

Peer Review File

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Reviewer A:

Comment 1: Please see the attached version of the manuscript with my edits. reference 3, describing a previous publication of proteomic data from this group, does not appear from this group. the authors have confused two different tolerance trials conducted at Northwestern in their discussion. There have been recent updates on the tolerance experiences, published in Human Immunology in 2018 that need to be used as the description of the trial outcomes in this manuscript are outdated.

Reply 1: Thanks for your encouraged comments. First, we corrected all errors you edited in the PDF. In the discussion about disease recurrence, we confused the two clinical trials in Northwestern. We corrected the text and appreciated your comment.

The ref 3 is a typo error, we are sorry about it.

Thanks for reminding us to update the results from Northwestern. We discussed more details and cited the article by Prof. Leventhal published in Human Immunology. In addition, we updated references and discussed their work.

Change in the text: Page 7 Line 9 “transient”. Page 18 Line 10 “unmanipulated”.

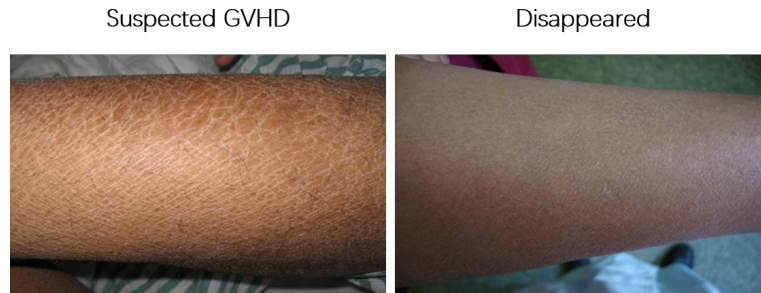
Page 19, Line 1-7. “At Northwestern University, 2/10 recipients had nephritis relapse in HLA identical living donor transplantation with serial infusion of CD34⁺ selected DHSCs (22). However, in their subsequent trial using facilitating cells, it has not seen disease recurrence in any of the 26 patients where durable chimerism was achieved without immunosuppression drugs (24). We believe relapse depends on the level of immune system reconstruction and reeducation in recipients, but the mechanism still needs to be explored.”

New references added:

- Leventhal JR, Mathew JM. Outstanding questions in transplantation: Tolerance. Am J Transplant 2020;20:348-54.
- Gallon L, Mathew JM, Bontha SV, et al. Intra-graft Molecular Pathways Associated with Tolerance Induction in Renal Transplantation. J Am Soc Nephrol 2018;29:423-33.
- Leventhal JR, Miller J, Mathew JM, et al. Updated follow-up of a tolerance protocol in HLA-identical renal transplant pairs given donor hematopoietic stem cells. Hum Immunol 2018;79:277-82.

Comment 2: Mention of possible GVHD in one subject that disappeared is confusing. It is not clear from the paper what the percentage of chimerism was in each subject over time. the figure illustrating chimerism in one subject (figure 4) is hard to understand and a table showing chimerism by STR analysis would be better

Reply 2: The 1st case is only one patient with possible GVHD. It is in deed disappeared. We are not sure it is GVHD, so we described “suspected GVHD” in the manuscript. Here is the picture showing the skin.



In Page 16, we indicated that chimerism was induced in the first recipient who is HLA-matched by 30% to 50%, which remained 4 to 6 weeks after kidney transplantation (Figure 4). At 6 months, the chimerism disappeared. The other 10 recipients induced less than 1% chimerism. As you suggested, we added Table 4 to describe the data and modified the title of Figure 4.

Change in the text: [Page 29, new Table 4. Page 32 Line 17](#) “Figure 4. Chimerism on D8S1179 loci in No.1 recipient.”

Comment 3: all the subjects had the same cause of ESRD? this is very surprising (GN).

Reply 3: In China, glomerulonephritis is the most common cause of end-stage renal disease. In our study, all patients were diagnosed as glomerulonephritis in the department of Nephrology, although some of them did not have renal biopsy.

Change in the text: no change.

Comment 4: table showing biopsy results does not indicate which subject had a graft loss...

Reply 4: Thanks for your suggestion. We added a column showing the graft outcome in the Table 5 (original Table 4).

