UNIVERSITA' DEGLI STUDI DI ROMA TOR VERGATA
FACOLTA' DI MEDICINA E CHIRURGIA
Dipartimento di Medicina Sperimentale e Chirurgia
U.O.C. Chirurgia Epatobiliare e Trapianti
Direttore: Prof. Giuseppe Tisone

Rome, 15th June 2019

Dear Editor,

We hereby submit the revised original manuscript entitled **"*De novo* malignancies after liver transplantation: the effect of immunosuppression. Personal data and review of literature. " Manuscript NO:** 48771

We thank the Editor and the Reviewers for the comments and for the opportunity to revise the article with the intent to strengthen its content.

A detailed point by point answer to all criticism has been provided.

Reviewer's code 00054465: This is an excellent review of the current literature regarding De-Nova malignancies occurring after liver transplantation in adults and children. The literature review is comprehensive and discussed appropriately. The authors include their own experience at the Tor Vergata Institute for weaning patients off immunosuppression drugs. The conclusion that an Immunosuppression Free State is a goal to go for makes sense with the medical knowledge at this time.

Authors: Thank you for your kind comments.

Reviewer's code 00050232: The subject of the article is the one with the greatest problems concerning liver transplantation. The review was performed using good and extensive bibliographic references. The Discussion Section is objective as well as the suggested perspectives. For these reasons the manuscript deserves publication.

Authors: Thank you for your kind comments.

Reviewer's code 02908399: 1 Title. Adequate 2 Abstract. Adequate 3 Key words. Adequate 4 Background. Adequate 5 Methods. Adequate 6 Results. The study increases our understanding of DNMs after liver transplant. There is promising evidence that patients can be suppression free at some stage in the post-transplant period. It would be worthwhile to know if any particular blood levels of immunosuppression like TACROLIMUS and CYCLOSPORIN were linked with DNMs. It would also be interesting to know if immunosuppression increased the risk of HCC recurrence in patients within or outside Milan criteria. There are no tables in the manuscript. Perhaps the frequencies of DNMs in adult and pediatric patients can be represented in tables or graphs. 7 Discussion. Adequate

Authors: Thanks for your revision. These are very interesting point that we tried to address the best way possible in our manuscript. Unfortunately most of the available studies did not sufficiently described Tacrolimus and CNIs blood levels, so it was not possible to assess any solid conclusion about this. However, further information with new bibliography about the aforementioned requests concerning HCC recurrence were cited besides the one already included. Tables about the incidence of DNMs are included in the manuscript.

Reviewer's code 03021264: De novo malignancies are serious complication that impairs the survival of transplant recipients. This review article examined about the DNMs and immunosuppression weaning both in adult and pediatric OLT recipients through literatures and clinical trials, revealed the incidence, main types, possible inducements and main treatment methods of DNMs after liver transplantation. It has a very positive reference value for clinical work. The paper cites a large number of effective references, rich in content, reasonable methods for processing data, and draws conclusions with high reliability. However, due to the relatively low incidence of DNM, the observation period is long, and it is difficult to provide large samples and prospective comparative studies in clinical practice. Therefore, observations of the effects of immunosuppression withdrawal on DNMs were only reported exclusively, and the data does not provide statistical comparison. Further improvement of related studies is required. In addition, liver-localized PTLD as a special type may be originating from the donor, the treatment effect is very different. The donor/host origin of PTLD may have prognostic significance because donor origin has different clinical and pathologic features compared with the case of host origin (as related report [Am J Surg Pathol,2000,24(5):733-41.]), authors may consider adding relevant content.

Authors: Thank you for your kind comments. Your suggestion were amended in the manuscript.

Reviewer's code 01221666: This systemic review aims to summary data regarding de novo malignancies after liver transplantation. Though the topics is not novel, it is still worth reporting in the era of growing field of transplant oncology. Comments The wordings throughout the manuscript needs a great revision. Specific words are suggested. For example, Methods in Abstract In addition, the review assesses the differences in adult and pediatric recipients by describing the adopted immunosuppression regimens, the different type of diagnosed solid and blood "tumors" as well as "the clinically tolerant cases". 1. blood malignancy is a better term for blood tumor 2. the clinically tolerant cases are not clear here. Page 8 3.2.1 PTLD Most PTLDs are due to EBV. Even if a clear cut-off range of EBV-DNA levels has not been well recognized, virus detection may be sufficient to reveal early "lymphoma". Do your "lymphoma" mean all

lymphoma or just "PTLD"? Line 9-10 The survival rate was significantly better in patients "treated" with Tacrolimus compared to CsA (81.2% against 50% after 5 years from the PTLD diagnosis). It can be misleading that tacrolimus and CsA are drugs that "treat" PTLD. Page 14 Line 8-10 In fact, physicians, in order to treat or avoid the rejection set the blood levels to the up threefold; this can lead to an impairment of the immune system and "expose recipients to DNMs". Do you mean expose recipients to the risk of getting DNM? Figure 2 is missing.

Authors: Thank you for your kind revision. Your suggestions were very useful and were addressed in the manuscript. We addressed the concern regarding figure 2.

Reviewer's code 02734287: This is a good review on the association of immunosuppression and malignancies. The part discussing the effects of weaning of immunosuppression on malignancies is especially valuable since there is not much data published dealing with this phenomenon.

Authors: Thank you for your comments.

