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A JOURNAL TO EXPLORE NEW HORIZONS OF THORACIC SURGERY

Peer Review File

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Review Comments

Reviewer A

1. **Atkinson and Sharma have written an interesting, comprehensive and well-written overview of immunosuppressive therapy currently used in LTx. It was a pleasure to read.**
Reply: We appreciate the reviewers' insightful comments.

I only have a few minor suggestions:
- 62,64: abbreviations can be removed as otherwise not used in abstract
Reply: Abbreviations removed from the abstract.

- 151: I think it is 80% induction of which about 70% IL-2RA?
Reply: This is correct based on the last ISHLT data available.

Changes in the text: Added a sentence specifying that slightly over 70% of induction recipients receive IL-2RA, lines 152. Correction made to line 198 which incorrectly stated that 80% of patients received IL-2RA.

- 172-179: it would be interesting if the authors can mention if there was a difference in infection rates between induction vs no induction, also note that Snell's study only used a single dose ATG instead of the typical 3-5 days
Reply: We have more thoroughly reviewed the data surrounding adverse events with induction therapy and added a section discussing this data. The data is expectedly mixed, but we tried to present objective representation of what it shows. We agree that it is important to note the atypical dosing protocol utilized by Snell et al and have added this to the text.

Changes in text: Added section commenting on risk of infection and malignancy with induction, lines 239-59. Made change noting that single dose ATG regimens also exist, line 177. Updated ATG dosing in Table 1. Added additional line when referencing the Snell study that a single dose regimen was used, lines 232-35.

- I miss some references for lines 137-143, 231-251, 305-314
Reply: References added.

Changes in text: Added expanded references for ATG section, lines 171, 182. Cyclosporine and Tacrolimus sections, lines 329, 334, 342, 345, 356. Azathiprione, line 425.

- For the target levels for CNI, I'd add depending on time after transplantation to be complete
Reply: Added to the text

Changes in text: Added sentences in the cyclosporine and tacrolimus sections indicating that target concentration may vary based on time from transplantation, with higher doses being considered in the first year. Lines 336-38, 352-54.

- Pirfenidone: I'd suggest moving this part to CLAD instead of adjunctive therapy, in which you can highlight that antifibrotic agents are under investigation for CLAD? The recent large multicenter EPOS trial could not demonstrate a significant beneficial effect of pirfenidone on pulmonary function decline in BOS patients, but I see the official paper still hasn't been published. Especially the results in RAS are eagerly awaited as we might perhaps expect more effect in these patients because there are more extensive fibrotic abnormalities.
Reply: We removed the dedicated CLAD section (condensed into the ACR, AMR, CLAD sections into one shorter Rejection section) to focus the review further. We added a comment about the EPOS trial and noted ongoing trials evaluating pirfenidone in RAS and nintedanib in BOS.

Changes in text: Added comments on EPOS trial, ongoing pirfenidone and nintedanib studies, lines 614-19.

- 511: I'd rather say respiratory infections (as some types of viral bronchiolitis may also contribute to CLAD onset)
Reply: Based on feedback from several reviewers, the ACR, AMR, and CLAD sections were significantly condensed, so this sentence was removed.

Changes in text: This sentence was removed.

- 518: there is indeed not a lot of data on TLI in LTx, but I'd refer to the data available. E.g.: PMID 31643104, 19545735, 19545735, 19545735
Reply: As above, the ACR, AMR and CLAD sections were significantly reduced based on reviewer feedback. However, we have provided references to the TLI data available in the text.

Changes in text: Added TLI references, lines 630, 640

Reviewer B

Overall the authors have done a good job of summarizing the current state of immunosuppression in lung transplant. However, I believe the article should

focus more the shortcomings of the current state and some of the future directions being investigated to help bridge the gaps that currently exist. Some key items to consider for inclusion could be discussion of extended release tacrolimus dosing forms, usefulness of CYP450 genetics, integration of belatacept into the immunosuppression armamentarium, use of dd-cfDNA assays to manage titration of immunosuppression and so on.

Reply: There are clearly many shortcomings, and we tried to highlight the general lack of good, prospective data to guide management decision. Mention of extended relief tacrolimus is certainly worthwhile. A comment on the availability of XR tacrolimus and ongoing trials evaluating its efficacy in lung transplant has been added. Multiple reviewers requested expansion of the section on belatacept. A recent trial from Huang et al was stopped early due to multiple deaths in the belatacept group, calling into question the role this agent has in lung transplant. This has been added to the belatacept section, which has been generally expanded. Due to expansion of the review in multiple other areas in response to reviewer comments, we elected to omit discussion of cc-DNA as the most compelling trials evaluating this modality of monitoring are ongoing i.e (ALAMO and PROSPERA).