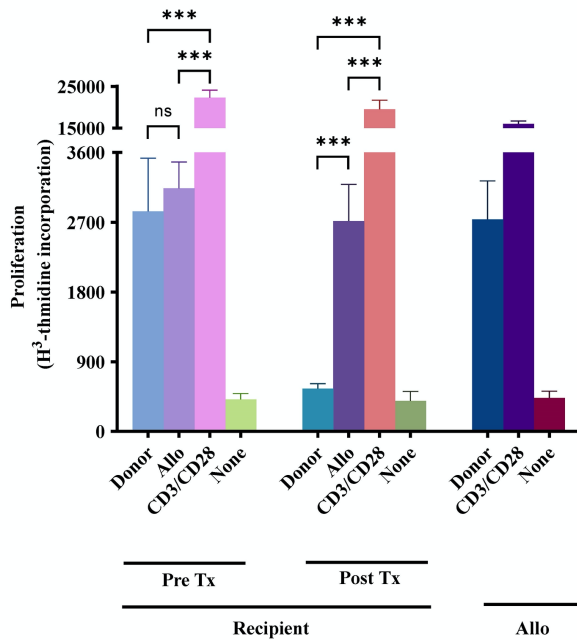
Change in the text: [Page 30, modified Table 5.](#)

Comment 5: Figure 5 showing MLR results are cut off partially. The authors may want to refer to previously published work from Northwestern and MGH in their discussion showing a lack of correlation of DSH with tolerance...

Reply 5: The Figure 5 was cut off in the merged PDF due to tech problem of the submission system. The original Figure 5 had been uploaded intact. Here is the Figure

5. Yes, we want to refer to patients demonstrated *in vitro* donor-specific hyporesponsiveness (DSH) by MLR post-transplant regardless of not predictive of successful immunosuppressants weaning and tolerance.

Figure 5



Change in the text: Page 18 Line 4-7 “Besides chimerism, we also found the DSH phenomenon in the recipient. Interestingly, DSH alone without successful donor chimerism might not be predictable for IS weaning and tolerance. It is consistent with previous study reported by Leventhal et al (27).”

New references added:

- Leventhal J, Abecassis M, Miller J, et al. Chimerism and Tolerance Without Gvhd In Mismatched Recipients Of Combined Hematopoietic Stem Cell/Kidney Transplants: Donor-Specific Hyporeactivity Is Not a Reliable Biomarker For Tolerance. *Blood* 2013;122:912.

Comment 6: there is no data showing long term recovery of leucocyte populations over time... this would add much to the MS if available.

Reply 6: In the results, we mentioned lymphocyte recovery. Page 12 Line 22 “All patients had persistent lymphopenia, and then the lymphocytes gradually recovered after 1 month. The lymphocytes count increased and returned to the normal level approximately 6 months post transplantation.”

Change in the text: no change.

Comment 7: Overall, this manuscript, detailing an attempt to induce tolerance using

TLI and unmodified mobilized DHSC for the first time in China is interesting but lacks depth in terms of data provided. additional information as requested would be very helpful for reconsideration

Reply 7: Appreciated again for your valuable comments for improving our manuscript.

Reviewer B:

Comment: Although these are negative results, it should be reported somewhere. The reasons of their failure to induce chimerism are too low radiation dose and too low stem cell numbers, comparing with the Stanford protocol. Since no chimerism is induced in most patients, It is difficult to prove the relevance of the protocol and the long-term allograft survival with low dose immunosuppression. The authors may need to compare with the results of comparable transplant recipients with conventional immunosuppression. Additionally, the authors should show DSA results as they often become positive if immunosuppression is too low. If these patients are truly better than conventional patients, what is the mechanism of long-term survival with low dose immunosuppression? Please discuss it little more in depth. Since there is no chimerism, is that simply the effect of TLI?

Reply: Thanks for the positive comments from the reviewer B. This is the first clinical trial using DHSCs infusion and TLI to induce tolerance in kidney transplantation in China. It is widely accepted that immune tolerance is the ultimate goal in organ transplantation, but the process is extremely difficult. When we noticed the report by MGH in NEJM, we initiated our clinical trial in China. Like other leading centers (Northwestern/Duke, MGH, Stanford), we didn't set a conventional IS control group during protocol design. We acknowledge that the dose of CD34⁺ cells in our protocol was much lower than that in the Stanford protocol. We can not deny the contribution of TLI to the long-term allograft survival with low dose of immunosuppression. However, we also can not deny the contribution of DHSCs infusion. In the revised manuscript, we listed the three cohorts of Stanford, compared and discussed more.

According to DSA, we are very sorry that we did not perform the DSA test.

Change in the text:

Page 16 Line 21; Page 17 Line 1-22; Page 18 Line 1-4. These texts are added.

