Induction tolerance with donor hematopoietic stem cell infusion in kidney transplantation: a single-center experience in China with 10-year follow-up

Xuanchuan Wang
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China

Cheng Yang
Zhongshan Hospital Fudan University https://orcid.org/0000-0003-4060-2746

Linkun Hu
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China. Department of Urology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China

Zheng Wei
Department of Hematology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Qunye Tang
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China

Bing Chen
Department of Radiation Oncology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Yuan Ji
Department of Pathology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Ming Xu
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China

Zhaochong Zeng
Department of Radiation Oncology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Ruiming Rong
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China. Department of Transfusion, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Tongyu Zhu (tyzhu_dr@163.com)
Department of Urology, Zhongshan Hospital, Fudan University; Shanghai Key Laboratory of Organ Transplantation, Shanghai 200032, China

Research

Keywords: Kidney transplantation, immune tolerance, hematopoietic stem cell, chimerism

Posted Date: March 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17158/v1

Version of Record: A version of this preprint was published at Annals of Translational Medicine on November 1st, 2020. See the published version at https://doi.org/10.21037/atm-20-2502A.

Abstract

Background. We performed the first clinical trial in China in which kidney transplantation was combined with donor hematopoietic stem cell (DHSC) infusion for tolerance induction. This study summarizes the 10-year follow-up results.

Methods. From 2009 to 2017, 11 cases of living-related kidney transplantation combined with DHSC infusion were performed. Two of them were HLA-matched, and nine were HLA-mismatched. The DHSCs were mobilized using granulocyte colony-stimulating factor and harvested one day before transplantation. The recipients received consecutive total lymphoid irradiation for 3 days before kidney transplantation. The induction drug was anti-thymocyte globulin. DHSCs were infused on the 2 nd , 4 th and 6 th postoperative days.

Results. One HLA-matched recipient induced 30-50% chimerism, and the others only induced less than 1% chimerism. Recipients had a low immune response to their donors while sustaining normal reactivity to non-donors in mixed lymphocyte reactions. All recipients were followed up for 717~3,918 days. One recipient lost allograft function, and 10 recipients had stable renal function. None of the 11 recipients had myelosuppression or graft-versus-host disease post transplantation. Our protocol did not increase the risk of infection. Allograft biopsy confirmed that one patient had mild acute rejection, and the other 10 recipients did not develop rejection. Five patients reduced the dose of immunosuppression.

Conclusions. This study shows that long-term stable kidney allograft survival may be achieved with low-dose immunosuppression maintenance using our protocol.

Trial registration: Chinese Clinical Trial Registry, ChiCTR-TNC-09000399. Registered 22 April 2009 - Retrospectively registered, http://www.chictr.org.cn

Background

Although immunosuppression (IS) and antibodies have been developing progressively, the long-term survival of renal allografts is still unsatisfactory [1]. The main reason is rejection, either acute or chronic. In addition, many side effects, such as cardiovascular diseases and metabolic disorders caused by IS, also threaten the lives of recipients. Therefore, tolerance induction is always the ultimate goal of organ transplantation.

In 2017, the third meeting of the International Clinical Tolerance Workshop was held in Stanford. This meeting summarized the updated progress of studies on kidney and liver transplant tolerance induction. Four centers, including Stanford, Northwest/Duke, Massachusetts General Hospital and Samsung Medical Center, reported their progress in kidney clinical tolerance studies. The patients were HLA-matched or HLA-mismatched. Some recipients underwent IS withdrawal post transplantation. The results are encouraging, but adverse events cannot be ignored [2].

From 2009, we performed the first clinical trial of kidney transplantation combined with donor hematopoietic stem cells (DHSCs) for immune tolerance induction in China. In 2012, we reported a proteomics study from 4 patients [3]. Here, for the first time, we report the long-term follow-up results of a kidney transplantation

clinical tolerance study in China. We attempted to induce transit chimerism through combined kidney transplantation with the infusion of donor $CD34^+$ selected hematopoietic stem cells.

Methods

Patients

From April 2009 to December 2019, 11 pairs of subjects were enrolled in this study. The average age of recipients was 33.2 ± 5.1 years, ranging from 22 to 42 years. The age of donors ranged from 31 to 62 years, with an average of 50.5 ± 11.1 years. Ten recipients received primary and one received secondary renal transplantation. The flow crossmatches were all negative. Initially, the preoperative panel reactive antibodies (PRAs) were set at < 10%. Seven pairs of donors and recipients were parent-child relationships, and four pairs were siblings. Two pairs were full HLA-matched at six HLA loci, and the others were 1 to 3 HLA-mismatched. The study was registered in the Chinese Clinical Trial Registry (http://www.chictr.org.cn, ChiCTR-TNC-09000399) with written informed consent. The general information of donors and recipients is detailed in Table 1.

Table 1
General information of donors and recipients.

No.	Recipient				Relationship	HLA mismatch	Donor			Follow-up days
	Gender	Age	ABO	Primary disease			Gender	Age	ABO	
1	M	36	A	GN	Sibling	0	M	41	A	3918
2	F	22	B	GN	P to C	3	F	46	O	3890
3	F	36	AB	GN	P to C	3	M	60	B	3883
4	M	32	A	GN	Sibling	1	M	31	A	3809
5	F	29	B	GN	Sibling	2	F	40	B	3792
6	M	33	A	GN	P to C	3	M	57	A	3671
7	M	31	A	GN	P to C	3	M	57	A	3664
8	M	37	B	GN	Sibling	0	F	40	B	3060
9	M	34	AB	GN	P to C	2	M	62	AB	3046
10	M	42	O	GN	P to C	2	F	60	O	2918
11	M	43	O	GN	P to C	1	F	62	O	1023

GN: glomerular nephritis; P to C: parent to child.

