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Reviewer A

General Comment:
While reading the manuscript it seems that the authors strengthen the concept of hypofractionated radiotherapy for HCC, while "real" SBRT (3-6 fractions) techniques and data are less mentioned or ignored (see specific comments also). Consequently most of the literature for SBRT in 3-6 fractions is missing. This might be a good idea if the patients included into this study do not qualify for shorter concepts for example due to lesions size or other factors, or if the institutional policy is simply different, but this is not mentioned or explained anywhere in the text. Moreover, a comparison of the current results with the results of 3-6 fraction SBRT series seems important anyway.

Reply to general comment: Thank you for the review and comments. The most commonly used fractionation schemes in our cohort of ablative RT were 50 Gy in 5 fractions and 60 Gy in 15 fractions. The institutional policy is to use a more protracted hypofractionation regimen to achieve ablative doses whenever the tumor is large, central or close to organs at risk. A protracted hypofractionated course also makes it easier for adaptive planning whenever serial cone beam images show organs at risk in the high dose region. SBRT (3-6 fractions) was used in some of the patients when an ablative dose could be achieved without compromising the nearby organs at risk.

As an institutional radiation therapy guideline for treating liver tumors, doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 are usually used for smaller peripheral lesions, away from the gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree.

As advised, we will elaborate on this further in our materials and methods section and also add some of the data on SBRT (3-6 fractions) to the introduction and discussion.

In the discussion, we compared our results to the sequential phase I and II trials by Bujold et al that used SBRT with doses of 24 to 54 Gy in six fractions and to a study by Yang et al that compared SBRT to conventional RT for HCC. We also added the review by Gerum et al, that reports on the studies on SBRT (3-6 fractions) used to treat HCC.

Changes in the text: The following elaboration on the institutional radiation therapy policy for treating liver tumors was added to the materials and methods section, radiation therapy details paragraph: "As an institutional radiation therapy guideline for treating liver tumors, doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 are usually used for smaller peripheral lesions, away from the

gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree." (page 8, Lines 9-19)

As advised, the following was added to the introduction: "Stereotactic body radiation therapy (SBRT) using 3-6 fractions has been shown to be effective in several retrospective series and phase I/II clinical trials with Local control of 65-100% at 1 year." (see page 5 Lines 7-9) and the text has been modified to: "However, the main challenge is usually delivering high enough doses to ablate the tumor without causing serious adverse effects to the organs at risk (mainly the gastrointestinal tract and the normal liver), and most importantly, preserving liver function in cases of large tumors or those in close proximity to organs at risk . This is where a more protracted hypofractionated ablative radiation therapy course can be more safely delivered." (page 5, Lines 9-15).

In the discussion, as advised, we added the following: " Our most commonly used ablative doses were 50 Gy in 5 fractions or 60 Gy in 10 fractions. Ablative RT doses can be achieved with 3-6 fractions when doses approach around 54 Gy. In a review by Gerum et al that included several retrospective and prospective phase I/II trials, SBRT (3-6 fractions) achieved good local control, 65-100% at 1 year. However doses have to be reduced whenever safety is a concern mainly in tumors close to GI structures or the biliary tree which leads to decreased efficacy. The need for limiting toxicity and improving the therapeutic ratio remains essential for safe dose escalation. A more protracted hypofractionated ablative RT offers the advantage of increasing BED to the tumor while limiting toxicity to adjacent organs at risk." (Page 14, Lines 17-25 and Page 15, Lines 1-2)

Comment 1: The author mentioned in the introduction: "To date, the majority of published studies on the use of hypofractionated ablative doses for HCC, using 10 or more fractions, have used protons (8–11)." There are many studies using hypofractionated photon techniques as well but usually with less than 10 fractions ? Why do the author strengthen the use of more fractions by ignoring the data for SBRT with less fractions?

Reply 1: Thank you for the comment. As mentioned in the reply to the general comment above, we added the review by Gerum et al that reports on the different studies in the literature that use SBRT in 3-6 fractions to the introduction and to the discussion. We also emphasized that our most commonly used regimens for ablative RT were either 5 fractions (50 Gy) or 10 fractions (60 Gy). We also detailed in the materials and methods section the institutional guideline on the choice of fractionation for liver tumors. We use more fractions whenever an ablative dose cannot be safely administered in 3-6 fractions due to the size of the tumor or due to its location (central or close to organs at risk).