“The dose of CD34⁺ cells in our protocol is 0.2-3.0×10⁶/kg, which was much lower than that in the Stanford protocol. In their first cohort of 6 HLA mismatched patients, the dose of CD34⁺ cells was 3.1-11.1×10⁶/kg, none of the 6 patients developed chimerism that persisted beyond 3 months. In the second cohort of 22 HLA matched patients, more CD34⁺ cells (4.3-17.5×10⁶/kg) were infused. There were 16 patients who developed chimerism for at least 6 months and had IS drugs successfully discontinued without

reinstitution, 7 had stable chimerism during and after IS drug withdrawal, and 9 eventually lost chimerism. This HLA matched cohort had a most chimerism induction rate. In their third cohort of 10 HLA haplotype matched patients, the dosage of CD34⁺ cells was the highest with $8-22 \times 10^6/\text{kg}$, which was nearly 8-10 folds to our protocol. Half patients were induced chimerism (5). But in Northwestern, the trial enrolled 37 HLA mismatched patients (1 in Duke), 26 patients achieved durable chimerism (T cell chimerism > 50%). Twenty-three patients developed “full” peripheral blood chimerism, with > 98% donor cells (14). Although the first patient induced chimerism, most of patients failed in our study. Relative low dose of CD34⁺ cells might be a reason. Stable chimerism has been considered as a key factor for tolerance induction so far. Since the chimerism induction was failure, we can not deny the contribution of TLI to the long-term allograft survival with low dose of immunosuppression. However, we also can not deny the contribution of DHSCs infusion. The detail mechanism is complex. Although durable donor chimerism is sufficient to establish tolerance, it is also indicating sustained chimerism is by no means absolutely necessary (25). We must achieve a balance between GVHD and chimerism. Northwestern investigated gene expression and microRNA expression profiles in renal biopsy samples from tolerance-induced bioengineered stem cell product (FCRx) recipients, paired donor organs before implant, and subjects under standard immunosuppression with and without acute rejection (26). They found some intragraft molecular pathways characteristics, but it is still too early to clarify the full mechanisms. For us, to find an appropriate dose of DHSCs in Chinese patients still needs further trials.”

Reviewer C:

In this study, the authors performed a creative and meaningful study. They reported the long-term outcomes of patients treated with donor hematopoietic stem cells and kidney transplantations. They provide precious experience in inducing tolerance of patients with kidney transplantations. However, some points should be improved, especially for article writing.

Reply: Thanks for the positive and constructive comments from the reviewer 3.

Comment 1: In the abstract, the authors summarized the study in the background. However, they should demonstrate the necessity of this research. Please refer to similar studies to learn how to write the background.

Reply 1: We have modified the abstract.

Change in the text: Page 4 Line 2-5. “Immunosuppression therapy after kidney transplantation for life increases risks of infection, cardiovascular diseases, metabolic diseases and cancer. So far, four centers (3 in the US, 1 in South Korea) have reported

clinical tolerance trails in kidney transplantation.”

Comment 2: In the abstract, the authors did not state the information about follow-up in the method.

Reply 2: The information is added in the revised manuscript.

Change in the text: Page 4 Line 14-15. “All patients are followed-up until now. Routine laboratory examinations, chimerism, biopsy and mixed lymphocyte reaction were performed.”

Comment 3: The topic of the study is the treatment of kidney transplantation combined with donor hematopoietic stem cells. However, they only emphasized the long-term stable kidney allograft survival with low-dose immunosuppression. The conclusion does not match the aim of the study.

Reply 3: Thanks for your comment. We modified the conclusion in the abstract and made it clearer and more specific.

Change in the text: Page 5 Line 3-5. “Low dose of immunosuppression with long-term stable kidney allograft survival could be achieved using our immune tolerance induction protocol by donor hematopoietic stem cell infusion and total lymphoid irradiation.”

Comment 4: Authors misunderstand the structure of instruction in the academic paper with advocacy reports. In the instruction, authors should summarize the current progress of this topic, point out the gap of previous studies, and state the necessity of their study.

Reply 4: Thanks for your suggestion. We revised the introduction section, and introduced the three leading centers’ trails in the US. Although encouraging results have been achieved, manipulatable immune tolerance induction still face many hurdles and mysteries. In China, we are the first and only center to perform tolerance induction in kidney transplantation with DHSCs infusion and TLI. No one knows whether these protocols are suitable for Chinese people.

Change in the text: Page 6 Line 13-22; Page 6 Line 1-4.