Changes in text: Added comment regarding availability of extended-release tacrolimus and the fact that there are ongoing trials evaluating this agent, line 348-50. Added additional information about the data for belatacept in lung transplant, focusing on the Huang trial. Reference also made to case reports of severe ACR with belatacept switched from CNI. Lines 519-31

It also seems that the potential scope of this review is very large. To increase focus of the review, it would be beneficial to significantly decrease or eliminate the discussion of ACR/AMR. This topic is of sufficient depth to warrant it's own review and more. Perhaps a pipeline/future directions section would increase the value of this review.

Reply: We agree that the scope of the review was too large. The ACR, AMR and CLAD sections have been condensed significantly.

Changes in text: ACR, AMR and CLAD sections have been condensed to one section on Rejection, lines 624-40. A future directions section was not added given the already broad scope of the review. We highlighted numerous future directions in the body of the text, notably XR tacrolimus, alemtuzumab induction with low dose everolimus and tacro, the recent negative data on belatacept, and the role of pirfenidone and nintedanib in BOS/CLAD.
Additional focused feedback has been included below.

Line 130-132: The authors should make a brief mention as to the reason why daclizumab was withdrawn from the market for completeness.

Reply: Added comment for completeness

Changes in text: Added comment regarding the development of severe inflammatory brain diseases with daclizumab, line 164-65.

Line 138: ATG is quite often administered intraoperatively. The authors should also mention the weight based dosing of this medication.
Reply: Added comment about intraoperative and weight-based dosing of ATG

Changes in text: As above, lines 175-76.

Line 151-153: The sentence beginning at the end of 151 and ending at the beginning of 153 is extemporaneous, consider removal.
Reply: We agree that this sentence is unnecessary, it has been removed.

Changes in text: Sentence from previous 151-53 removed.

Line 263: "reasonably large" is overly ambiguous. Replace this with the number of patients in the trial.
Reply: Agree that this is ambiguous, updated to include number included in the trial

Changes in text: Changed to include number of patients in the trial, line 368.

Line 265: I believe "or" should be "of"
Reply: This has been corrected.

Changes in text: Changed “or” to “of”

Line 268-272: This information can be minimized or omitted.
Reply: Condensed information from other solid organ transplants.

Changes in text: Removed specific referral to McAlister study, indicated that there is data demonstrating superiority of tacrolimus over cyclosporine in other solid organ transplants, lines 376-77.

Line 387-392: Belatacept may see increased growth in the lung transplant populations for multiple reasons beside renal sparing. It may be interesting to consider expanding this section. You may consider Pham et al opinions presented in: Belatacept dosing in Lung Transplantation: is there a Method to the Madness?
Reply (as from above): Multiple reviewers requested expansion of the section on belatacept. A recent trial from Huang et al was stopped early due to multiple deaths in the belatacept group, calling into question the role this agent has in lung transplant. This has been added to the belatacept section, which has been generally expanded.

Changes in text: Added additional information about the data for belatacept in lung

transplant, focusing on the Huang trial. Reference also made to case reports of severe ACR with belatacept switched from CNI. Lines 519-531

Reviewer C

The authors present a narrative review on the status of immunosuppression after lung transplantation.
Although quite informative, I have some criticisms.
First, the authors should reflect on which reader they want to target. The review, indeed, is to some extent too "simple" and superficial to address experts in the field. I would suggest improving the immunologic background of the immunosuppressive agents and to provide more evidence behind each strategy. Moreover, I would omit immunosuppression for ACR/AMR/CLAD.
Reply: We have expanded upon the immunologic background of many of the agents discussed (see below for specifics) and included more data, including from recent studies and trials. We elected to condense the ACR/AMR/CLAD section in order to briefly highlight the use of the discussed agents in those entities.

Changes in text: Additional immunologic background provided for ATG, lines 173-75, alemtuzumab, lines 187-89.
Added additional data for the following:
Risks of induction, lines 239-259
Alemtuzumab vs basilixmab, lines 277-284
Aletuzumab vs ATG, 285-288
Tacrolimus vs cyclosporine, lines 371-75
Belatacept, lines 519-531
Inhaled steroids, 562-69
Pirfenidone, lines 615-19

Second, the review suffers from the personal opinion of the authors. Although the effort must be acknowledged, I would suggest to remove the institutional preference of the authors and to improve objectivity of the presentation.
Reply: Institutional preference removed from the text.