Clinical Protocol

Before collecting DHSCs, the donor received a 5-day course of subcutaneous injections of granulocyte colony-stimulating factor (G-CSF) at a dose of 7.5 mg/kg/day. G-CSF mobilized donor mononuclear cells into the peripheral blood. These cells were harvested by COM. TEC Blood Cell Separator (Fresenius AG, Bad Homburg, Germany). $CD34^+$ and $CD3^+$ cells were assessed by flow cytometric analysis. One day before transplantation, DHSCs were harvested and stored at − 70 °C.

Recipients received total lymphoid irradiation (TLI) on days − 3, -2 and − 1 before kidney transplantation. We used the Siemens ONCOR Impression LINAC System (Siemens, Berlin, Germany), a 6MV photon beam with a multileaf collimator (MLC), and a pattern of 480 ~ 510 cGy/3Fx/3D, 160 ~ 170 cGy/1Fx per single fraction. Patients received irradiation to all major lymphatic regions using anterior-posterior-posteroanterior (AP/PA) fields. The supradiaphragmatic or “mantle” field encompassed the low cervical, supraclavicular, infraclavicular, axillary, mediastinal, and pulmonary hilar nodes, as well as the thymus.

During kidney transplantation, recipients received 50 mg of rabbit anti-thymocyte globulin (ATG). On postoperative days 2, 4, and 6, the recipients received an intravenous infusion of cryopreserved DHSCs, which contained $CD34^+$ cells (0.2-3.0 × 10^6/kg) and $CD3^+$ cells (Table 2). The recipients were treated with a triple immunosuppressive regimen including calcineurin inhibitors (tacrolimus or cyclosporine A), antimetabolic drugs and corticosteroids (Fig. 1).

Table 2

The total number of infused $CD34^+$ and $CD3^+$ cells.

Patient No.	$CD34^+$cells (× 10^6/kg)	$CD3^+$cells (× 10^8/kg)
1	1.69	14.39
2	0.75	0.31
3	0.31	0.31
4	2.35	2.98
5	0.20	0.63
6	3.00	3.77
7	2.17	2.0
8	0.37	2.25
9	1.39	3.02
10	0.22	1.73
11	0.22	1.73

Patient Follow-up Assays

Chimerism was detected by means of DNA genotyping of simple sequence-length polymorphic markers that encode short tandem repeats (AmpFl STR Identifiler PCR Amplification Kit, Applied Biosystems, USA). The routine blood examination was assessed every day after TLI. The percentage of lymphocyte subsets was tested by flow cytometry. Peripheral blood mononuclear cells were stained with fluorochrome-labeled anti-CD3, CD4, CD8, CD19, CD56 and CD16 antibodies (eBioscience, San Diego, CA, USA). A mixed lymphocyte reaction was performed to detect T cell function in recipients. The recipients' transplanted kidney function was monitored by regular follow-up, and the kidney pathologic status was assessed by biopsy.

T-cell Responses To Antigens

The mixed lymphocyte reaction was performed by culturing peripheral-blood mononuclear cells as responder cells with irradiated donor or irrelated third part allogeneic mononuclear cells as stimulator cells and measuring 3^{H}-thymidine incorporation. Round-bottom culture plates were stimulated with plated-coated anti-CD3 antibody and soluble anti-CD28 antibody (both from eBioscience) in RPMI 1640 medium and then were incubated for 5 to 7 days in a humidified 37 °C, 5% CO_2 incubator. Next, 3^{H}-thymidine was added 18 h before the end of the culture period. The cells were then harvested onto glass fiber mats for the measurement of 3^{H}-thymidine incorporation.

Statistics

Data are presented as the mean ± standard deviation. Statistical analysis (SPSS 18.0 software, SPSS, Inc., Armonk, NY, USA) was performed using the two-tailed independent Student's t test (paired or unpaired) after a demonstration of homogeneity of variance with the F test. Statistical significance was set as $p < 0.05$.

Results

Donor bone marrow mobilization of hematopoietic stem cells

Before stem cell harvest, G-CSF (7.5 μg/kg/d) was used for 5 consecutive days in donors. The average baseline values of white blood cells (WBCs), neutrophils, lymphocytes and peripheral blood mononuclear cells (PBMCs) were $(6.94 \pm 1.98) \times 10^9$/L, $(4.48 \pm 1.92) \times 10^9$/L, $(1.84 \pm 1.11) \times 10^9$/L and $(0.43 \pm 0.14) \times 10^9$/L, respectively. After mobilization, the average WBC, neutrophil, lymphocyte and PBMC counts were $41.65 \pm 13.37 \times 10^9$/L, $35.79 \pm 11.24 \times 10^9$/L, $3.06 \pm 1.12 \times 10^9$/L and $1.90 \pm 0.69 \times 10^9$/L, respectively. All the above cells were significantly increased. The details are shown in Fig. 1. In the harvested DHSCs, the total numbers of $CD34^+$ cells and $CD3^+$ cells were $0.2\text{-}3.0 \times 10^6$/kg and $0.31–14.39 \times 10^8$/kg, respectively (Table 2).

Change Of Blood Cells And Lymphocyte Subsets After Tli

After TLI, a dramatic decrease in lymphocyte counts occurred in all patients immediately, reaching 0 after ATG by day 0. Before TLI (D-4), the lymphocyte count was $(1.70 \pm 0.61) \times 10^9$/L with a rapid decrease after 3

days (D-1). However, the number of white blood cells and neutrophils remained stable. After TLI at D-1, the proportions of $CD19^+$ B cells (3.23 ± 1.10 vs. 12.80 ± 5.58) significantly decreased and $CD56^+CD16^+$ NK cells (10.9 ± 2.84 vs. 15.36 ± 4.70) increased compared to those before TLI (D-1). The percentage of $CD8^+$ T cells increased, while $CD3^+$ T cells and $CD4^+$ T cells had no significant change (Table 3). Each individual data point is shown in Fig. 3. All patients had persistent lymphopenia, and then the lymphocytes gradually recovered after one month. The lymphocyte count increased and returned to the normal level approximately 6 months post transplantation.