“In brief, Northwestern established persistent chimerism with CD34+ DHSCs, T cells and facilitating cells infusion. Their nonmyeloablative conditioning included fludarabine, cyclophosphamide and total body irradiation (3). They also performed a separate trail of induction tolerance in HLA matched patients, and 6/15 patients achieved tolerance (4). Stanford used total lymphoid irradiation (TLI) and anti-thymocyte globulin (ATG) as conditioning regimen, and infused CD34+ DHSCs, T cells for immune tolerance induction (5). Massachusetts General Hospital carried out two trials in which patients were with or without hematologic malignancy. In the trial

that enrolled non-hematologic malignancy patients with HLA mismatched, they used local lymphoid irradiation, cyclophosphamide and anti-CD2 mAb as conditioning regimens. Whole bone marrow was infused to induce tolerance (6). All the results from these leading centers are encouraging, but adverse events cannot be ignored (2). Besides, manipulatable immune tolerance induction still face many hurdles and mysteries. Whether these protocols are suitable for Chinese people is unknown.”

Comment 5: In patients of methods, authors should better list the inclusion and exclusion criteria of their study. If criteria have been stated in the published article, authors should provide the reference.

Reply 5: Thanks for your comment. We have added the inclusion and exclusion criteria.

Change in the text: Page 8 Line 7-10.

“The inclusion criteria are as follows: patients who were eligible to receive renal transplant, age>18 years, with negative flow crossmatches, panel reactive antibodies (PRAs)≤10%. The exclusion criteria are ABO incompatible, active infection, history of malignant tumor within 5 years, tuberculosis and pregnancy.”

Comment 6: In patients' follow-up assays, the authors stated that the recipients' transplanted kidney function was monitored by regular follow-up. What does regular follow-up mean? What is the interval of regular follow-up?

Reply 6: The patients were followed up once a week in the first 3 months after renal transplantation, every two weeks after three months, and once a month after half a year. We have added the description in the methods.

Change in the text: Page 10 Line 8-9. “The patients were followed up once a week in the first 3 months after renal transplantation, every two weeks after three months, and once a month after half a year.”

Comment 7: Adverse events should be defined in methods.

Reply 7: Thanks for your suggestion. Adverse events were defined as diarrhea after TLI, graft-versus-host disease (GVHD), severe infection, myelosuppression and other related symptoms during TLI and DHSCs infusion.

Change in the text: Page 9 Line 12-13. “Adverse events are defined as diarrhea after TLI, graft-versus-host disease (GVHD), severe infection, myelosuppression and other related symptoms during TLI and DHSCs infusion.”

Comment 8: Detailed demographic data at baseline should better be listed.

Reply 8: The demographic data in the Table 1 has been modified. We added the peak PRAs and current SCr.

Change in the text: Page 26, see new Table 1.

Comment 9: Only one recipient had mild rejection (Banff IA grade, Banff 07 criteria). Please provide the reference of Banff criteria here. It's better to use 'mild rejection with Banff IA grade according to Banff 07 criteria'.

Reply 9: The reference has been cited in the revised manuscript. According to the suggestion, we modified the expression to 'mild rejection with Banff IA grade according to Banff 07 criteria'.

Change in the text: Page 14 Line 10-11. "Only one recipient had mild rejection with Banff IA grade according to Banff 07 criteria (8)".

New reference added:

- Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant 2008;8:753-60.

Comment 10: Please improve the conclusion in the manuscript according to No.3

Reply 10: Yes, we rewrote the conclusion section.

Change in the text: Page 19 Line 12-18. "This is the first report in China that induction tolerance in kidney transplantation with DHSCs infusion. Although most of patients failed to induce chimerism and none patient achieved immunosuppression free, all of them reduced immunosuppression dosage with stable allograft function. Our protocol still needs improvement and adjustment in terms of changing DHSCs dosage, frequency in the future. We will also follow up these patients. We hope to establish a more effective protocol for immune tolerance induction in Chinese people."

Comment 11: English writing is relatively poor. Many grammatical errors could be found in the article, such as 'have been developing'. The English writing should be improved by professional editors.

Reply 11: We used Springer Nature Editing Service as the editor suggested.

Change in the text: Corrections are highlighted with yellow background.

Comment 12: I suggest performing a case-control study of this topic. The study design in this research is weak evidence strength.

Reply 12: The same question is also raised by the reviewer B. Like other leading centers (Northwestern/Duke, MGH, Stanford), we didn't set a conventional IS control group during protocol design. But thanks for your suggestion again, we will set a control group in next manuscript.

Change in the text: No change.