

DETAILED RESPONSE TO REVIEWERS

Title: Radiofrequency combined with immunomodulation for hepatocellular carcinoma: State of the art and innovations

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Dear Editors and Reviewers,

We would like to thank the reviewers for the constructive criticism regarding our manuscript entitled “**Radiofrequency combined with immunomodulation for hepatocellular carcinoma: State of the art and innovations**”.

A point-by-point response to the reviewers’ comments was included below. The alterations performed in the manuscript are highlighted in yellow. All authors have read and approved the revised version of this manuscript. The authors have no financial interest and no conflicts of interest to disclose.

We look forward to hearing from you concerning on the suitability of the revised manuscript for publication.

Yours sincerely,

Nagy Habib

Reviewer #1 (ID: 03699961)

1) General Comments

In this review article, the authors summarized the evidence that we have now in the combinatorial treatments of hepatocellular carcinoma using radiofrequency ablation and

immunotherapy. Although the enough information is included, some of them are not along with guidelines and well-known facts in clinic. In addition, it is better to show how each evidence is confident. The followings are concerns that the authors may wish to consider:

We appreciate the reviewers' comments. We have addressed the reviewers concerns in the sections below.

2) Specific comments

Major concerns:

1. The authors should indicate the evidence level of each study that is listed in the Table 1.

Answer: We have included the evidence level of each study in Table 1.

2. In the reference 30, a substantial number of cases were treated with not only RFA, but also cryoablation and/or TACE. The reference 30 is not a suitable manuscript in this review, in which the authors focus on RFA but on other locoregional treatments.

Answer: We have removed reference 30 from Table 1, but kept this study in the body of our manuscript in order to enrich the discussion.

Minor concerns:

1. American Association for the Study of Liver Diseases, The Japan Society of Hepatology, European Society for Medical Oncology, and European Association for the Study of Liver, all recommend the treatment using RFA for the patients with HCC of 3 cm or less in diameter and 3 nodules or less in number. HCCs over 3 cm should not be treated using RFA.

Answer: The indication of RFA is for patients with HCC with solitary tumor ≤ 5 cm or up to three nodules ≤ 3 cm. You can find this information here:

Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet 2018; 391:1301.

2. A reduction in HCV viral load after immunotherapy is too primitive to mention and out of focus in this review article. It is well known that the eradication of HCV does

not significantly alter the recurrent rate at the first time after treatment of initial HCCs. The recurrence rate is getting lower at the second and later recurrences.

Answer: We have removed the data regarding HCV viral load from Table 1. Thank you for pointing.

3. In terms of aflatoxins and aristolochic acid, it would be helpful if correspondent references are provided.

Answer: The correspondent reference for this information is ref #5, which is placed right at the end of the sentence regarding aflatoxins and aristolochic acid, which is at the end of the first paragraph of Introduction section.

Reviewer #2 (ID: 00054672)

1) General Comments

In this review article Carneiro da Costa et al. summarized the published data regarding the combination of radiofrequency ablation (RFA) and immunotherapy for hepatocellular carcinoma. This is an important subject as the results and the outcomes of HCC treatments are far from optimal. RFA is a recommended method of treatment for small HCC (>3 cm) however it has been burdened with the progression/recurrence of the disease, therefore addition of immunotherapy is a promising attempt to improve the outcomes of HCC treatment as presented in this review based on the eight selected articles. This is a well-written review, and I would recommend it publication.

We appreciate the reviewers' comments. We have addressed the reviewers concerns in the sections below.

2) Specific comments

1. Data on HCV viral load are unnecessary and out of the scope of this manuscript;

Answer: We have removed the data regarding HCV viral load from Table 1. Thank you for pointing.

2. Minor typing mistakes throughout the text should be corrected;

Answer: We have revised the text and corrected all typing mistakes.

3. Throughout the text there are several references missing when referring to the specific data and should be added accordingly;

Answer: We have included all correspondent references along the manuscript text.

4. References are not prepared according to the journal requirements.

Answer: We have adjusted references according to the journal requirements.

Reviewer #3 (ID: 00006459)

1) General Comments

The topic of this review, improving treatment of hepatocellular carcinoma (HCC), is very important due to the low survival from HCC. It is logical that the new immunotherapies and indeed some other therapies will synergise with Radiofrequency Ablation (RFA), so it is important to evaluate such combination approaches, as is done here. This is a good review. It needs some improvements to structure, clarity and English expression.

We appreciate the reviewers' comments. We have addressed the reviewers concerns in the sections below.

2) Specific comments

1. Most important is to largely confine the quoted data to HCC. The papers on colorectal cancer [CRC], refs 11 and 15, must be kept separate and made clear that they are on CRC.

Answer: We have removed all references on CRC from our manuscript.

2. CAR-T and vaccines are mentioned, but need more detail to be understood.

Answer: We have provided more details regarding CAR-T therapy and vaccines at the end of the section "IMMUNOTHERAPY AND HCC".

3. This review would be improved by adding sections commenting upon other methods that may or might synergise with RFA. I suggest mention of lytic virus anti-tumour therapy approaches. I suggest mention of potential future directions. For example, theranostics, such as with isotopes on FAP inhibitors that target tumour stroma [J Calais - Journal of Nuclear Medicine, 2020; Lindner, T., et al. (2019). "Targeting of activated fibroblasts for imaging and therapy." EJNMMI Radiopharmacy and Chemistry 4(1):16.]

Answer: We agree that mentioning all therapeutic modalities that synergize with RFA must be studied and intensely discussed. However, our objective in this review was purely to describe the results presented in the literature regarding the

association of immunomodulators and RFA, which is a very incipient subject, with an extremely limited number of studies around. If we start discussing other interventions, such as lytic virus antitumor therapy and others, we might distance ourselves from our objectives. We are thankful for the literature recommendations that you suggested and we agree that these topics really need high-level discussions; however, we think that this is out of the scope of our current manuscript. If you do not agree with us, we can review this question and find the best way. Thank you very much for your suggestions.

4. An image relevant to RFA would improve this review.

Answer: We have included an Image (Figure 1) illustrating the use of RFA for HCC.

5. For a broad GE audience, I suggest an explanation of when you would choose to use RFA versus TIPS. This would explain the place of RFA into context with HCC therapy as a whole.

Answer: Actually, these interventions (RFA and TIPS) are indicated for different purposes. RFA is an interventional radiology modality of treatment for HCC, providing ablation of the tumor and, thus, has curative purpose. On the other hand, TIPS is a procedure indicated only to contain the complications related to portal hypertension. We did not include this information in the manuscript text due to not being objective of our analysis. We would rather clarify this information here and not include it in the manuscript. However, if needed, we can do it.

6. The abstract needs improved clarity and English. Also, The third page is the page most in need of improved English. Eg ‘a way of antigen to the induction’ and nearby sentences. Also, mid-page is a sentence that includes the word, ‘evidence’, but needs a reference cited.

Answer: We have revised the whole manuscript, correcting typing and grammatical mistakes, trying to make it clearer. We have also included the correspondent reference for the extract that you pointed.

7. Page 6 and Table 1: ‘number of white cells...” needs explanation of whether these WBC were intratumoral or in blood or other location.

Answer: The increase in WBC that is exhibited in Table 1 is related to its blood concentration. We have added this information in the manuscript by saying that there was an increase in “circulating white blood cells”.

8. Page 8: near mid- page is a sentence ending, “carcinoma in mice.” Please specify which reference (s) is relevant here.

Answer: We have removed this paragraph from the manuscript body.