Table 3
Lymphocytes change after TLI.

	Days post transplantation				**P value***
	-4	-3	-2	-1	
Counts (10^9/L)					
WBC	5.16 ± 1.28	4.76 ± 0.87	4.48 ± 1.29	3.80 ± 1.21	0.004
Neutrophils	2.95 ± 1.02	3.23 ± 0.84	3.23 ± 0.88	3.04 ± 0.92	0.766
Lymphocyte	1.65 ± 0.60	1.05 ± 0.37	0.87 ± 0.40	0.53 ± 0.33	< 0.001
Percentage					
$CD19^+$ B cells	12.73 ± 5.33	7.20 ± 3.22	3.85 ± 2.24	3.12 ± 1.11	< 0.001
$CD3^+$ T cells	74.71 ± 5.76	76.67 ± 6.40	75.47 ± 5.33	78.24 ± 5.15	0.193
$CD4^+$ T cells	48.16 ± 6.31	47.23 ± 6.39	42.17 ± 8.94	47.08 ± 6.50	0.547
$CD8^+$ T cells	23.46 ± 4.78	25.99 ± 6.95	28.30 ± 7.37	28.03 ± 7.67	0.007
$CD56^+CD16^+$ NK cells	11.09 ± 2.78	13.58 ± 5.16	19.21 ± 5.97	16.42 ± 5.59	0.021
WBC: white blood cell. *: comparison between D-4 and D-1.					

Adverse Events

During TLI, 1 patient experienced fatigue, and 5 patients had mild diarrhea. During DHSC infusion, 1 patient exhibited oxygen saturation decline, chest tightness and discomfort. After stopping the infusion, the symptoms improved, while the rest of the recipients did not show any adverse events. Previously, suspected graft-versus-host disease (GVHD) was reported. After one month of follow-up, skin symptoms disappeared, and GVHD did not occur in the other 10 recipients. The infection risk did not increase compared with that of the routine protocol. No bone marrow suppression was observed in 11 recipients.

Chimerism Induction

The first recipient with HLA matching induced 30–50% chimerism, which remained at 30–50% 4 to 6 weeks after kidney transplantation (Fig. 4). At 6 months, the chimerism disappeared. The other 10 recipients induced less than 1% chimerism.

T Cell Reactivity To Donor And Non-donor Allo-antigens

At 6 months after renal transplantation, we detected the T cell reactivity of recipients to donor and non-donor allo-antigens. Compared to pretreatment, T cell proliferation in recipients upon donor antigens was significantly reduced post transplantation but remained similar to that upon non-donor antigens. We also set a positive internal control using anti-CD3 and anti-CD28 antibody stimulation. For the external control, we tested T cell reactivity from a healthy volunteer (Fig. 5). These results demonstrated that our protocol induced a donor-specific low response of T cells in recipients.

Long-term Follow-up Results

By December 31, 2019, the median follow-up period was 3,671 days. Nine recipients underwent protocol biopsy 1–3 years after transplantation. Only one recipient had mild rejection (Banff IA grade, Banff 07 criteria), and the remaining 10 had no rejection (Table 4). The first recipient presented renal allograft dysfunction at 4 years post transplantation. Proteinuria and poor control of hypertension occurred in the fourth year. The biopsy indicated glomerulonephritis relapse without rejection. Therefore, this patient returned to immunosuppressive drug treatment. Unfortunately, he had to receive hemodialysis at 4 years after kidney transplantation due to allograft loss. Ten patients had stable allograft function. Among them, 5 patients reduced the dosage of immunosuppressive agents, 4 patients used tacrolimus 1.0 to 3.0 mg/d, and 1 patient used rapamycin 1 mg/d, mofetil mycophenolate 0.5 to 1 g/d and prednisone 5 mg/d.

Table 4
Pathology results of kidney biopsy.

Patient No.	1st biopsy		2nd biopsy		3rd biopsy		4th biopsy	
	POD	Result	POD	Result	POD	Result	POD	Result
1	41	Normal	1218	Suspected CNI toxicity				
2	91	IA	201	AR IA	369	Normal		
3	53	Normal						
4	92	Normal	185	Normal	365	Normal	731	Normal
5	3534	Normal						
6	41	Normal	194	Normal	360	Normal		
7	NA							
8	45	Normal	249	Normal				
9	94	Normal						
10	380	Normal	1777	Normal				
11	NA							

POD: postoperative day; CNI: calcineurin inhibitor; AR: acute rejection

Discussion

In this clinical trial, through kidney transplantation combined with DHSC infusion as well as TLI pretreatment, we induced mixed chimerism in one patient and reduced the dosage of immunosuppressive agents in four patients. Only one patient experienced mild rejection.

In 2008, the Massachusetts General Hospital of Harvard University and Stanford University were the first institutions to report the successful induction of immune tolerance in living kidney transplantation [4, 5]. In 2012, Northwestern University reported the clinical trial result of induction immune tolerance using hematopoietic stem cells combined with promoting cells (FCRx) in living kidney transplantation [6]. At Massachusetts General Hospital, operational tolerance was induced in 7 of 10 patients, 4 of whom remained off IS [4, 7]. The Stanford University protocol found that 24/29 HLA-identical living donor kidney transplant recipients withdrew IS. However, this protocol has not been successful in HLA-mismatched donor/recipient pairs [8, 9]. A total of 26/37 HLA-mismatched, unrelated and related living donor kidney transplant recipients were removed from IS at Northwestern University [6, 10].