Changes to text: Removed induction institutional preference, originally lines 205-207. Removed CNI institutional preference, originally lines 274-276. Removed cell cycle inhibitor institutional preference, originally lines 336-338.

Third, the authors should include the last reports and analyses published in 2021.
Reply: We have included references for the 2020 and 2021 lung transplant reports. We have not changed the text, as the 2020 and 2021 reports utilized the same patient cohort as the 2019 report.

Changes to text: We have included references to the above reports, line 111.

Minor revisions:

- The rationale of induction therapy is not only to avoid T-cell mediated alloreactivity but also to reduce doses of maintenance immunosuppression. Please correct

Reply: We appreciate the reviewer's important comment. However, changes in maintenance immunosuppression after induction (basiliximab) is not a common practice. We do acknowledge that recent single center studies have implicated potential reductions in maintenance immunosuppression with alemtuzumab, but these are not widely accepted at this juncture and future multicenter studies are needed to better inform this practice. We have included a statement indicating that this can be considered part of the rationale behind induction therapy.

Changes to text: Added sentence indicating that induction may aid with reducing doses of maintenance immunosuppression, line 142-43

- ATG has effects on B cells and monocytes.

Reply: Will expand on ATG's immunologic impact.

Change in text: Added sentence expanding on ATG's additional immunologic impact (beyond T-cell depletion), lines 173-75. Did mot explicitly mention monocytes, but rather indicated the effect ATG may have on leukocyte-endothelium interactions.

- I personally doubt that the "BEST" data come from registry analysis. These data are not granular, often not fully correct and very superficial. Please correct.

Reply: We agree with the reviewer. However, the intent of the authors was to convey that currently the data to support induction is from large retrospective studies due to the absence of high quality prospective trials, and the mixed results those trials have produced.

Changes in text: Changed "best" to "most robust", line 210.

- The authors have a clear bias against alemtuzumab. 1) alemtuzumab has depletional effect on monocytes, macrophages and DC. 2) alemtuzumab seems to promote a tolerogenic environment. 3) prospective evidence from Jaksch and colleagues. 4) ABSOLUTELY NO EVIDENCE of myelosuppression and consequently increased rates of infection or malignancy. This is only unfounded reluctance. I invite the authors to correct the sentence and provide objective data.

Reply: We appreciate the reviewers' comments. Of note, the authors practice in one of the few lung transplant centers around the world that use alemtuzumab in various

settings post lung transplant and share the observations commented by the reviewer. We also acknowledge that the section on alemtuzumab was not detailed in comparison to the corresponding IL-2RA and ATG sections. We have provided dosing information and adverse effects for alemtuzumab, as we did for basiliximab and ATG. Regarding specific comments: 1 and 2: We expanded the immunologic impact of alemtuzumab. 3. We discuss evidence in favor of alemtuzumab in lines 270-291. We have added evidence from the Jaksch trial looking at alemtuzumab vs ATG in lines 285-88. 4. There is robust evidence outside of lung transplant that alemtuzumab confers a significant risk of infection, and to a lesser degree, myelosuppression. We agree that the lung transplant literature does not demonstrate this infectious risk consistently, though there are some reports that do suggest the possibility of increased infectious risk with alemtuzumab. For example, in Furuya *et al*'s UNOS review, a non-CMV infectious cause of death was significantly more common in the alemtuzumab group compared to basiliximab or no induction, as was a malignant cause of death. We have included this data in a section dedicated to evaluating risk of infection with induction therapy, per the request of other reviewers. Benazzo *et al* also showed that while aletuzumab was associated with lower infectious risk compared to ATG or no induction through the first year, it was associated with greater risk of infection at greater than one year. The quality of these data is certainly low, and other studies have failed to reproduce these findings, and thus multicenter data is required to better inform this practice. We agree that the final sentence of the alemtuzumab section was overly subjective, and largely not in line with the data provided, so this has been removed.

Changes in text: Added sentence describing additional immunologic effects of alemtuzumab, lines 187-89. Added dosing information and adverse effects, lines 190-194. We have added evidence from the Jaksch trial looking at alemtuzumab vs ATG in lines 285-88. Removed the sentence regarding concerns for infectious risk and myelosuppression with alemtuzumab as it was subjective.

