

October 22, 2019

Dear Dr. Vassilios Papalois,

Editor-in-Chief of *World Journal of Transplantation*,

Thank you for the opportunity to review our manuscript. We present to you this revised version of "Therapeutics Administered During Ex Vivo Liver Machine Perfusion: An Overview" (Manuscript No: 51316) for the special issue on machine perfusion. We are grateful for your initial invitation and continued consideration.

We thank the three reviewers who critically reviewed our manuscript and provided insightful, constructive comments to enhance our work. Careful consideration was made regarding the manuscript revision process, and we are excited to present this revised version of our manuscript.

Thank you,

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**Therapeutics Administered During Ex Vivo Liver Machine Perfusion: An Overview**  
**Response to the Reviewers**

**Reviewer #1 (02492984) Comments:**

**This is the interesting review regarding organ protection during ex vivo liver perfusion before transplantation. They raised three categories of therapeutics including RNAi pathway, defatting cocktails and vasodilators. They summarized currently available literature and this is very good information for readers. Although almost every studies they discuss is preclinical, they are promising therapeutic options for this types of specific conditions. One more limitation would be that ex vivo liver perfusion itself is not standard therapy in several countries at the moment.**

**Response:**

We agree with this comment and have further clarified this limitation in the section entitled "Current Ex Vivo Machine Perfusion Clinical Trials." While this review was focused more on the recent basic science advances made in the area of therapeutics administered during ex vivo liver machine perfusion, we did feel it was important to highlight some of the most current clinical trials utilizing this method of machine perfusion. We therefore clarified this perspective and mentioned that these studies have only been performed in a select number of countries to date due to it being a new method of organ preservation.

We thank Reviewer #1 for the insightful comments and believe these improvements have greatly enhanced our revised manuscript.

**Reviewer #2 (02446061) Comments:**

**The manuscript content is interesting. Please edit to be clearer about the two trends to therapeutics in EV-LMP (RNA and vasomodilators). Other therapies are not mentioned! This is important as your work included the trends from basic research.**

**Response:**

We agree that it would be helpful to have a side-by-side comparison of the mentioned therapeutics and have therefore created Table 3 which proposes advantages and limitations/future considerations of each therapy. We hope that this additional table further clarifies the new trends in administered therapeutics during ex vivo machine perfusion. In addition, this review focused on the basic science research surrounding therapeutics specifically added during ex vivo liver machine perfusion to reduce ischemia-reperfusion injury. While there are other studies examining additional endpoints of ex vivo machine perfusion in liver transplantation than we have covered, we felt as though including therapeutics specifically concerning the context of our review from seven different categories addressed the current research in this field of liver preservation as best as possible.

Thank you to Reviewer #2 for constructive comments regarding the revision of our manuscript, and we believe the suggestions improved our revision.

**Reviewer #3 (00502954) Comments:**

**This is a very good review that has covered most of treatment potential for liver injury. It will surely help reader to understand prevention and treatment strategies to preservation injury in liver transplantation. It will be helpful for reader to comprehensively understand this field if authors can provide one illustration for the crosstalk (cons and pros) between the proposed therapeutic treatments and the methods of organ presentation. Authors should discuss more about targeting few candidate molecules that play important roles in prevention of liver injury. In addition, it will be important to discuss if low temperature would reduce effectiveness of siRNA or other drugs treatment considering enzyme function is temperature dependent.**

**Response:**

We believe the suggestion of including a pro and con illustration covering the therapeutics was a wonderful idea to further clarify our review and have therefore created a third table. In this Table 3, we have summarized the advantages and limitations/future considerations of each of the seven mentioned therapeutics while also including additional references to help enhance this table. While many of the mentioned therapeutics are new in the field of liver machine perfusion, we have also included future considerations in the limitations column to communicate some of the current research that is being conducted to enhance our knowledge of the role of specific therapeutic agents and their inclusion in liver machine perfusion.

We also agree with the comment about further clarifying and discussing targeting the candidate molecules implicated in prevention of liver injury. We have included an

additional paragraph in the introduction which highlights some of the key mediating pathways involving in liver ischemia-reperfusion injury while also mentioning that identification of a single, specific pathway implicated in hepatic ischemia-reperfusion injury may not be a realistic approach to mitigate damage due to the multiple, perhaps redundant, pathways involved in ischemia-reperfusion injury. In addition, we do mention the specific, yet diverse, targets of the therapeutics that various groups have explored, and this information is included after each discussed study.

In addition, we have further clarified the point about temperature conditions on siRNA effectiveness by mentioning that although preliminary studies demonstrate the uptake of siRNA in both hypothermic and normothermic conditions, further studies will address the effect of temperature on siRNA efficacy in target silencing.

Thank you to Reviewer #3 for the insightful comments and suggestions for improvement regarding the above points. We believe these comments greatly enhanced our manuscript revision.