Peer Review File

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Reviewer A

The review is well written one. Here are some minor comments for consideration:

Comment 1) Please add a brief discussion on the latest evidence on the dose, fractionation, and sequence in combining RT and immunotherapy.
Reply 1) We have added the following at Page 17, Lines 389-398. "As mentioned before, RT can induce immunogenic cell death, release tumor antigens, and modulate the tumor microenvironment, while immunotherapy can enhance immune responses against cancer cells in HCC. The optimal dose, fractionation, and sequence of these treatment modalities are still being explored. The selection of dose and fractionation depends on various factors, including tumor size, location, and liver function. In general, lower doses of radiation (hypofractionation) are thought to be more immunogenic, potentially enhancing the immune response.[122,123] However, higher doses (conventional fractionation) may also have immunomodulatory effects.[123,124] The optimal approach may involve a balance between tumor control and immune activation, which requires further investigation in clinical trials specific to HCC."

Comment 2) Role of RT in Early-stage HCC (BCLC A), please consider adding J Hepatol. 2021 Mar;74(3):603-612 into the results.
Reply 2) We added the following at Page 10, Lines 206-211. "In a randomized phase III-controlled trial comparing PBT to RFA in the treatment of recurrent HCC (size < 3 cm, number ≤ 2), PBT demonstrated non-inferiority to RFA in terms of 2-year local progression-free survival (LPFS) rates, with 94.8% for PBT and 83.9% for RFA.[82] The intention-to-treat analysis also showed comparable LPFS rates between the two treatment arms. The progression-free survival (PFS) and OS rates were not significantly different between the two arms."

Comment 3) Reference 77 refers to BCLC C patients and should not be put under the section of BCLC B.
Reply 3) We deleted the following at Page 11, Line 240. "A recent open-label randomized trial demonstrated that EBRT with TACE for *advanced HCC (with portal vein invasion) was well-tolerated and improved PFS, objective response rate (ORR), time to progression, and OS compared to sorafenib treatment."*

We added this line " An open-label randomized trial demonstrated that EBRT with TACE for advanced HCC (with portal vein invasion) was well-tolerated and improved PFS and OS compared to sorafenib treatment.[103]"under the section of RT for Late-Stage HCC at Page 14, Lines 311-313. This reference now became number 102.

Also, this reference 102 was added at the bottom of Table 3 on Page 37.

Table 3 Radiation Therapy for Late-Stage HCC

Trial	Type of Study	Prospective vs. Retrospective	Radiotherapy	Local Control rate at 2 years	Overall Survival rate at 2 years
Kang et al.[96]	Single-site,	Prospective	SBRT	94.6%	68.7%
Andolino et al.[97]	Single site,	Prospective	SBRT	90%	48%
Kwon et al.[67]	Single-site,	Prospective	SBRT	67.5%	77.3%
Huang et al.[98]	Single-site	Prospective, matched-pair	SBRT	75%	72.6%
Bujold et al.[99]	Single-site	Prospective	SBRT	87% at 1 year	55% at 1 year
Sanuke et al.[100]	Single-site	Retrospective	SBRT	91% at 3 years	70% at 3 years
Jang et al.[101]	Single-site	Prospective	SBRT	87%	63%
Yoon et al.[102]	Single-site	Prospective	EBRT+TACE	None reported	55.4% at 48 weeks or roughly 28% at 2 years

SBRT, stereotactic body radiotherapy; EBRT, external beam radiotherapy; TACE, transarterial chemoembolization

Comment 4) Reference 72 should not be put under the section of RT for late-stage HCC.

Reply 4) The following was deleted under RT for Late Stage HCC originally from Page 12, Lines 252-256. "According to another retrospective study looking at patients (ECOG PS not mentioned) with inoperable nonmetastatic HCC (CPC A, B, C), SBRT achieved better LC rates compared to radiofrequency ablation (RFA) for treating tumors larger than 2 cm.[72] This study suggested that SBRT could potentially have an advantage over RFA, which needs validation from prospective studies."

The following was deleted from RT for Late-Stage HCC, originally from Page 12, Lines 261-263. "The one and two-year freedom from local progression for these tumors was

83.6% and 80.2% for RFA and 97.4% and 83.8% for SBRT."

This reference 72 was also deleted from Table 3.

Comment 5) Under the section of RT for late-stage HCC, should discuss the RTOG 1112 study and reference 77.

