Novel diagnostics and therapeutics to prevent injury in native and transplanted kidneys

Groeneweg, K.E.

Chapter 6

Autologous bone marrow derived mesenchymal stromal cell therapy with early tacrolimus withdrawal: the randomized, prospective, single-center, open-label, TRITON study

Marlies E.J. Reinders, Koen E. Groeneweg, Sanne H. Hendriks, Jonna R. Bank, Geertje J. Dreyer, Aiko P.J. de Vries, Melissa van Pel, Helene Roelofs, Volkert A.L. Huurman, Paula Meij, Dirk J.A.R. Moes, Willem E. Fibbe, Frans H.J. Claas, Dave L. Roelen, Cees van Kooten, Jesper Kers, Sebastiaan Heidt, Ton J. Rabelink, Johan W. de Fijter

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Abstract

Background

After renal transplantation, there is a need for immunosuppressive regimens which effectively prevent allograft rejection, while preserving renal function and minimizing side effects. From this perspective, mesenchymal stromal cell (MSC) therapy is of interest.

Methods

In this randomized prospective, single-center, open-label trial, we compared MSCs infused 6 and 7 weeks after renal transplantation and early tacrolimus withdrawal with a control tacrolimus group. Primary end point was quantitative evaluation of interstitial fibrosis in protocol biopsies at 4 and 24 weeks posttransplant. Secondary end points included acute rejection, graft loss, death, renal function, adverse events, and immunological responses.

Results

Seventy patients were randomly assigned of which 57 patients were included in the final analysis (29 MSC; 28 controls). Quantitative progression of fibrosis failed to show benefit in the MSC group and GFR remained stable in both groups. One acute rejection was documented (MSC group), while subclinical rejection in week 24 protocol biopsies occurred in seven patients (four MSC; three controls). In the MSC group, regulatory T cell numbers were significantly higher compared to controls (p=0.014, week 24).

Conclusions

In conclusion, early tacrolimus withdrawal with MSC therapy was safe and feasible without increased rejection and with preserved renal function. MSC therapy is a potentially useful approach after renal transplantation.

Introduction

Over the last two decades significant progress has been achieved in short-term survival of kidney transplants.[1,2] Unfortunately, these advancements have not led to a similar improvement in long-term kidney transplant survival rates. Various factors, including donor graft quality, ischemia/reperfusion (I/R) injury, alloreactivity, viral infections, and drug therapy, may adversely affect renal structure causing graft scarring and compromising long-term function.[3] The intensity of current immunosuppressive drugs, albeit efficacious in preventing rejection, is associated with increased risk for (viral) infections and malignancies. Calcineurin inhibitors (CNIs) are the cornerstone of current immunosuppressive therapy, but they have direct nephrotoxic effects. It has been demonstrated that CNI withdrawal should be undertaken before month 6 to prevent the occurrence of irreversible tubulointerstitial damage.[4,5] So far, early CNI withdrawal studies have proven to be risky and invariably lead to increased rejection and even loss of grafts.[6] Consequently, there is a need for immunosuppressive regimens that can prevent allograft rejection, while preserving renal function and promoting patient and graft survival in the long term. MSCs have immunosuppressive properties and roles in tissue repair, and various (mainly experimental) studies have demonstrated that MSCs may increase regulatory T cell (Treg) levels and polarize the immune system toward tolerance.[7,8] In renal transplantation, early studies using MSCs focused on safety and feasibility.[9-12] Although most of these studies were not designed as efficacy trials, there were indications that MSCs possess immunosuppressive properties, as evidenced by an increase in Tregs and downregulation of cytotoxic $CD8T^{+}$ cells in a small number of patients. We performed a randomized, prospective, single-center, open-label study in living-donor kidney transplant recipients in which we compared autologous bone marrow (BM)-derived MSC therapy (infused at weeks 6 and 7) with concomitant early tacrolimus withdrawal (at week 8) to standard tacrolimus dose. Primary end point was quantitative evaluation of interstitial fibrosis and secondary end points included biopsy-proven acute rejection, graft loss, death, renal function, adverse events, and immunological responses at week 24. We chose to perform the study on a background of alemtuzumab-based induction to minimize the risk for acute rejection[13] and mTOR inhibition, since experimental studies demonstrated tolerogenic properties in combination with MSCs.[14] In a post hoc long-term analysis, peripheral blood immune cell composition was also obtained at week 52 in patients with sufficient follow-up. In addition, the efficacy end point (biopsy-proven acute rejection (BPAR), graft loss, or death) was obtained up to 5 years in patients who had a longer follow-up.

