Answer to the reviewers

Answer to the Chinese reviewer

Thanks for the positive assessment of the work. I proceeded, as requested, in changing with numeric characters the number of patients presented in the first section of the abstract Materials and Methods: "*Drug-eluting bead transarterial chemoembolization (DEB-TACE) using a new generation of microspheres (Embozene TANDEM, 40 µm) preloaded with 100 mg of doxorubicin was performed on 48 early or intermediate hepatocellular carcinoma (HCC) patients with compensated cirrhosis*".

General comments for the reviewers (French and Japanese)

Thanks for the interesting comments that you provided on the paper. They helped to improve and emphasize the true purpose of the study.

I proceeded to change some small parts of the work and the abstract upon review of your observations. Your feedback brought me to the realization that the purpose of the study was not adequately showed.

After re-reading the text in the light of your comments, I noticed that it often referred to transplant patients. In fact, I can understand why most of your remarks had "transplant" as the main theme.

We are a group of interventional radiologists working in a multidisciplinary team focused on the care and treatment of HCC as well as being liver transplant center. Our hospital is a reference oncology center at the national level.

As interventional radiologists we have long experience in endovascular and percutaneous treatments for the loco regional therapy of HCC. We also carry out a large number of c-TACE, and DEB-TACE and as such have a lot of experience using particles with different diameters

(300-500, 100-300, 70-150 microns), assessing risks and benefits of each one. We also worked for years with the TARE, using 30 microns Yttrium-90 particles, gathering some experience and sensitivity in the use of small diameters. We understand inherent risks related to them and to the shunts between hepatic, gastrointestinal, splenic and pulmonary circulation. Therefore we think that undertaking a safety assessment of this new generation particles in DEB-TACE is an obvious next step. We would then like to publish our initial experience on usage in terms of safety, radiological and histological evaluation of effectiveness.

Without doubt, there is some opposition to this coming from western countries regarding the usage of smaller particles (less than 100 microns) in DEB-TACE for HCC. Many experts in this field of the treatment suggest that the particles smaller than 100 microns have an unfavorable risk-benefit ratio, if compared to bigger particles, due to the risk of migration into the microcirculation which is the primary cause of intra and extra hepatic non-target embolization.

Meanwhile, they don't believe there is a clear demonstration of superiority of efficacy of these microspheres compared to bigger microspheres which are handled with more ease. In fact, the theoretical advantage of the distal tumor penetration with reduced post-ischemic angiogenesis remains to be proven. Possibly a study with two arms with patients undergoing DEB-TACE using particles above 100 micron and 40 micron particles with histological comparison of nodules treated could be developed in the future. This would help in proving the theoretical better distribution of particles in the tumor and its pathological vessels.

In any case, a first series devoid of complications in the use of these new microspheres is of interest to the scientific community. There is a possibility to become the starting point to assess in the future the potential benefits in terms of effectiveness, feasibility and safety of the procedure on a larger sample.

The histologic data of patients undergoing transplantation reported in the manuscript looked very interesting in the efficacy assessment. It will be useful to quantify the degree of necrosis

induced by the microspheres, and also to verify their theoretical greater penetration in the distal vascular network. Your comments on the liver transplant, the number of patients inside the Milan criteria, the duration of the waiting list and the indications for transplantation were all appropriate. As a result of your input, you have allowed me to focus the research, the study design and its purpose.

Limitations of our work are the choice of having only a single arm, the small sample size and retrospective analysis of data. However, in our opinion, the data related to safe use of the particles, considering we didn't have complications related with their use, could be really interesting.

Individual Answer to the French Reviewer

Thanks for the general opinion expressed and the comments showed about the work.

With regards to "Radiological response in non-transplanted patients" you're right in your comments. The paragraph refers to all 48 patients of the series. It was a mistake derived from an earlier version of the paper in which the results were in only one paragraph, after I added the paragraph referring to the histological response of the transplanted patients. Therefore I made the change suggested. Now in Results you can see the paragraph named "*DEB-TACE and Radiological Tumor Response*" intended for all the 48 patients.

The responses according to RECIST and mRECIST have been reported for all treated nodules of the entire population, organized according to the measure of the nodules in the respective Tables 3 and 2.

Regarding the patients initially included in the Milan criteria, drop-outs and possible contraindications to transplant, as explained in the *general response to the reviewers*, was a data not reported because of the purpose of the study was to evaluate the safety of the particles so we didn't focused on patients in the transplant list.

Individual Answers to the Japanese Reviewer

Thanks for the general opinion expressed and the comments showed about the work.

As explained in the *general response to the reviewers*, thanks to your comments, I made changes in the text in order to clarify the purpose of the study; evaluating the safety and efficacy of the use of smaller particles for DEB-TACE in treating HCC patients at intermediate early stage. I hope now the aim will be better understood.

In our series there were 48 HCC patients in early and intermediate stage, and according to BCLC, not eligible to alternative therapies (RFA, MWA) or surgery because of stage of disease, lesion site, comorbidity, age and/or anesthetic risks. Some of them were on the transplant waiting list and received TACE as a bridging treatment, others came out for disease progression. This data has not been developed in detail because the goal was not an assessment of TACE as bridging or down staging treatment. I hope I have clarified, with the revision and explanations provided, the study design.

As I mentioned before, and in order to clarify better, we use selection criteria according to BCLC in order to choose patients in early and intermediate stage, not eligible for alternative therapies or surgery due to different complications previously reported.

You are right when you assert that on DEBTACE there are a lot of works in literature but nothing about the safety and any possible advantage reported by the use of 40 microns drug eluting beads.

The waiting list in our center stands at 4.8 months. The Milan Criteria was used to assess any decision to transplant.

As expressed before, we understand the weaknesses due to its retrospectiveness, its single arm approach and its small sample but it represents a first experience on the safety profile in

the use of this new generation particle, actually perceived with skepticism for the scientific community in the treatment of HCC.

The primary objective of the study, therefore, remains the evaluation of the safety of these new particles, and their effectiveness in terms of locally induced necrosis evaluated by imaging (CT / MRI) in conjunction with the histological diagnosis in cases of transplant patients.