Peer Review File

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Reviewer A

In this manuscript Gouchoe et al. dissect the role of CD38 in ischemia reperfusion injury in the context of cardiopulmonary bypass and thoracic transplantation. Hereby the authors highlight the mechanisms of CD38 signaling involved in NAD+ homeostasis and inflammatory signaling.
This study is of interest to both the thoracic surgery as well as transplant community and appeals clinically relevant providing an important overview of CD38 regulated pathways promoting IRI. There is a gap of evidence of CD38 signaling in transplantation which the authors target to address.

Overall, the manuscript is well written and the transition from a clinical perspective to molecular studies was achieved elegantly. The scientific work that has been performed on CD38 signaling in organ transplantation is scarce but summarized appropriately by the authors. These paragraphs may benefit from some outlooks on the impact of CD38 on allo-immune responses.

Comment 1: Since there are oral CD38 inhibitors available, it would be interesting to specify the reasons impeding clinical transition in more detail. What are the main reasons / risk factors that occurred in the experimental studies that have impeded the initiation of clinical in-human studies? Are there – by now – any efforts made to try those drugs in the clinics?

Reply 1: The majority of oral inhibitors of CD38 are monoclonal antibodies, while there is one recently developed oral CD38 inhibitor (MK-0159) that has recently been purchased by Astellas (https://www.nmn.com/news/astella-drug-may-reduce-damage-caused-by-stroke) for the purpose of reducing heart IRI. We believe that CD38 inhibition in hearts is not particularly novel, however has not gained mainstream attention in the scientific community and therefore MK-0159 will be one of the first attempts at large to possible demonstrate clinical efficacy. In terms of transplantation, our lab is actively studying the role of oral CD38 inhibitors in transplantation (both heart and lung) – though our data is only preliminary and not ready for publication. Overall, we cannot point to specific reasons why more attempts have not been made with the FDA approved inhibitors to reduce cardiac/transplant related IRI, and we hope this review will improve attention to the subject.

Changes in the text: NA

Comment 2: Since IRI constitutes a crucial event instigating the allo-immune response towards allogeneic allografts, the transplant paragraph should include some mechanisms on how targeting CD38 signaling may alter the allo-immune response. Altering NAD homeostasis through targeting CD38, for instance, may also impact the allo-immune response. Elkhal et al. demonstrated that increased NAD+ levels promote CD4+ T cell derived IL-10 production leading to improved allograft survival (PMID: 26928119). There is a bunch of literature (PMID: 33329591) about the involvement of CD38 in further inflammatory processes which could be discussed in the context of transplantation to broaden the paragraphs.

Reply 2: We agree with the above comment and have added necessary text within our manuscript under the section ‘Improving Donor Viability in Thoracic Transplantation’, subheading ‘Immune Modulation’

Changes in text: Line 326-345

Comment 3: Moreover, it may be elegant to add an outlook on CD38 as a target to desensitize recipients towards the donor allograft. Indeed, inhibiting CD38 has evolved as a therapeutic approach to compromise the production of donor specific antibodies. As shown by Kwun et al (2019) (PMID: 31227636) targeting CD38 with daratumumab, a monoclonal antibody against cd38 reduced anti-HLA-antibodies and anti HLA-donor specific antibodies in a non-human primate model and 2 clinical transplant cases.

Reply 3: We agree with the above comment and have added necessary text within our manuscript under the section 'Improving Donor Viability in Thoracic Transplantation', subheading 'Immune Modulation'

Changes in text: Line 326-345

Reviewer B

In general, the authors have written a comprehensive review on this topic.

Comment 1: This is a narrative review. I do not understand why you describe in the Methods a search strategy that is rather reflecting the foundation of a systematic review. A search strategy is not a goal in itself. Since you subsequently eliminate a whole series of papers based on criteria that are at least in part subjective, the search strategy becomes an irrelevant element. Systematic reviews may be relevant to the extent that they are implementing a meta-analysis, which is not applicable to this specific topic.

Reply 1: In accordance with the Journal of Thoracic Disease's requirements for reviews, our 'expert review' is in fact a Narrative Review, and thus we must comply with journal requirements for sections in the manuscript, as well as reporting guidelines/checklists, search strategies, limitations of our review, etc. Please refer to 'Guideline for Authors' if further questions: https://jtd.amegroups.org/pages/view/guidelines-for-authors. We agree that our search strategy did remove papers from our review, and have listed that as a limitation.

Changes in the text: N/A

Comment 2: I suggest to drop all mentions of 'narrative review'. The methodology of systematic reviews has been overrated tremendously. More important is the actual knowledge of the authors on the topic and their familiarity with the literature. The selection process as such is not interesting.

Reply 2: Please see above comment for the necessity to include the methodology of our review.

Changes in the text: N/A

Comment 3: The key issue is provided in line 215: 'Inhibition is a tradeoff in the heart.' Therapeutic interventions are far from trivial.

Reply 3: We agree therapeutic interventions are far from trivial and have reworded this section accordingly.

Changes in the text: line 272-273

Comment 4: Limitations section. This is not to the point. I really do not understand why there is such an emphasis on the search process, which cannot be a goal in itself. A good review is an expert review, which implies that the authors are really familiar with the topic.

Reply 4: Please see above comment for the necessity to include the limitations section of our review.