Our protocol was similar to that of Stanford University, but the donor hematopoietic cell product did not control for the dose of $CD34^+$ and $CD3^+$ cells. We used TLI 3 days before kidney transplantation. Each dose was 160 ~ 170 cGy, and the total dose in 3 days was 480 ~ 510 cGy. However, Stanford University used TLI

after kidney transplantation (80 ~ 120 cGy × 10 times, a total of 800 ~ 1,200 cGy) [8, 9]. As we were the first to carry out clinical studies on kidney transplantation immune tolerance induction in China, we integrated irradiation protocols of Harvard and Stanford and developed our protocol for safety and efficacy. Learning from the theory of re-education of autologous or allogeneic hematopoietic stem cell transplantation in autoimmune diseases and leukemia [11–15], we infused a small dose of hematopoietic stem cells three times to prevent GVHD. Our long-term follow-up results showed that none of the patients had GVHD.

It is widely believed that successful induction of chimerism is essential for the induction of immune tolerance [3, 9, 16, 17], and the three major centers in the United States also induce different degrees of chimerism. Harvard University induces transient chimerism [18], Stanford University induces mixed chimerism, and Northwestern University induces complete chimerism [19–21]. The Stanford University program successfully achieved continuous mixed chimerism in HLA-matched living kidney transplantation and completely withdrew immunosuppressive drugs. This protocol has also successfully achieved continuous mixed chimerism in HLA-mismatched living kidney transplantation; however, complete discontinuation of immunosuppressive drugs has not been achieved. Although immune tolerance was not induced in HLA-mismatch recipients, they found that persistent mixed chimerism prevented both acute and chronic rejection [8, 9]. In our study, 2 donors were HLA-matched, 9 were HLA haplotype matched, and 1 recipient induced 30–50% chimerism. Unfortunately, chimerism disappeared after half a year. In addition, the chimeric rate of the other recipients was low. This may be the reason complete immune tolerance was not induced.

The ATG dose in our protocol was relatively low compared with the corresponding doses used at the three US centers, and they also used cyclophosphamide, anti-CD2 antibody and fludarabine; second, we used noncontrolling hematopoietic stem cells. The number of $CD34^+$ cells infused was lower, but the number of $CD3^+$ cells was higher. Stanford University controlled hematopoietic stem cell transplantation with $CD34^+$ cells (10×10^6/kg) and $CD3^+$ cells (1×10^6/kg). Our long-term follow-up results showed that 11 patients did not have GVHD or severe infection, and two patients had slight leukopenia for long-term follow-up, suggesting that our TLI protocol is safe. Therefore, we can increase the strength of pretreatment in the future, including increasing the TLI and/or ATG dose.

The risks posed by our protocol are mainly infection and myelosuppression. To prevent infection, all recipients were placed in the laminar flow chamber after kidney transplantation (for approximately one week in the first five patients). Next, we attempted to reduce the time in the laminar flow chamber to 2–3 days. Only one recipient had pulmonary infection. In this study, the first patient had recurrence of nephritis and loss of renal function 4 years after kidney transplantation. At Northwestern University, 2/10 recipients had nephritis relapse [20], which also suggested that we should be strictly screened when enrolling recipients and that caution should be taken when reducing or discontinuing immunosuppressive agents. Although the remaining 10 recipients were followed up for stable allograft function, we did not monitor the DSA. In the future, we should test DSA in all recipients.

Conclusion

Induction of immune tolerance in kidney transplantation remains very difficult, especially in recipients with HLA mismatches. This study shows that, in China, our protocol is safe and feasible without increasing the

risk of infection.

Abbreviations

DHSC
donor hematopoietic stem cell
IS
immunosuppression
PRA
panel reactive antibodies
G-CSF
granulocyte colony-stimulating factor
TLI
total lymphoid irradiation
MLC
multileaf collimator
AP/PA
anterior-posterior-posteroanterior
ATG
anti-thymocyte globulin
WBC
white blood cell
PBMCs
peripheral blood mononuclear cells
GVHD
graft-versus-host disease
CNI
calcineurin inhibitor
AR
acute rejection

Declarations

Ethical approval and consent to participate

The study was registered in the Chinese Clinical Trial Registry (http://www.chictr.org/, ChiCTR-TNC-09000399) with written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors have no competing interests to declare.

Funding

This study was supported by the National Natural Science Foundation of China (81800659 to XW, 81770746 to CY, 81770747 and 81970646 to RR), National Key R&D Program of China (2018YFA0107502 to CY, 2018YFA0107501 to RR), Shanghai Rising-Star Program (19QA1406300 to CY), the Medical and Health Talents Training Plan for the Excellent Youth of Shanghai Municipal (2018YQ50 to CY), Project ELITE: A Special Supportive Program for Organ Transplantation by COTDF (2019JYJH05 to CY) and the Science and Technology Commission of Shanghai Municipality (16431902300 to TZ).

Authors' contributions

Xuanchuan Wang and Cheng Yang drafted the manuscript. Tongyu Zhu and Ruiming Rong conceived the study. Bing Chen, Zhaochong Zeng and Zheng Wei participated in the protocol design. Yuan Ji provided the pathologic assessment. Ming Xu, Ruiming Rong and Tongyu Zhu performed the surgery. Qunyu Tang and Linkun Hu provided clinical laboratory examinations. All authors read and approved the final version of the manuscript.

Acknowledgments

Not applicable.

References

1. Coemans M, Susal C, Dohler B, Anglicheau D, Giral M, Bestard O, Legendre C, Emonds MP, Kuypers D, Molenberghs G, et al: **Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015.** *Kidney Int* 2018, **94:**964-973.