Materials and methods

Study design and patients

The TRITON study is a 24-weeks investigator-initiated, randomized, prospective, open-label, single-center, clinical study, performed at the Leiden University Medical Center (LUMC), the Netherlands. The trial design has been published previously.[15] The trial protocol, available at the Appendix S1 and S2 section, was approved by the local ethics committee at the LUMC, Leiden, and by the Central Committee on Research involving Human Subjects (CCMO) in the Netherlands. The trial was performed in accordance with the principles of the Declaration of Helsinki. In total, 70 de novo renal recipients of a kidney from a living donor, 18–75 years of age, were recruited from the transplant clinics of the LUMC. The inclusion/exclusion criteria were described previously.[15] Written informed consent was obtained from all participants.

Randomization and masking

Patients were randomly assigned before transplantation to either the MSC or control group in a ratio 1:1 (*Figure S1*). A patient was randomized only after verification of eligibility and informed consent. The randomization procedure was designed and implemented by the IMO (Informatie Management Onderzoek) department of the University Medical Center Groningen (UMCG), the Netherlands, using a web-based system (ALEA). Investigator or authorized delegate from the study staff received an individual login code with which they could randomize their patients. The web application returned the allocated treatment. As a confirmation, the web application also sent an e-mail with the randomization information to selected users. Patients maintained this randomization number throughout the study. Because of the nature of the intervention (BM biopsy and MSC infusions), participants and physicians were not masked to treatment assignment.

Procedures

All patients in the study received alemtuzumab (anti-CD52),15 mg subcutaneously, at days 0 and 1 as well as tacrolimus (Prograft®), everolimus (EVL; Certican®), and low-dose prednisone, as maintenance therapy (*Figure S1*).[15] Patients in the MSC group received two doses of autologous BM MSCs, intravenously at weeks 6 and 7 after transplantation. Autologous MSCs were chosen instead of third-party MSCs to prevent alloimmunization. The dose of tacrolimus was reduced to 50% at the time of the second MSC infusion and completely withdrawn 1 week later. Patients received a higher dose of prednisolone (15 mg instead of 10 mg) for 14 days after the second infusion to diminish risks of tacrolimus withdrawal. In patients in the control group, the trough level of tacrolimus was lowered to a target of 6–8 ng/ml 8 weeks after transplantation. BM was aspirated from the posterior iliac crest of all patients in the MSC group under general anesthesia during the renal transplantation, as described previously.[15] This protocol was approved by the local ethics

committee (P13.283) and by the CCMO (NL4371200013). Processing of the MSCs took place at the Interdivisional Good Manufacturing Practice (GMP) Facility of the LUMC (*Table S1*).[15] The MSC product was infused via peripheral infusion within 30 min with a target dose of 1.5×10^6 per/kg body weight IV (range $1–2 \times 10^6$), according to our previous study.[15] Monitoring of the patients occurred according to the assessment schedule, as described in the protocol (page 28).

Outcomes

The primary end point was the quantitative progression of interstitial fibrosis between the 4- and 24-week protocol biopsies as measured by morphometric analysis of collagen deposition. Interstitial collagen fibers in protocol biopsies were visualized by Sirius Red (SR) staining and quantified as a percentage of total tubulointerstitial tissue (glomeruli and large vessels excluded) by quantifying positive pixels in five representative locations at 40× magnification with a macro created in ImageJ version 1.50i.[16] Included secondary end points were composite end point efficacy failure (BPAR, graft loss, or death); proteinuria, Banff scores at the protocol biopsies, renal function as measured by estimated (e) glomerular filtration rate (GFR), (serious) adverse events ((S)AE), including (viral) infections, the presence of de novo donor-specific antibodies (dnDSA), and peripheral blood immune cell composition. Scoring of renal biopsies was performed in a blinded fashion by a renal pathologist from our center after completion of the study, using the most recent Banff classification.[17] Findings in a protocol biopsy with evidence of rejection were reported as subclinical acute rejection (SCAR). Renal function was calculated by the eGFR (ml/min/1.73 m^2) using the CKD-EPI formula.[15] AEs and SAEs were documented according to Medical Dictionary for Regulatory Activities (MedDRA®); the international medical terminology developed under the auspices of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Tacrolimus and EVL quantification was assessed using a previously validated LC–MS/MS assay.[18]