Changes in the text: N/A

Comment 5: The authors mention several clinical trials, which in itself is relevant. However, I do not see the relationship between these trials and the topic of this review. Potential changes in CD38 are not obligatory a mediator of potential beneficial effects of these interventions.

Reply 5: The role of including these trials highlight that CD38 activation is being actively studied in current ongoing clinical trials – we have reworded some of this section to make it clearer. We agree potential changes in CD38 are not obligatory a mediator of potential beneficial effects of these interventions, and therefore we have to continue in both clinical and pre-clinical studies to further elucidate CD38's role in IRI in cardiac surgery and thoracic transplant.

Changes in the text: line 232 to 247

Reviewer C

The authors are to be congratulated for cogently summarizing the potential implications of CD38 inhibition in ischemia reperfusion secondary to cardiopulmonary bypass and in thoracic transplantation. Provocative data is accumulating for a role of CD38 in ischemia reperfusion, but several important questions remain unanswered. CD38 is a conserved cellular mediator of calcium signaling and secondary messengers that mediate endothelial injury and cell death. As an interconnected and pleiotropic mediator of a cascade of inflammation, cellular injury and apoptosis, the utility of CD38 as a therapeutic target is critically dependent on timing and targeting of CD38 in specific organs, as well as the specificity for the Type II versus Type III forms of CD38, depending on the indication and desired effect.

Comment 1: The authors have thoroughly reviewed the known literature on the effects of CD38 in inflammation, ischemia-reperfusion, and cellular injury or apoptosis. However, they should acknowledge the importance and challenge of tissue/cellular targeting of therapeutic CD38 inhibition, and they could propose some strategies to achieve sophisticated targeting of CD38 blockade, as above. One potential option in transplantation for targeted CD38 blockade would be administration of the potential drug to the allograft alone while it is isolated during ex vivo perfusion. The potential for this type of approach should be addressed. Also, although it may seem obvious to some readers, it is worth discussing the option of using a CD38 inhibitor in the preservation perfusate for organ procurement (cardioplegia for heart transplant or Perfadex for lung allografts), as well as using a CD38 inhibitor in cardioplegia solution in non-transplant cardiac surgery. Another example would be an inhaled CD38 inhibitor for the lung during cardiopulmonary bypass. These are just some obvious approaches which the authors could expand on to demonstrate the potential to overcome the limitation of a conserved, ubiquitous molecule that is expressed in many different tissues and cells and likely has an important role in these cells. Targeted drug delivery would be a strategy to decrease toxicity and increase efficacy.

Reply 1: We agree with the reviewer's critique and have added necessary information into a new section titled 'Therapeutic Delivery'

Changes in the text: 396- 412

Comment 2: One of the most interesting challenges related to targeted drug delivery for this particular target is related to the two relevant types of CD38, Type II and Type III. This is worth discussing and likely could be addressed using different drug characteristics. In some cases, inhibition of both Type II and Type III CD38 might be of benefit. This is touched upon in lines 268-269 but this idea could be developed further.

Reply 2: We agree with the reviewer's critique and have added necessary information into a new section titled 'Therapeutic Delivery.' We agree finding ways to both inhibit Type II or III CD38 presents unique, challenges and is a large area of focus of our lab. However, these designs are intellectual property and under-development, and therefore we are unable to offer specifics at this point.

Changes in the text: 396- 412

Comment 3: One area of discussion that is a bit of a stretch is the relevance of CD38 activation in the lungs during cardiopulmonary bypass. There is certainly some mild ischemia of the lungs during cardiopulmonary bypass, but the fact that there is perfusion of the lung continuously through the bronchiolar arteries, and unless complete heart bypass is needed, there is usually some pulmonary artery blood flow as well. All of this seems that it could be addressed with low tidal volume ventilation with a low level of FiO2 as is being tested in the ongoing trials. CD38 activation may be measured in the FOCUS trial but is unlikely that the solution to this problem will require CD38 blockade.

Reply 3: We agree that CD38 inhibition will not be the only solution to IRI (for both heart and lungs) during CPB and will involve many different aspects of treatment. For the heart during CPB we believe CD38 inhibition could possibly have a great therapeutic benefit due to the nature of CPB itself, while this effect on the lungs due to alternative blood lung will be lessened.

Changes to the text: N/A

Comment 4: Overall, this is an interesting review that highlights a promising area of drug development for mitigation of ischemia reperfusion injury of the heart in cardiac surgery, as well as in heart and lung allografts. Much of the potential strategy for this application could be further developed in the review. There are a few minor comments about syntax and grammar that are delineated in the comments to the authors. Minor comments:

Line 101: "…did not result in PubMed." I know what you mean but this sounds awkward. Is there another way to phrase this? "did not yield a search result in PubMed", perhaps?

Line 104: "was obtained it a majority" should be "was obtained if a majority"

Line 152: "without trigger an unwanted" should be "without triggering an unwanted"
Line 175-176: "and ventilating with a high fraction of inspired oxygen during cardiopulmonary bypass"—do you mean "…during weaning and separation from cardiopulmonary bypass"? These aforementioned trials aside, most times there is no ventilation during cardiopulmonary bypass.

Line 183/184 should the text in quotes and italics be in parentheses?

Reply 4: The above changes have been made, and/or clarified.

Changes in the text: lines 141, 145, 202, 229, 243-244.