2. Kawai T, Leventhal J, Wood K, Strober S: **Summary of the Third International Workshop on Clinical Tolerance.** *Am J Transplant* 2019, **19:**324-330.
3. Zuber J, Sykes M: **Mechanisms of Mixed Chimerism-Based Transplant Tolerance.** *Trends Immunol* 2017, **38:**829-843.
4. Kawai T, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL, Shaffer J, Preffer FI, Ding R, Sharma V, et al: **HLA-mismatched renal transplantation without maintenance immunosuppression.** *N Engl J Med* 2008, **358:**353-361.
5. Scandling JD, Busque S, Dejbakhsh-Jones S, Benike C, Millan MT, Shizuru JA, Hoppe RT, Lowsky R, Engleman EG, Strober S: **Tolerance and chimerism after renal and hematopoietic-cell transplantation.** *N Engl J Med* 2008, **358:**362-368.
6. Leventhal J, Abecassis M, Miller J, Gallon L, Ravindra K, Tollerud DJ, King B, Elliott MJ, Herzig G, Herzig R, Ildstad ST: **Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation.** *Sci Transl Med* 2012, **4:**124ra128.
7. Spitzer TR, Sykes M, Tolkoff-Rubin N, Kawai T, McAfee SL, Dey BR, Ballen K, Delmonico F, Saidman S, Sachs DH, Cosimi AB: **Long-term follow-up of recipients of combined human leukocyte antigen-matched bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease.** *Transplantation* 2011, **91:**672-676.
8. Scandling JD, Busque S, Shizuru JA, Lowsky R, Hoppe R, Dejbakhsh-Jones S, Jensen K, Shori A, Strober JA, Lavori P, et al: **Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation.** *Am J Transplant* 2015, **15:**695-704.
9. Scandling JD, Busque S, Lowsky R, Shizuru J, Shori A, Engleman E, Jensen K, Strober S: **Macrochimerism and clinical transplant tolerance.** *Hum Immunol* 2018, **79:**266-271.
10. Leventhal JR, Miller J, Mathew JM, Kurian S, Tambur AR, Friedewald J, Charrette J, Abecassis MM: **Updated follow-up of a tolerance protocol in HLA-identical renal transplant pairs given donor hematopoietic stem cells.** *Hum Immunol* 2018, **79:**277-282.
11. Down JD, White-Scharf ME: **Reprogramming immune responses: enabling cellular therapies and regenerative medicine.** *Stem Cells* 2003, **21:**21-32.
12. Talmadge JE, Singh R, Ino K, Ageitos A, Buyukberber S: **Mechanisms of immune dysfunction in stem cell transplantation.** *Int J Immunopharmacol* 2000, **22:**1041-1056.
13. Ullah MA, Hill GR, Tey SK: **Functional Reconstitution of Natural Killer Cells in Allogeneic Hematopoietic Stem Cell Transplantation.** *Front Immunol* 2016, **7:**144.
14. Picardi A, Mengarelli A, Marino M, Gallo E, Benevolo M, Pescarmona E, Cocco R, Fraioli R, Tremante E, Petti MC, et al: **Up-regulation of activating and inhibitory NKG2 receptors in allogeneic and autologous hematopoietic stem cell grafts.** *J Exp Clin Cancer Res* 2015, **34:**98.
15. Abrahamsson S, Muraro PA: **Immune re-education following autologous hematopoietic stem cell transplantation.** *Autoimmunity* 2008, **41:**577-584.
16. Sasaki H, Oura T, Spitzer TR, Chen YB, Madsen JC, Allan J, Sachs DH, Cosimi AB, Kawai T: **Preclinical and clinical studies for transplant tolerance via the mixed chimerism approach.** *Hum Immunol* 2018,

79:258-265.

17. Yolcu ES, Shirwan H, Askenasy N: **Mechanisms of Tolerance Induction by Hematopoietic Chimerism: The Immune Perspective.** *Stem Cells Transl Med* 2017, **6:**700-712.
18. Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E, Tolkoff-Rubin N, Preffer F, Crisalli K, Gao B, et al: **Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression.** *Am J Transplant* 2014, **14:**1599-1611.
19. Leventhal J, Abecassis M, Miller J, Gallon L, Tollerud D, Elliott MJ, Bozulic LD, Houston C, Sustento-Reodica N, Ildstad ST: **Tolerance induction in HLA disparate living donor kidney transplantation by donor stem cell infusion: durable chimerism predicts outcome.** *Transplantation* 2013, **95:**169-176.
20. Leventhal JR, Mathew JM, Salomon DR, Kurian SM, Suthanthiran M, Tambur A, Friedewald J, Gallon L, Charette J, Levitsky J, et al: **Genomic biomarkers correlate with HLA-identical renal transplant tolerance.** *J Am Soc Nephrol* 2013, **24:**1376-1385.
21. Leventhal JR, Elliott MJ, Yolcu ES, Bozulic LD, Tollerud DJ, Mathew JM, Konieczna I, Ison MG, Galvin J, Mehta J, et al: **Immune reconstitution/immunocompetence in recipients of kidney plus hematopoietic stem/facilitating cell transplants.** *Transplantation* 2015, **99:**288-298.