Changes in the text:
Materials and methods: we added the following: "As an institutional radiation therapy

guideline for treating liver tumors, doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 are usually used for smaller peripheral lesions, away from the gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree." (page 8, Lines 9-19).

We added the following to the Introduction: "Stereotactic body radiation therapy (SBRT) using 3-6 fractions has been shown to be effective in several retrospective series and phase I/II clinical trials with Local control of 65-100% at 1 year." (see page 5, Lines 7-9) and the text has been modified to: "However, the main challenge is usually delivering high enough doses to ablate the tumor without causing serious adverse effects to the organs at risk (mainly the gastrointestinal tract and the normal liver), and most importantly, preserving liver function in cases of large tumors or those in close proximity to organs at risk . This is where a more protracted hypofractionated ablative radiation therapy course can be more safely delivered." (page 5, Lines 9-15).

Discussion was modified as follows: " Our most commonly used ablative doses were 50 Gy in 5 fractions or 60 Gy in 10 fractions. Ablative RT doses can be achieved with 3-6 fractions when doses approach around 54 Gy. In a review by Gerum et al that included several retrospective and prospective phase I/II trials, SBRT (3-6 fractions) achieved good local control, 65-100% at 1 year. However doses have to be reduced whenever safety is a concern mainly in tumors close to GI structures or the biliary tree which leads to decreased efficacy. The need for limiting toxicity and improving the therapeutic ratio remains essential for safe dose escalation. A more protracted hypofractionated ablative RT offers the advantage of increasing BED to the tumor while limiting toxicity to adjacent organs at risk." (Page 14, Lines 17-25 and Page 15, Lines 1-2)

Comment 2: For BED calculations and comparison of doses used in different studies, it seems important to have more details how the dose was prescribed. Is 50 Gy in 5 fractions the maximum or isocenter dose ? Is it the dose to a surrounding isodose and if so, to which one ? Or was the maximum dose specified and a specific isodose (for example 80%) surrounded the PTV ? Please describe the dose prescription more detailed. Why was BED >= 80 defined as ablative ? Usually BED > 100 is defined as ablative. Please explain. Moreover, how was the PTV created ? Was an ITV based on 4D CT ?, was a CTV margin added, and if so so how much ?, How was the PTV margin ?

Reply 2: Prescription dose (50 Gy in 5 fractions for example) is the dose prescribed to the periphery of the PTV with the aim to have 80-90% of the GTV covered with the prescription dose (GTV: V100% of prescription dose =90% in an ideal scenario, this can go down to V100=80%) and 95% of the PTV covered by >=90% of the prescription dose (PTV D95% >=90% of the prescription dose).

GTV was contoured on the contrast-enhanced simulation scans (for DIBH cases:

contrast is injected, 1st breath hold is 30 seconds post start of injection, 2nd breath hold is 90 seconds post start of injection. For 4DCT cases, contrast is injected at the start of the scan).
Deep inspiration breath hold technique was preferably used for motion management. For those patients unable to comply with DIBH, 4D CT was used and an internal target volume (ITV) was created. Nearby organs at risk (OAR) were contoured and expanded by 3-5 mm for PRV.
PTV was created by expanding GTV/ITV by 5 mm subtracted by the OAR expansion. In cases with more than one dose level, CTV low dose is a 1 cm expansion on GTV/ITV and PTV low dose is CTV low dose +5 mm. CTV high dose is 5 mm expansion on GTV/ITV subtracted by OAR expansion and PTV high dose =CTV high dose.

BED >=80 was chosen as an ablative RT dose given that HCC is a radiosensitive tumor and extrapolated from the study by Tao et al (JCO 2016) on another liver tumor, intrahepatic cholangiocarcinoma (IHCC), where they specified a BED of 80.5 Gy as ablative.