Reply 5) For Reference, 77, this reference was changed to Reference 102. The following was added at Pages 14-15, Lines 311-327. "The trial explored the combination of EBRT with TACE vs sorafenib in treatment naive HCC patients with macrovascular invasion. Ninety patients were randomized in 1:1 fashion. The primary endpoint of the trial was 12-week PFS rate. In this head-to-head comparison, the PFS rate was significantly higher in the TACE-EBRT group (86.7% vs 34.4%, p<0.001). Higher rates of radiologic response were also seen in the TACE-EBRT group when compared to sorafenib (33.3% vs 2.2%) at the 24-week mark. Modified progression free survival (mPFS) and OS were 31 and 55 weeks for TACE-EBRT group and 11.7 and 43 weeks for sorafenib. Notably, patients with macrovascular invasion, a prognostic predictor of poor overall survival, were evaluated in this trial.[103] The trial allowed for treatment crossover and were evaluated with intention-to-treat analysis. The superior mPFS of the TACE-EBRT group can make a case for treating patients initially with this combination prior to starting treatment with sorafenib. One limitation of the study is that the majority of the patients (85%) had hepatitis B associated HCC, and the results cannot be extrapolated to the general HCC population."

For RTOG 1112, the following was added at Page 15, Lines 329-341. "The recent results from the NRG/RTOG 1112 further indicate the role of combination strategies for treating HCC.[104] The combination of sorafenib with SBRT vs then standard-of-care sorafenib was evaluated in treatment naive advanced HCC patients. Trial accrual was closed early with new therapies approved for first line use in advanced HCC. Patients treated with SBRT + Sorafenib had improved OS of 15.8 months (90% CI, 11.4-19.2) vs 12.3 months (90% CI, 10.6 -14.3) with sorafenib alone (HR, 0.77; 90% CI, 0.59-1.01; 1-sided *P* = .55). Notably, 75% and 73% of patients in the SBRT/sorafenib and sorafenib monotherapy arms had macrovascular invasions, respectively. Also, the SBRT with sorafenib combination achieved a higher mPFS of 9.2 months (95% CI, 7.5-11.9) vs 5.5 months (95% CI, 3.4-6.3) in those treated with sorafenib alone (HR, 0.55; 95% CI, 0.40-0.75; *P* = .0001).[104] The improved PFS and OS might indicate future role of combination strategies involving RT with newly approved IO strategies in management of HCC."

Reviewer B

Major

Comment 1) The paper is well reviewed and described but various radiotherapies like SBRT, EBRT, cyber knife and SIRT should be classified and explained their benefit, merit & demerit for the treatment of HCC in one table.

Reply 1) We have added language to include additional forms of radiotherapy Page 8, Lines 172-175. "In addition to EBRT for the treatment of HCC, other forms of radiotherapy include stereotactic body radiotherapy (SBRT), including CyberKnife, selective internal radiation therapy (SIRT), carbon iron radiation therapy (CIRT), and proton beam therapy (PBT).[66-71] (Table 2)"

We also made mention of this table 2 on Page 9, Lines 189-190. "The general description, advantages and disadvantages of each form of radiotherapy are covered in Table 2."

The following Table 2 was made and placed on Pages 35-36.

Table 2 Advantages and disadvantages of various RTs for the treatment of HCC

Treatment Method	Description	Delivery Method	Main Advantages	Main Disadvantages
External beam radiation therapy (EBRT)[68]	A traditional form of radiation therapy that uses external beams of radiation to treat cancer.	External beam directed at the tumor, with intensity modulated to spare healthy tissue.	Can treat a larger area and is not limited by the size or shape of the tumor.	Requires many treatments over several weeks. More exposure of healthy tissue to radiation, which can lead to side effects.
Stereotactic body radiation therapy (SBRT)[69]	A radiation therapy that delivers very high doses of radiation to a small, precise area of the body.	External beam targeted precisely to the tumor.	Can treat smaller, well-defined tumors. Fewer treatments needed than traditional radiation. Minimizes exposure to healthy tissue.	May not be suitable for larger or less well-defined tumors. Can have side effects such as fatigue, skin changes, and other symptoms depending on the location of the treatment.