Figures

Figure 1

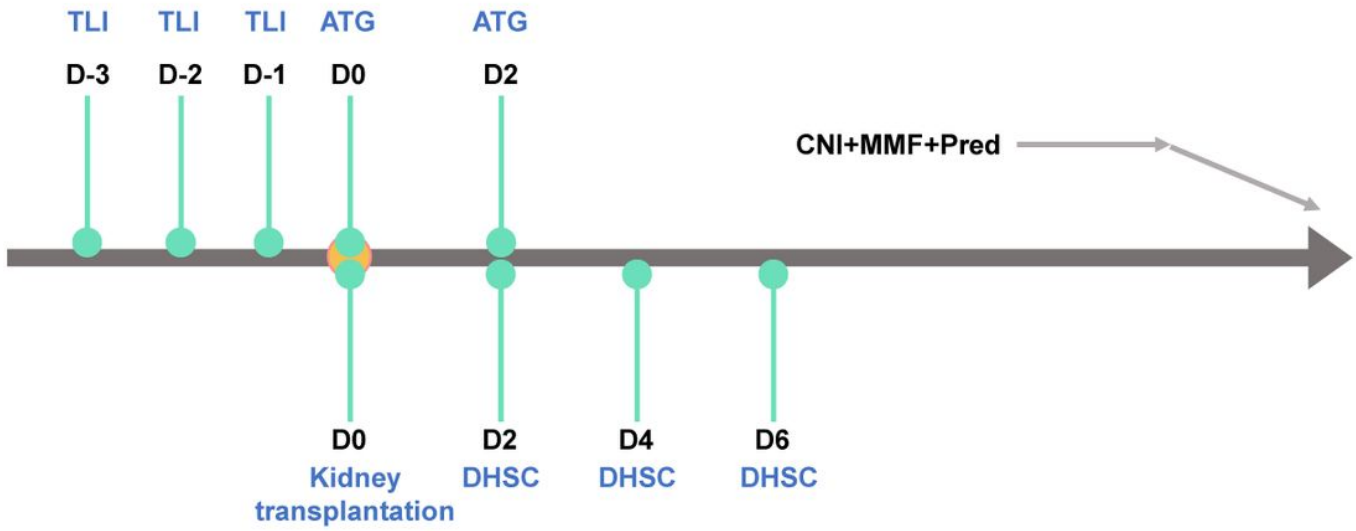

Figure 1

Treatment of recipients. Before kidney transplantation, recipients received TLI 3 times at D-3, D-2 and D-1. The induction drug was ATG, which was administered at D0 and D2. After transplantation, DHSCs were infused at D2, D4 and D6. The maintenance IS consisted of CNI, MMF and prednisone, which were gradually reduced.

Figure 2

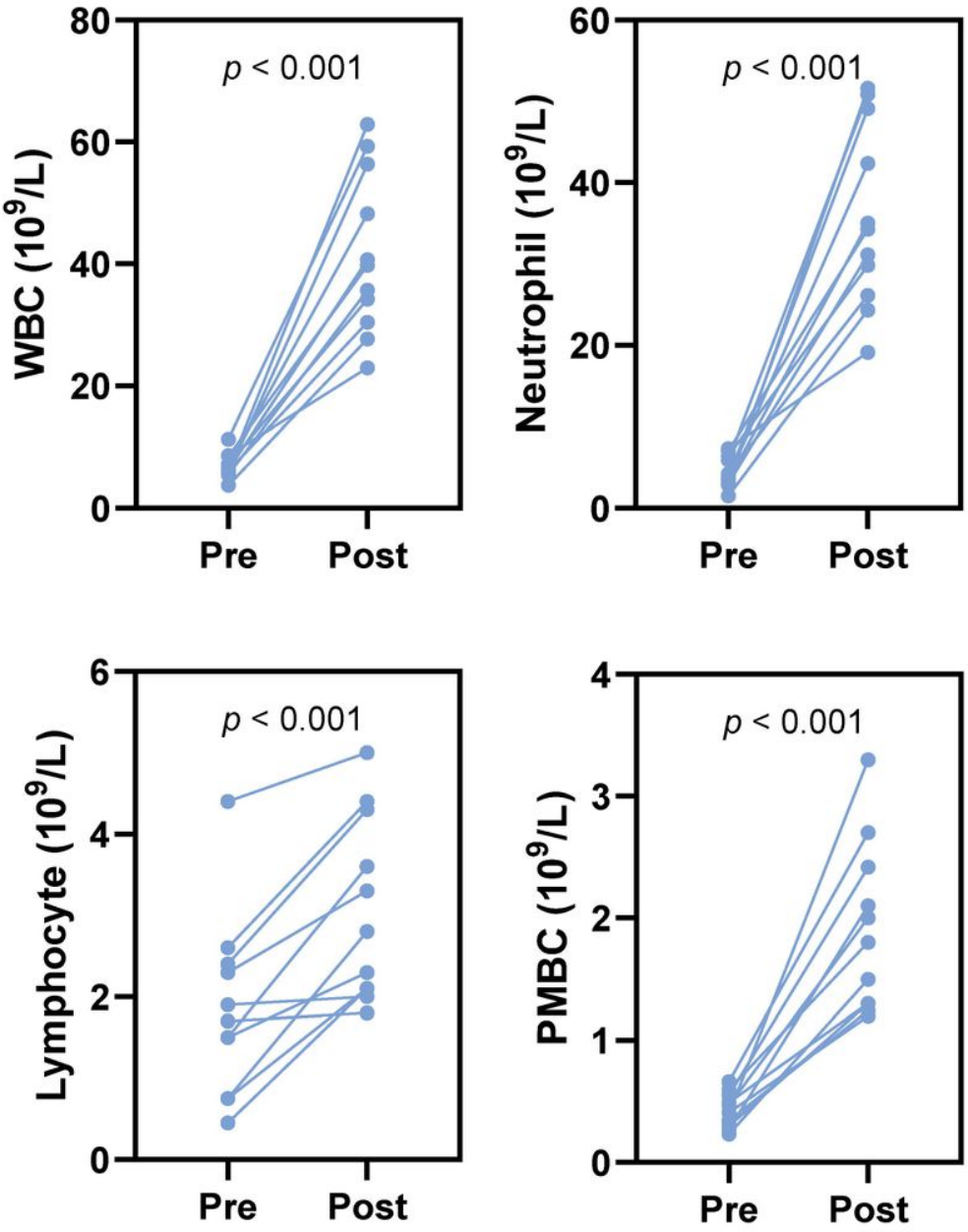

Figure 2

Changes in WBCs, neutrophils, lymphocytes and PBMCs in donors. After bone marrow mobilization, the number of WBCs, neutrophils, lymphocytes and PBMCs significantly increased in donors. WBC: white blood cell; PMBC: peripheral blood mononuclear cell.