Changes in the text: The following was added to the materials and methods section: First paragraph of radiation therapy details, we added: “BED >=80 was chosen as an ablative RT dose given that HCC is a radiosensitive tumor and extrapolated from the study by Tao et al (JCO 2016) on another liver tumor, intrahepatic cholangiocarcinoma (IHCC), where they specified a BED of 80.5 Gy as ablative (7).” (Page 7, Lines 9-12) Second paragraph of Radiation therapy details: we added: “GTV was contoured on the contrast-enhanced simulation scans. Deep inspiration breath hold technique was preferably used for motion management. For those patients unable to comply with DIBH, 4D CT was used and an internal target volume (ITV) was created. ” (Page 7, Lines 15-18) and “PTV was created by expanding GTV/ITV by 5 mm subtracted by the OAR expansion. In cases with more than one dose level, CTV low dose is a 1 cm expansion on GTV/ITV and PTV low dose is CTV low dose +5 mm. CTV high dose is 5 mm expansion on GTV/ITV subtracted by OAR expansion and PTV high dose =CTV high dose.” (Page 7, Lines 22-24 and Page 8, Lines 1-2)
“Prescription dose was the dose prescribed to the periphery of the PTV with the following aims: GTV: V100% of prescription dose =90% in an ideal scenario, this can go down to V100 (GTV)=80% and PTV D95% >=90% of the prescription dose.” has been added to the third paragraph of Radiation therapy details. (Page 8, Lines 4-7)

Comment 3: Usually MRI is the gold standard for imaging of HCC, why was CT used instead during follow-up ?

Reply 3: In this retrospective study, not all patients had MRI at baseline and at every follow up. Since multiphasic liver CT scans were performed at every follow up, they were used to assess response using RECIST criteria version 1.1.

Changes in the text: The sentence that patients had available multiphasic CT scans at follow up was added to the materials and methods, outcomes and toxicities paragraph (see page 9, lines 2-3).

Comment 4: RECIST is usually used for response evaluation not for determination of local control, what does it mean RECIST was used for local control ? How was local control exactly defined ?

Reply 4: Thank you for the comment. RECIST was used for response evaluation and the response was recorded as stable disease, partial response, complete response and disease progression. Local control was defined as no progression of disease. Lesions that showed partial response, complete response or stable disease were considered to be locally controlled.

Changes in the text:
As advised, the following was added to the materials and methods section, outcomes and toxicities paragraph: "RECIST version 1.1 was used to assess whether lesions showed partial response, complete response, stable disease, or disease progression. Lesions that showed no progression of disease were considered to be locally controlled" (page 9, Lines 6-9).

Comment 5: Why have patients with extrahepatic disease have been treated with local RT ? For palliative reasons ? If so, why were they not excluded from the comparison if present only in one group ?
Why have patients with CP C been included ? This is usually an exclusion criteria for local therapies. It would further be of interest to subdivide CP B patients as most data on liver SBRT indicated that patients with early CP B are candidates for SBRT (although at higher risk for toxicity than CP A), but patients with a score of >= 8 are poor candidates for SBRT and should be treated with extreme caution…

Reply 5: Patients with extrahepatic disease who were offered local RT were mainly to palliate or to central tumors that were threatening to cause biliary obstruction. We agree that the presence of more advanced liver disease and extrahepatic disease in only one of the groups (non-ablative RT group) are among the limitations of the study. This was stated in the limitations section of the discussion and it sheds light on the importance of patient selection for ablative RT.
CP C was one patient in the non-ablative RT group that had no other treatment options. Sample size was a limitation to subdivide CP B patients. We will add this important detail to the limitations of the paper in the discussion section.

Changes in the text: This was added to the first paragraph of the results: "One patient had CP C in the non-ablative RT group and had no other treatment option." (Page 10, Lines 12-15).
Discussion has been modified to state the following: "The main limitation is that the two groups treated with ablative vs. non-ablative RT were different in terms of more extrahepatic disease, more advanced liver disease, more TVT, and larger median tumor sizes in the non-ablative RT compared to the ablative RT group. Another limitation is our inability to further subdivide patients with CP B score due to their low number." (Page 15, Lines 20-25)

Comment 6: Information about the size of the lesions is necessary for any comparison with other RT techniques (for example 3-6 fraction SBRT) or with other ablative treatment options. One may include diameters of the lesions, or GTV volume or PTV volume, just to have an idea about the lesions character in the two groups…especially because they have been treated with hypofractionated RT in more fractions usually used for SBRT

Reply 6: Information on the size of the lesions can be found in table 1. As advised, we added this information to the text in the first paragraph of the results section. The size of the lesions in the non-ablative RT group ranged from 1.1 to 18.3 cm with a median of 8.1 cm compared to a median of 3.8 cm (2.8-4.65) in the ablative RT group.