CyberKnife[136,137]	A type of SBRT. It uses a robotic arm to deliver highly focused beams of radiation.	External beam targeted precisely to the tumor, capable of adjusting to patient movement.	Offers real-time tracking and adjustment for patient movement, making it highly precise. Can treat tumors in challenging or risky areas.	Limited by the size of the tumor and can be more costly. Requires sophisticated equipment and experienced clinicians.
Selective Internal Radiation Therapy (SIRT)[70,138]	A form of internal radiation therapy where radioactive microspheres are injected into the blood vessels that feed the tumor.	Internal, via catheter directly into blood vessels feeding the tumor.	Delivers radiation directly to the tumor, sparing healthy liver tissue. Can be used in patients with larger or multifocal liver disease.	Risk of radiation exposure to non-targeted tissues and can be more costly. More invasive procedure. Requires a specialized interventional radiology team.
Carbon Ion Radiation Therapy (CIRT)[71,139]	A type of particle therapy that uses carbon ions to treat cancer.	External beam directed at the tumor.	Carbon ions are heavier and have a higher linear energy transfer (LET), leading to more precise targeting and potential for greater tumor kill.	Limited availability as it requires specialized equipment and can be more costly. More research is needed to fully understand the long-term effects and comparative effectiveness.
Proton Beam Therapy[66,140]	A type of radiation therapy that uses protons to treat cancer.	External beam directed at the tumor.	Protons stop at the tumor site, reducing exposure of healthy tissue beyond the tumor. Can be more precise than traditional radiation.	Requires specialized equipment and can be more costly. More research is needed to fully understand the long-term effects and comparative effectiveness.

External beam radiation therapy, EBRT; Stereotactic body radiation therapy, SBRT; Selective Internal Radiation Therapy, SIRT; Carbon Ion Radiation Therapy, CIRT

Comment 2) Figure 1 detail should be larger and restructured to easy understanding of immunomodulator and radiation.

Reply 2) Figure 1 has been updated to be larger and more detailed for increased. Moreover, in the figure legend, we added descriptions with color codes and changed some wording on Pages 39-40.

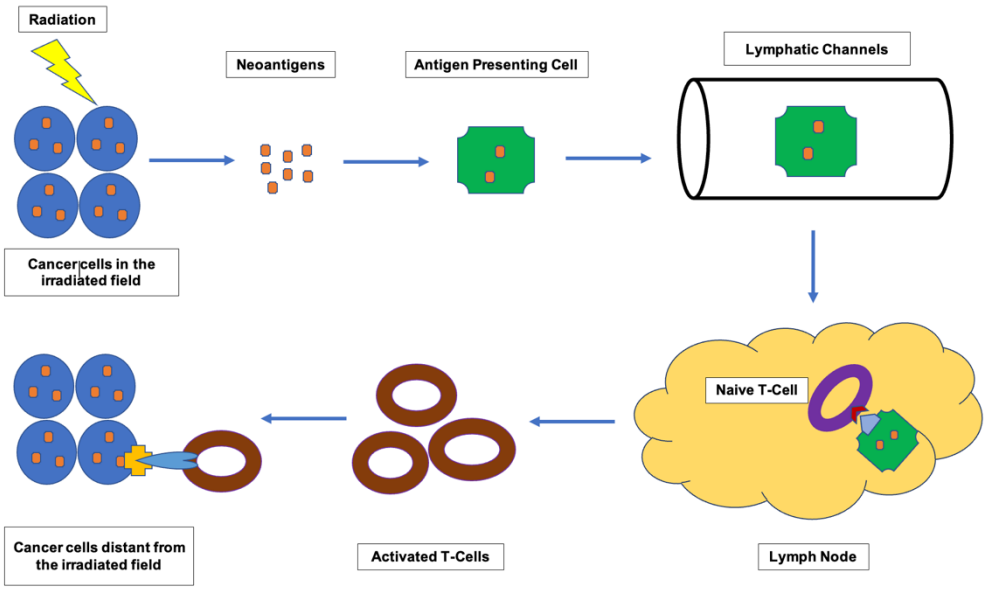

Figure 1. The Abscopal Effect Radiotherapy can lead to immunogenic cell death in irradiated cancer cells, leading to the release of neoantigens. The neoantigens (orange) are taken up by the antigen-presenting cells (APCs) (green). These APCs process the neoantigens and migrate through lymphatic channels to lymph nodes (yellow). In the lymph nodes, the APC presents the neoantigen to naive T-cells (purple) via the major histocompatibility complex and other stimulatory signals. Subsequently, these T-cells become activated and proliferate. The activated T cells (brown) leave the lymph nodes and migrate to distant sites. They execute their anti-tumor function by killing abscopal cancer cells distant from the irradiated field.

Comment 3) If the differences to potentiate the immunotherapy existed or speculated in various radiotherapy, explain it even though it is still in basic study step.