Figure 3

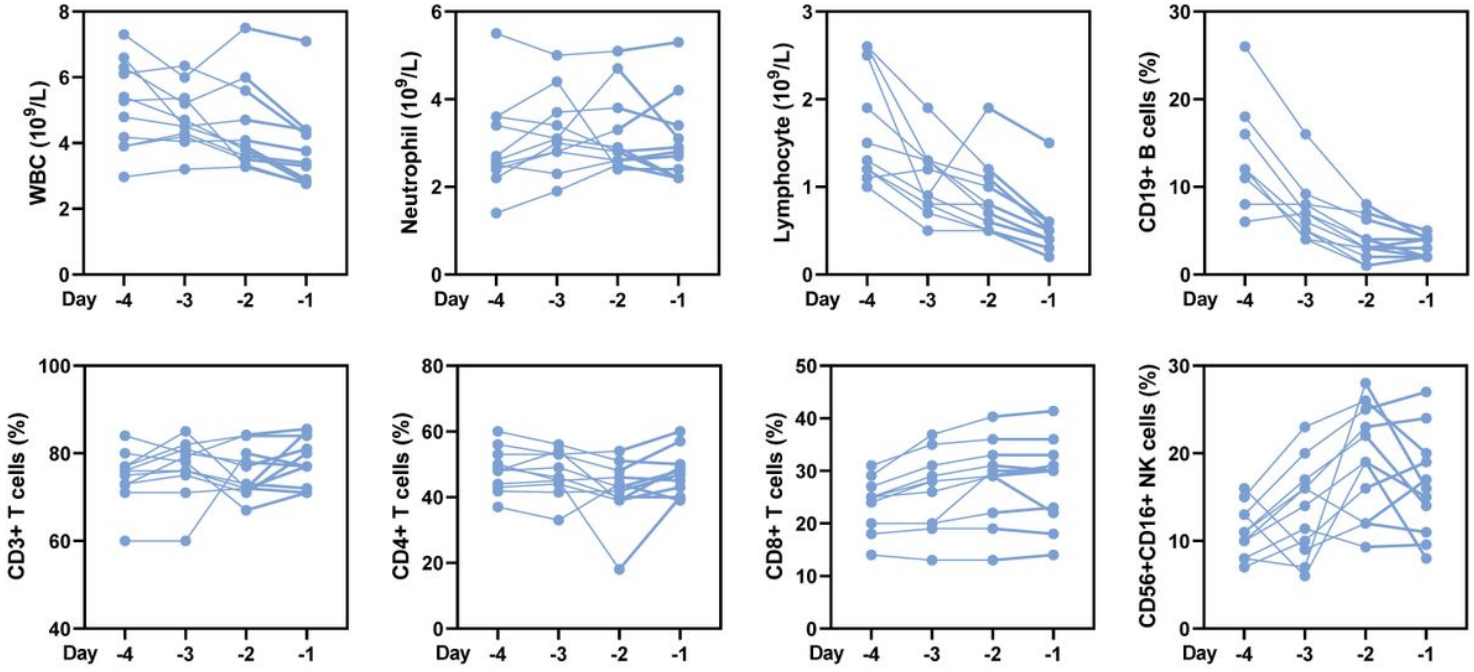

Figure 3

Immune cell changes in recipients after TLI. This figure shows the change in WBC, neutrophil and lymphocyte counts, as well as T cells, B cells and NK cells (% in lymphocytes) before and after TLI. The data at D-4 represented the baseline level before TLI. WBC: white blood cell.

