Author’s response to reviews

Title: Retrospective analysis of the immunogenic effects of intra-arterial locoregional therapies in hepatocellular carcinoma: A rationale for combining selective internal radiation therapy (SIRT and immunotherapy

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Author’s response to reviews:
Corrections for the manuscript BCAN-D-19-00648:
Specific answers to reviewers’ comments:
In the new version, the modified sections are indicated in red.

Reviewer 1

1. In Fig 2, the authors should provide typical pictures showing the distribution and expression of CD3+, CD4+, CD8+, and Granzyme B:
   Answer:
   Figure 1 shows CD4+ and CD8+ T cells in representative cases: Fig 1a in untreated patient, Fig 1b in preoperative SIRT-treated patient, and Fig 1c in a preoperative TACE-treated patient. We have added additional panels (Fig 1d, e, and f) to figure 1 showing Granzyme B expression in representative untreated, preoperative SIRT-treated, and preoperative-TACE patients. The legend for the figure was adapted accordingly.

2. Despite the increased infiltration of immune cells in SIRT group, why is tumor response between two groups not different (Table 2), or even higher in TACE group?
   Answer:
   Indeed, the pathological response, as evaluated on the basis of necrosis in the tumor, is significantly higher in the TACE group than in the SIRT group. Several reasons may explain this observation. First, among intra-arterial therapies, the embolic effect is much higher in TACE than in SIRT due to the larger size of the particles injected. Second, the mechanisms leading to cell death are different: TACE promotes ischemic cell death (as indicated by large amounts of cellular necrosis) whereas SIRT leads to radiation-induced cell death. The quantification of the pathological response on HE samples may preferentially reveal the first mechanism. Furthermore, it is possible that these different ways to induce cell death have different kinetics and that pathological samples taken at 15 weeks after treatment are too early to demonstrate radiation-induced cellular necrosis. In that regard, it has been shown that maximal response after SIRT is obtained after a prolonged period, even though the half-time of 90Yttrium is very short (Vouche, et al. Hepatology 2014; 60:192, Salem, et al. Gastroenterology 2012; 138:52).

   Of note, even if TACE produces significant tumor necrosis, it has been clearly shown that preoperative TACE before surgical resection of HCC does not improve long-term postoperative outcomes (references 5-7, 40-42).

   We have added additional text to the discussion (page 14, line 301):
   This discrepancy between cellular necrosis and immune infiltrates in patients treated with TACE or SIRT could be related to several factors. First, the mechanisms leading to cell death are different in these two approaches. TACE uses larger microspheres (embolized at the arteriolar level) and produces ischemia-induced cell death, whereas SIRT uses smaller radioactive microspheres (embolized at the capillary level), and produces radiation-induced immunogenic cell death. Second, the kinetics of these two treatments may be different and it is possible that 15 weeks between intra-arterial treatment and surgery is not long enough for optimal visualization of significant tumor necrosis after SIRT [44, 45].

3. The reason for the increase of immune cell infiltration in SIRT group should be discussed:
   Answer:
   A sentence has been added to the discussion (page 13, line 283):
   The mechanisms leading to immune activation after SIRT remain hypothetical. In fact, several
observations have suggested that some of the clinical effects of radiation therapy could be related to stimulation of the antitumor immune response, including in the rare cases where a response is induced at distant sites after radiotherapy (the so-called abscopal effect) [39-41]. More specifically, it has been shown that tumor irradiation may increase the release of tumor antigens and the diversity and activity of TILs [42], suggesting potential synergistic activity with immunotherapy, such as immune checkpoint inhibitors [32].

Reviewer 2
1. Needs some language corrections before being published:
   Answer:
   The manuscript has been reviewed by a professional English-speaking reviewer (see certificate). The changes that have been made are indicated in red in the revised manuscript.

Reviewer 3
1. I think that the results of the study are overstated in the discussion and mainly in the conclusion. This is a basic research only about what can happen in HCC nodules after preoperative treatment. However, there is no correlation with any immunotherapy and the possible effect of SIRT and TACE if far to be understood:
   Answer:
   We fully agree that the potential effects of SIRT and TACE on antitumor immune responses are yet to be understood. The results that we reported essentially suggest that SIRT, producing intratumor immune cell recruitment/activation, could be a better candidate to be combined with immunotherapy than TACE. In fact, these results are concordant with those reported by Chew et al. (reference 38). Furthermore, this approach, of combining SIRT and checkpoint inhibitors, is currently being investigated in a prospective trial (reference 32). Of course, a key limitation for the interpretation of our results is the limited number of patients evaluated and the retrospective nature of the study.
   A sentence has been added at the beginning of the conclusions section (page 15, line 323): These results should be interpreted cautiously due to the limited number of patients and the retrospective nature of the study. However, …

2. The language needs to be reviewed:
   Answer:
   The manuscript has been reviewed by a professional English-speaking reviewer (see certificate).

Additional corrections
We have proposed a new format for Figure 1.
Some references have been added and the reference numbers adapted accordingly.