Reply 4) The following was added at Page 7, Lines 147-165. "Translational research has explored the potentiation of immunotherapy by RT. In a study investigating the antitumor effect of a combination of immune checkpoint blockade and RT, the authors found that *in vitro* radiation application upregulated PD-L1 protein expression in HCC murine and human cell lines.[62] *In vivo* radiation exposure also upregulated PD-L1 expression in a dose-dependent manner in murine HCC tumor tissue. Investigation into the signaling pathways responsible for radiation-induced upregulation of PD-L1 suggests that radiation initially induces the production of IFN-γ, which then signals downstream expression of STAT3, ultimately increasing PD-L1 expression in HCC tumor cells. Mice receiving a combination of anti-PD-L1 treatment and radiation demonstrated significantly suppressed tumor growth and increased survival rate

compared to those receiving anti-PD-L1 or radiation alone. These findings support the reasoning that radiation increases the susceptibility of tumor cells to anti-PD-L1 treatment by increasing induction of PD-L1."

It is also important to note that enhanced PD-L1 expression can promote T cell exhaustion and subsequent dysfunction through the upregulation of inhibitory receptors such as PD-1 and CTLA-4. The authors found that combination treatment not only increased the percentage of CD8 T cells but also increased IFN-γ CD8 T cells, CD107a CD8 T cells, and Ki67 CD8 T cells, indicating an increased effector cell cytotoxicity and proliferation, when compared to those treated with monotherapy.[62]

Minor.

Comment 4) Carbon iron radiotherapy and proton beam should be cited in any parts of the manuscript.

Reply 4) A mention of carbon iron radiotherapy and proton beam therapy was added at Page 8, LInes 172-175. "In addition to EBRT for the treatment of HCC, other forms of radiotherapy include stereotactic body radiotherapy (SBRT), including CyberKnife, selective internal radiation therapy (SIRT), carbon iron radiation therapy (CIRT), and proton beam therapy (PBT).[66-71] (Table 2)"

We also added additional information about cabon iron radiation therapy at Page 10, Lines 223-232. "A comparison study was conducted to evaluate the efficacy of CIRT versus TACE in the treatment of single hepatocellular carcinoma (HCC).[83] The study included 31 patients who received CIRT and 23 patients who received TACE. After propensity score matching, 17 matched pairs of patients from each treatment group were analyzed. The median follow-up durations after CIRT and TACE were 43 and 32 months, respectively. The 3-year overall survival, local control, and progression-free survival rates in the CIRT group were significantly higher than those in the TACE group, with rates of 88% versus 58% ($p < 0.05$), 80% versus 26% ($p < 0.01$), and 51% versus 15% ($p < 0.05$), respectively.[83] These results suggest that CIRT may be a more effective treatment option for single HCC than TACE."

We also added PBT information at Page 10, Lines 206-211. " In a randomized phase III-controlled trial comparing PBT to RFA in the treatment of recurrent HCC (size < 3 cm, number ≤ 2), PBT demonstrated non-inferiority to RFA in terms of 2-year local progression-free survival (LPFS) rates, with 94.8% for PBT and 83.9% for RFA.[82] The

intention-to-treat analysis also showed comparable LPFS rates between the two treatment arms. The progression-free survival (PFS) and OS rates were not significantly different between the two arms."

W3 also add additional PBT information at Page 10, Lines 213-221. "In a recent study, PBT was utilized as a primary treatment modality in HCC patients, who either could not undergo other localized therapies or opted against them.[83] Notably, the use of PBT led to encouraging outcomes, presenting a 5-year freedom from local progression (FFLP) rate of 94.5% and a 5-year overall survival (OS) rate of 76.7% in patients classified as BCLC 0/A/B, where the majority were stage A (92.3%). A comparable study evaluated the same treatment modality, PBT, in a cohort of treatment-naive HCC patients. They documented a 5-year FFLP and OS rates of 94% and 69%, respectively, in BCLC 0/A patients (n = 30).[84] The studies affirm the promising potential of PBT as a viable treatment option for HCC patients for BCLC 0/A."

Additional PBT study information, was added at Page 11, Lines 240-242. "In a study looking at treatment-naive HCC patients with PBT, patients categorized as BCLC B (n = 34) had 5-year FFLP and OS rates, 87% and 66% respectively that were slightly lower than those seen with BCLC 0/A.[84]"

Carbon iron radiation therapy and proton beam therapy, were also added in Table 2.

Carbon Ion Radiation Therapy (CIRT)[71,138]	A type of particle therapy that uses carbon ions to treat cancer.	External beam directed at the tumor.	Carbon ions are heavier and have a higher linear energy transfer (LET), leading to more precise targeting and potential for greater tumor kill.	Limited availability as it requires specialized equipment and can be more costly. More research is needed to fully understand the long-term effects and comparative effectiveness.
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