Figure 4

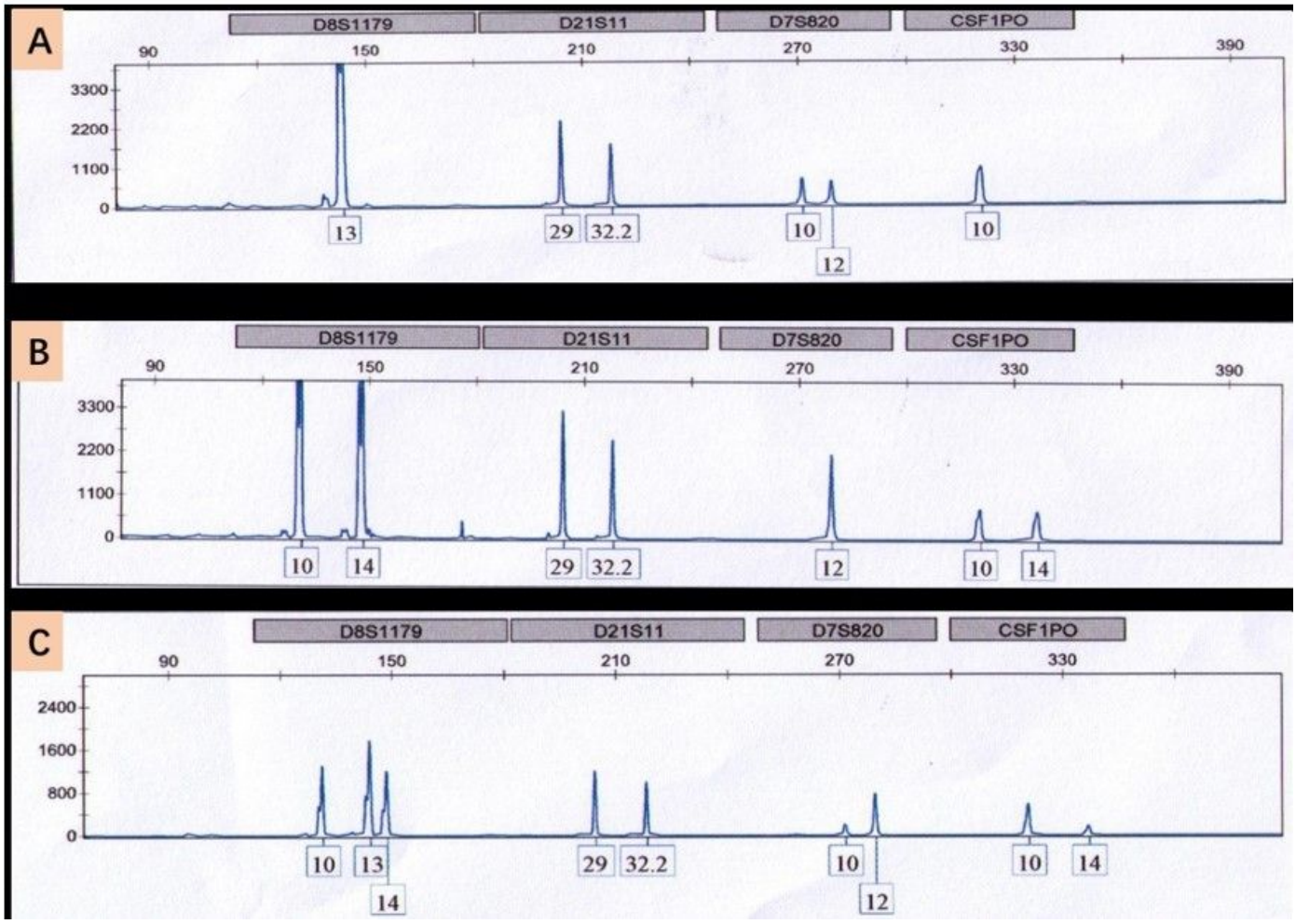

Figure 4

Chimerism on D8S1179 loci. (A) Loci 13 was observed in the donor. (B) Loci 10 and 14 were observed in the recipient before transplantation. (C) Two weeks after kidney transplantation, loci 13 from the donor were detected in the recipient.

Figure 5

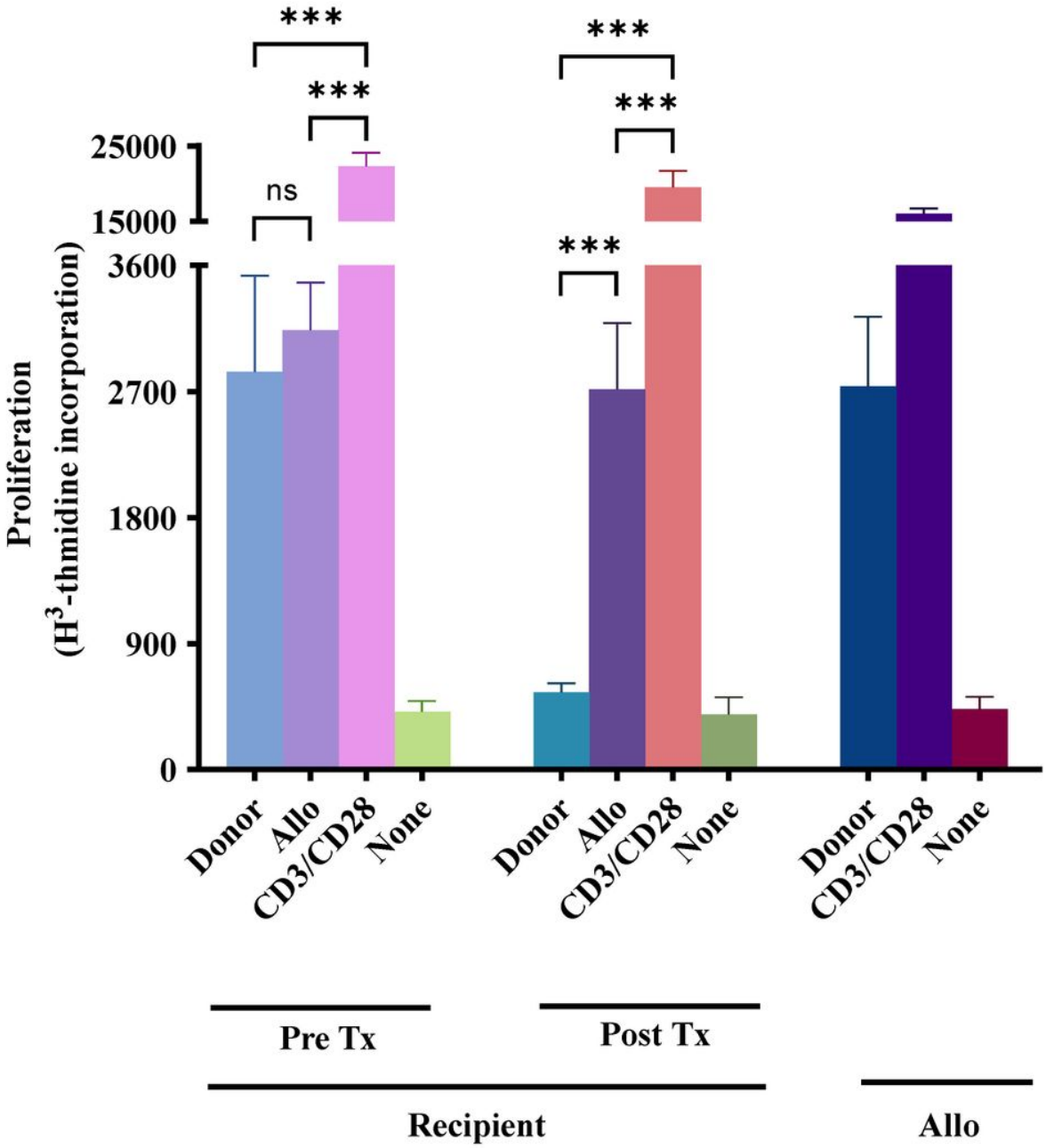

Figure 5

Mixed lymphocyte reaction. This figure represents the T cell reactivity of patient #2. Before kidney transplantation and pretreatment, recipient T cell proliferation significantly increased upon donor or third-part alloantigen stimulation, but after transplantation, T cell proliferation upon donor antigen stimulation significantly decreased compared to that of the third-part alloantigen, suggesting a low immune response of T cells specifically to the donor. To test internal quality control, we examined other T cells from a healthy

volunteer. These T cells demonstrated reactivity under both the donor and third part allo-antigens. CD3/CD28 stimulation was used as a positive control.