the preliminary antitumor activity requires further evaluation. In the long-term result of NRG-GI002, the addition of veliparib to TNT showed no significant differences in tumor response or 3-year outcomes (11).

To enhance local responses and long-term outcomes following TNT, it is essential to innovate radiation sensitizers and develop treatment regimens (1, 13). It has been demonstrated that radiotherapy and chemotherapy induce genomic and chromosomal instability. The DDR plays a pivotal role in this process. Consequently, DDR inhibitors act as sensitizers in cancer cells, enhancing the efficacy of radiotherapy and chemotherapy(12). Additionally, genomic and chromosomal instability can serve as a trigger for the activation of innate immune pathways and adaptive immunity, thereby initiating antitumor responses. The concurrent administration of chemotherapy, radiotherapy, and DDR inhibitors has been demonstrated to enhance the eradication of tumors by stimulating the activation of IFN-dependent T cells. (13). It is therefore possible that a new treatment option for patients with locally advanced and metastatic tumors may be combination therapy based on the theory that DNA damage-inducing therapy and ICIs with a synergistic effect on tumor regression.

The findings of the trial for DDR inhibitors and radiotherapy (3, 11) for patients with LARC are intriguing, encouraging investigators to conduct a more thorough data analysis to identify the underlying biomarkers for the complete response. Indeed, the combination of DDR inhibitors with radiotherapy may be optimized by a comprehensive molecular analysis including whole exon sequencing (WES) and whole transcriptome sequencing (WTS) of circulating tumor DNA/RNA, spatial transcriptome sequencing, WES/WTS of tissue samples, plasma proteomic profiling and germline analysis, conducted via supercomputing system (14).

The latest edition of the guidelines adopts a biomarker-driven approach with regard to patients with LARC (15). Immune checkpoint inhibitor monotherapy in LARC patients with deficient mismatch repair (dMMR) leads to clinical complete response, organ preservation and sustained quality of life (16). Moreover, the OPRA trial demonstrated the approximately 50% potential of NOM following TNT for patients with proficient MMR (pMMR) as an option to reduce morbidity without sacrificing disease control in LARC (2). Accordingly, a more precise ability to predict eventualities for the purposes of guiding treatment strategies at diagnosis and/or restaging would be beneficial. To facilitate the precision oncology in LARC, international collaborations(1) have been already established to integrate multi-omics data from ongoing TNT clinical trials. These include the JANUS Rectal Cancer Trial (A022104/NRG-GI010/NCT05610163), the ACO/ARO/AIO18.1 trial (NCT04246684), and the ENSEMBLE trial (NCT05646511) (17, 18, 19) . This collaboration will encompass multi-omics data, including genomics, transcriptomics, proteomics, pathomics, radiomics, clinical features, and quality of life (QOL) data. The deployment of an AI-based model for precise targeted treatment of LARC through a supercomputing system will facilitate the delivery of precision medicine to individual patients (1, 14). A comprehensive spatio-temporal multi-omics analysis employing supercomputers will bring about an era wherein individual patients will receive an appropriate treatment strategy utilizing chemotherapy, radiotherapy, immune checkpoint inhibitors, DDR inhibitors, and other methodologies.

With the progress of “precision oncology”, for patients with dMMR rectal cancer, immune checkpoint inhibitors have opened the door to a cure. For patients with pMMR rectal cancer, a keyhole to cure without surgery is being opened through a multidisciplinary approach that includes DNA-PK inhibitors as radiotherapy sensitizers.

3. If JGO regulations permit, it would be a good idea to include a summarized table for the ongoing trials mentioned in the text.

Thank you very much for your supporting comment. We created Table.1 Selected clinical trials for pepsertib as below,

Table 1. Selected clinical trials for peposertib

Cancer types	Phase	Combination	Clinical trial ID*
Rectal Cancer	Ib	Radiotherapy, Capecitabine	NCT03770689 (3)
Advanced Solid Tumors	I	Radiotherapy, Cisplatin	NCT02516813
Advanced Solid Tumors	I	Radiotherapy, Avelumab	NCT03724890 (9)
Glioblastoma	I	Radiotherapy, Temozolomide	NCT04555577
Renal Cell Carcinoma	I	177Lu-TLX250	NCT05868174
Small Cell Lung Cancer	I	Cisplatin, Etoposide	NCT03116971
Advanced Solid Tumors, Chronic Lymphocytic Leukemia	I		NCT02316197 (7)
Advanced Solid Tumors	I	M1774	NCT05687136
Advanced/Metastatic Solid Tumors, Cholangiocarcinoma, Gallbladder Carcinoma	I/II	Radiotherapy, Avelumab	NCT04068194
Neuroendocrine Tumors	I	Lutetium Lu 177 Dotatate	NCT04750954
Endometrioid Adenocarcinoma	I	Pegylated Liposomal Doxorubicin Hydrochloride	NCT04092270
Head and Neck Squamous Cell Carcinoma	I	Radiotherapy	NCT04533750
Head and Neck Squamous Cell Carcinoma	I	Radiotherapy ± Cisplatin	NCT02516813(8)
Leiomyosarcoma, Sarcoma	I	Pegylated Liposomal Doxorubicin Hydrochloride	NCT05711615
Prostate Carcinoma	I/II	Radium-223 dichloride ± Avelumab	NCT04071236

*Clinical trials are accessible at https://clinicaltrials.gov/.