Changes in the text: As advised, the following sentence was added to the first paragraph of the results section: "The median tumor size in the ablative RT group was 3.8 cm (2.8-4.65) compared to a median of 8.1 cm (1.1-18.3) in the non-ablative group." (Page 10, Lines 14-15)

Comment 7: Most of the recent literature regarding SBRT in 3-6 fractions is missing (reviewed for example in Gerum et al. World J Gastrointest Oncol 2019 May 15; 11(5): 367-376) in the discussion. Probably the patients included into this cohort had larger tumors, multifocality, or other risk factors preventing them from shorter and more ablative approaches, however this is not clearly stated anywhere in the manuscript and should be explained.

Reply 7: For the risk factors preventing patients from shorter ablative courses, we elaborated on the institutional choice of the fractionation regimen for HCC in the materials and methods section by adding that shorter ablative doses are usually delivered as doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 for smaller peripheral lesions, away from the gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, or whenever normal organs dose constraints can't be respected with shorter ablative doses, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree. We also added the reference to the review by Gerum et al to the introduction and discussion sections.

Changes in the text: As advised, the following was added to the materials and methods section: "doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 are usually used for smaller peripheral lesions, away from the gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree". (Page 8, Lines 9-19)
Introduction: "Stereotactic body radiation therapy (SBRT) using 3-6 fractions has been shown to be effective in several retrospective series and phase I/II clinical trials with Local control of 65-100% at 1 year." (see page 5 Lines 7-9)
Discussion: In a review by Gerum et al that included several retrospective and prospective phase I/II trials, SBRT (3-6 fractions) achieved good local control, 65-100% at 1 year. (Page 14, Lines 19-21)

Reviewer B

Comment 1: In MM section you must better describe the radiation procedure, the doses' constraints. You mention the criteria about what Ablative SBRT was done ... you muste better describe them ... what about the role and place of a tumour board ?

Reply 1: Thank you for the comment. More details on the ablative radiation procedure and the choice of the fractionation regimen were added to the materials and methods section, Radiation therapy details paragraph. We also elaborated more on the simulation and contouring.
GTV was contoured on the contrast-enhanced simulation scans (for DIBH cases: contrast is injected, 1st breath hold is 30 seconds post start of injection, 2nd breath hold is 90 seconds post start of injection. For 4DCT cases, contrast is injected at the start of the scan).
Deep inspiration breath hold technique was preferably used for motion management. For those patients unable to comply with DIBH, 4D CT was used and an internal target volume (ITV) was created. Nearby organs at risk (OAR) were contoured and expanded by 3-5 mm for PRV.
PTV was created by expanding GTV/ITV by 5 mm subtracted by the OAR expansion. In cases with more than one dose level, CTV low dose is a 1 cm expansion on GTV/ITV and PTV low dose is CTV low dose +5 mm. CTV high dose is 5 mm expansion on GTV/ITV subtracted by OAR expansion and PTV high dose =CTV high dose.
Prescription dose (50 Gy in 5 fractions for example) is the dose prescribed to the periphery of the PTV with the aim to have 80-90% of the GTV covered with the prescription dose (GTV: V100% of prescription dose =90% in an ideal scenario, this can go down to V100=80%) and 95% of the PTV covered by >=90% of the prescription dose (PTV D95% >=90% of the prescription dose). We also added the institutional policy on choosing the fractionation regimen.

A table with the dose constraints for 3, 5, 10, 15 and 25 fractions has also been added.

We also added that HCC cases selected for ablative RT were localized and unresectable. They were discussed at the institutional multidisciplinary tumor board prior to selection for ablative RT.

Changes in the text: As advised, the following was added to the materials and methods section, Radiation therapy details: “localized unresectable HCC” has been added to the sentence: In 2016 we started using ablative radiation doses with a stereotactic hypofractionated technique for the treatment of localized unresectable HCC. (Page7, Lines 2-3)

“HCC cases are discussed at a multidisciplinary tumor board prior to referral for ablative RT” (Page 7, Lines 4-5)

“GTV was contoured on the contrast-enhanced simulation scans. Deep inspiration breath hold technique was preferably used for motion management. For those patients unable to comply with DIBH, 4D CT was used and an internal target volume (ITV) was

created. ” (Page 7, Lines 15-18)
“PTV was created by expanding GTV/ITV by 5 mm subtracted by the OAR expansion. In cases with more than one dose level, CTV low dose is a 1 cm expansion on GTV/ITV and PTV low dose is CTV low dose +5 mm. CTV high dose is 5 mm expansion on GTV/ITV subtracted by OAR expansion and PTV high dose =CTV high dose.” (Page 7, Lines 22-24 and Page 8, Lines 1-2)