- Lastly, some interesting data on belatacept after lung transplantation have been published. Please elaborate.

Reply: Multiple reviewers requested expansion of the section on belatacept. A recent trial from Huang et al was stopped early due to multiple deaths in the belatacept group, calling into question the role this agent has in lung transplant. This has been added to the belatacept section, which has been generally expanded.

Changes in text: Added additional information about the data for belatacept in lung transplant, focusing on the Huang trial. Reference also made to case reports of severe ACR with belatacept switched from CNI. Lines 519-531.

In summary, I congratulate the authors for the work done and I hope to read the revised version of this interesting review.

Reviewer D

I would thank Drs. Atkinson and Sharma for their fine contribution entitled "Immunosuppression in Lung Transplantation: A Narrative Review", the Authors did great job in reviewing of lung transplant immunotherapy. The article is fluent , UpToDate and we summarize the major aspects of immunosuppression in lung Transplantation.

These are some points and comments which might make the article better:

1) **Please remove the abbreviation of eth abstract.**

Reply: Abbreviations removed from the abstract.

Changes in text: Abstract was amended according to review guidelines

2) **Would be great if they compare the outcomes differences between Induction vs. no Induction protocols.**

Reply: The induction section has been expanded considerably, to more fully address clinical outcome differences and adverse events with induction vs no induction.

Changes in text: Induction section expanded, with specific data for induction vs no induction reviewed from lines 210-259, including a new section on risks of infection and malignancy with induction vs no induction.

3) **Please revise the article according to the Article review guidelines.**

Reply: Articles has been amended based on review guidelines.

Changes in text: Amended according to directions below.

Again, I congratulate the authors for their well-rounded article and good review of literature and including their center/personal experience insights, I am pretty sure this article will be a good addition to lung transplantation literature.

Reviewer E

This review by Drs Atkinson and Sharma provides a comprehensive and detailed description of immunosuppression in lung transplantation, which would be of great benefit to clinicians, researchers and patients. Following are some suggestions to improve the article.

1. The mTOR inhibitor sirolimus has been found to be associated with severe airway complications in several small, observational studies, and a relatively high proportion of patients have fatal consequences, which are briefly described in the article.

Reply: The data describing this frequent and catastrophic complication has now been added to the manuscript.

Changes in text: Added data describing airway complications and mortality, lines 504-09.

2. The description of the treatment plan for antibody mediated rejection (AMR) is superficial. The prognosis of AMR is extremely poor. Most of the treatments are for individual cases and small samples. And the treatment plans also vary from institutions to institutions. Besides, dosage and administration time are different in a diverse therapies, such as plasmapheresis, intravenous immunoglobulin (IVIG), Rituximab, Bortezomib, etc. It is recommended that the author make an effective summary and give readers a hint of best medication strategy.

Reply: Numerous reviewers felt that the scope of the review was too large already and felt as though the ACR/AMR/CLAD sections should be condensed. We have condensed this section, and thus treatments described for AMR remains very superficial.

Changes in text: ACR/AMR/CLAD sections condensed into one Rejection section, lines 621-640.

3. There are also some literatures on inhaled glucocorticoid therapy in the treatment of chronic lung allograft dysfunction (CLAD), such as F (Fluticasone) A (Azithromycin) M (montelukast), P (budesonide/formoterol) M (montelukast) N (n-acetylcysteine). These strategies are not mentioned in the article, and it is recommended to add relevant contents.

Reply: We added a section for inhaled corticosteroids, and eludicated some of the data available for treatment and prevention of BOS. We did not explicitly mention NAC, due to limited data and need to focus review to a degree (though we did reference trials that used NAC as a part of combination therapy). Azithromycin is discussed in lines 577-589, montelukast 591-96.

Changes to text: Added section on inhaled CS, lines 562-569.

4. There have been some small cohort clinical studies of pirfenidone in the treatment of CLAD, and some results have been obtained. Although some of them are statistically non-significant, the decline of FVC and FEV1 has been significantly slowed down from a clinical point of view. It is recommended to include and introduce these studies in this review to provide some new ideas for the treatment of CLAD.

Reply: we have expanded the pirfenidone section to note the EPOS trial (though still awaiting formal publication of data), and future trials evaluation pirfenidone and nintedanib in CLAD.

Changes in text: Expanded pirfenidone section as above, lines 615-619.