Reviewer's code 03372021: With the wide application of orthotopic liver transplantation in patients with end-stage liver disease, the number of long-term survival recipients is increasing, and the de novo malignancies (DNMs) are gradually receiving widespread attention. This article reviewed the incidence and the characteristics of DNMs in the adult and pediatric populations of orthotopic liver transplants (OLTs). Then the authors evaluated the role of immunosuppression minimization and withdrawal in liver transplant patients. However, there is currently insufficient evidence to confirm that immunosuppression withdrawal can reduce the incidence of DNMs. There have been some similar studies on DNMs after liver transplantation. This review provides limited new ideas for this field. In addition, some major issues need to be clarified. 1. In the ABSTRACT, the authors argued that 'the reconstitution of the immunological pathway could decrease the incidence of DNMs and may also help in treating liver transplanted patients suffering from cancers', this conclusion lacks sufficient research evidence and needs to be confirmed by further high-quality researches. 2. The structure of the article is unreasonable, and there are a few mistakes, for example, there is no 3.1 in part 3. The reviewer suggest it be divided into six major parts: introduction, literature research, de novo malignancies in the OLT population, the role of IS minimization and withdrawal in liver transplant patients, discussion, and conclusion and future prospects. 3. 'Several studies have demonstrated that the tolerogenic potential of the liver allows IS discontinuation and a permanent IS-free state (IFS)…' What is the tolerogenic potential of the liver? The authors should explain this sentence in detail and annotate relevant references. 4. The authors should describe Figure 1 in detail. How many

studies and patients were included in this article, and how many of them were about adult or pediatric OLT recipients. What were the age and sex distribution of these patients? 5. Abbreviations in the text should be noted where they first appeared, such as ALD, HNC, and LFTs. 6. In Page 6, the '2.4 Aim' should be introduced in the INTRODUCTION part, and this article is a review, the 'primary end point' and the 'secondary end points' are mainly used for clinical trials. 7. In Page 7, 'OLT recipients experience the highest onset rate of lymphomas (57%)', 'Overall, skin cancers are the most diagnosed DNMs', these two sentences contradict each other. 8. In Page 8, 'Over the years, albeit the mortality remains high (up to 85% and 69% respectively after one and five years) PTLDs are decreasing'. The mortality after one year was higher than that after five years? What is the reason for the decrease of PTLDs? 9. In Page 10, 'Despite being identified at earlier stages amongst OLT patients, the prognosis of colorectal metastasis is still worse than the general population', why the prognosis of OLT patients with colon cancer is reduced. 10. In Page 12, 'Data from…children with post-transplant non-PTLDs DNMs are older than recipients developing PTLDs malignancies (7.9 vs. 13.2 years of age, $P < 0.0001$)', the age was 13.2 vs. 7.9? 11. In Page 13, mTORi, CNIs, antilymphocyte medications and corticosteroids play different roles in the development of DNMs, and mTORi might play a slight protective role, so why should the patients stop all drugs, rather than selectively stop carcinogenic drugs? 12. In Page 13, 'IS drugs activate different pathways in the immune system and need to be carefully selected.' It would be better for the authors to list the pathways that are activated by IS and explain their relationship to DNMs. 13. Figure 2 is poorly organized, the resolution was low, and it was easily confused. It may be better to use a percentage for each malignancy, not the number of cases, and each malignancy should be arranged in the same order, such as the US, Sweden, and Israel. In addition, the incidences of DNMs vary greatly among different registries. What are the possible reasons? 14. Table 2 listed the incidence of malignancies after pediatric Solid Organ Transplantation, but this study mainly focused on the malignancies after liver transplantation, so I think table 2 is useless and meaningless for the conclusion of this article.

Authors: Thank you for your comments. We addressed your concerns and corrected the manuscript according to your kind suggestions whenever possible. Unfortunately in figure 1 we agree with your suggestion but in some studies the details about sex were not clearly stated so

we preferred not to describe them. We produced figure 2 with a more clear layout. The studies regrouped in Table 2 summarize the incidence of malignancies after all solid organ transplantation in children since the available database also include them with no clear distinction. Therefore, we believe it could still be helpful to the present manuscript since it includes data deriving from pediatric liver transplantations.

Reviewer's code 00052926: The authors conducted an interesting review and reported their personal observations about the De novo malignancies development after liver transplantation. The manuscript is written concisely and the data are convincing. I have the following minor issues with the manuscript: 1. mTOR inhibitors might play a slight protective role reducing the incidence of DNMs compared to calcineurin inhibitors in patients with OLT. However, many patients take mycophenolate mofetil in combination with CNI. What is the role of mycophenolate in DNM development? Are they any data? Please comment. 2. Alcohol abuse, smoking and PSC diagnosis correlates with increased risk of developing DNMs. How the authors explain the high probability of developing DNMs in these 3 groups? 3. PTLD are the second more prevalent DNMs after skin carcinoma in adults with OLT. What types of PTLD do the adults develop? What is the time mediating from OLT to the development of PTLD? 4. Skin cancer is the most prevalent DNM in adults with OLT. What is the prevalence of skin cancer? What is the time mediating from OLT to the diagnosis of skin cancer? 5. Please do not use acronyms like "IS" in the title of chapters. 6. Please make correct use of commas throughout the manuscript.

Authors: Thank you for your review. We amended your comments and included further information as requested when it was possible to describe them with solid data.

Sincerely Yours

Tommaso M. MANZIA, MD PhD
Corresponding author