“Prescription dose was the dose prescribed to the periphery of the PTV with the following aims: GTV: V100% of prescription dose =90% in an ideal scenario, this can go down to V100 (GTV)=80% and PTV D95% >=90% of the prescription dose.” has been added to the third paragraph of Radiation therapy details. (Page 8, Lines 4-7)

“doses of 50-60 Gy in 5 fractions, 45-75 Gy in 3 fractions, or 60-70 Gy in 10 are usually used for smaller peripheral lesions, away from the gastrointestinal (GI) tract. For large (>5cm) or central (within 2 cm from the porta hepatis) tumors, two or three dose levels are used as follows: 37.5/67.5 Gy or 37.5/60 Gy in 15 fractions with an optional 90 Gy hotspot created by contracting the PTV67.5 Gy by 0.5-1 cm, if away from the biliary tree. If the liver or gastrointestinal (GI) dose constraints could not be met with the above fractionation, 45/75 Gy in 25 fractions is used with an optional 100 Gy hotspot created by contracting PTV75 Gy by 0.5-1 cm, also if far enough from the proximal biliary tree”. (Page 8, Lines 9-19)

Table 1 showing dose constraints for ablative liver RT using 3, 5, 10, 15 and 25 fractions has been added (please see the first page in the HCC tables word document).

Comment 2: In Results section, you must provide CT images with isodoses illustrating the 2 radiation treatments ... you must add a table summarizing the toxicities.

Reply 2: As advised, a figure showing isodose lines of ablative and non-ablative RT treatments and a table summarizing toxicities were added.

Changes in the text: Toxicities were summarized, as advised, in Table 7 (see the last Table of the Tables word document).
Figure 1 was added with the following caption: isodose lines distribution of radiation therapy (RT) treatment for HCC showing ablative RT course in (A) 37.5 Gy/ 60Gy in 15 fractions (PTV 37.5 Gy in blue and PTV 60 Gy highlighted in orange) and a non-ablative RT course in (B) 30 Gy/40Gy in 5 fractions (PTV 40 Gy highlighted in blue and PTV 30 Gy in green) (Page 21 , Lines 2-6)
The following sentence was added to the main text, results section: “Figure 1 illustrates isodose lines distribution of RT treatment for HCC showing ablative RT course in (A), 37.5 Gy/ 60Gy in 15 fractions, and a non-ablative RT course in (B), 30 Gy/40Gy in 5 fractions.” (Page 10, Line 25 and page 11, Lines 1-2)

Comment 3: In the Discussion section ... you describe in few words an ongoing clinical trial comparing photon vs proton ... what about the next step of the present work ... a phase II randomized study ? At least you must improve the number of your patients included in the ablative group in order at least to re inforce your statistical power ...you must explain on which criteria one could select patients to receive an ablative SBRT

Reply 3: Regarding the next step of our current work, our institution has partnered

with a proton center and there's a plan to open the mentioned ongoing randomized trial (NRG GI003) of photons versus protons for the treatment for HCC at our institution. The patients selected for ablative RT at the multidisciplinary tumor board should have unresectable or locally recurrent HCC, no extrahepatic disease (defined as extrahepatic metastases or malignant nodes > 3.0 cm, in sum of maximal diameters) CP score of A or B7, and 3 or fewer single or multinodular tumors.

Changes in the text: As advised, the last paragraph of the discussion has been modified as follows: "There is an ongoing clinical trial randomizing patients with unresectable or locally recurrent HCC for photon versus proton RT in 5 or 15 fractions (ClinicalTrials.gov Identifier: NCT03186898). Our institution has partnered with a proton center and there's a plan to open the mentioned ongoing randomized trial for the treatment for HCC at our institution. The patients selected for ablative RT at the multidisciplinary tumor board should have unresectable or locally recurrent HCC, no extrahepatic disease (defined as extrahepatic metastases or malignant nodes > 3.0 cm, in sum of maximal diameters) CP score of A or B7, and 3 or fewer single or multinodular tumors. This will provide more definitive results on the use of ablative photons or protons RT for HCC." (Page 16, Lines 10-18)