Immunological monitoring

For human leukocyte antigen (HLA) antibody analysis, serum samples were screened using Luminex screen assay (Lifecodes, Immucor) and analyzed with a Luminex 200 reader. Definitions of the negative/positive discriminations were used as suggested by the provider. When positive, a single antigen bead (SAB) assay (Lifecodes, Immucor) was performed as standard-of-care. Assignment of positivity was assessed according to the manufacturer's instructions. Since MSCs are suggested to have immunomodulatory properties, we performed phenotypical analysis of leukocyte subpopulations on fresh whole blood. Staining, acquisition, and data analysis were performed strictly adhering to “The One” study protocol.[19] Absolute cell counts were obtained using the BD Multitest kit (BD Biosciences).

Post hoc analysis

Phenotypical analysis of leukocyte subpopulations was, in addition to the 24-week time point, also performed 52 weeks after renal transplantation. Assessment of composite end point efficacy failure (BPAR, graft loss, or death) and renal function by eGFR was also obtained in patients with a follow-up up to 5 years in a post hoc analysis (n = 52 at 1 year, n = 40 at 2 years, n = 24 at 3 years, n = 17 at 4 years, and n = 13 at 5 years, *Table 4*).

Statistical analysis

The study was designed to have a sample size of 25 in each group, or 50 in total, to have a power to detect a relative difference in mean percentages of fibrosis of at least 25% using an independent sample t test with a 0.05 two-sided significance level (α), as described previously.[15] We anticipated that 70% of the included patients would have valid measurements (withdrawal included) and therefore included 70 patients. Data analysis was performed using SPSS version 25.0 (SPSS, Inc.) and all graphs were created using GraphPad Prism version 8.0 (GraphPad Prism Software, Inc.). Parametric data were described as mean ± SD, nonparametric data as median and interquartile range (IQR), and categorical data as numbers and percentages. $p<0.05$ were considered statistically significant. The slopes of eGFR data were calculated and analyzed using a linear regression analysis. Immune monitoring data were analyzed using the Mann–Whitney test with Bonferroni correction for multiple testing. A data safety monitoring board (DSMB) monitored the safety of subjects. The trial is registered with ClinicalTrials.gov, NCT02057965.

Results

Patients

Between March 3, 2014 and January 17, 2020, 70 patients, aged 19 to 74 years, were enrolled in the study: 36 patients were randomly assigned to the MSC group and 34 to the control group (*Figure 1*). Thirteen patients did not receive allocated treatment, because of abnormal MSC growth (defined as karyotypic abnormalities in the final product; *n*=4), contra indication for MSC infusion due to the COVID-19 pandemic (*n*=1), impossibility of obtaining a baseline renal biopsy (*n*=2 in MSC and *n*=1 in control group), withdrawn informed consent (*n*=4 in control group) and (relative) contra indication for prednisone usage (*n*=1 control group). In total, 29 patients were assigned to the MSC and 28 to the control group (*Figure 1*). Patient baseline characteristics were similar in both groups (*Table 1*). Of the 29 patients in the MSC group, 28 patients received two infusions of MSCs, all within the proposed range. One patient received one dose of MSCs within the proposed range. The second dose was not given because of the COVID-19 pandemic. This patient gave informed consent to continue the study. All patients had stable vital signs before and after MSC infusion monitored using MEWS (*Table S1*). In 28 patients in the MSC group and 23

patients in the control group, two renal biopsies could be obtained (*Figure 1*), in order to assess the quantitative progression of interstitial fibrosis.

Quantitative progression of fibrosis score

The quantitative progression of fibrosis score in the biopsies was similar in both groups (MSC group 1.0 ± 7.9; control group 0.3 ± 7.8, *p*=0.755). The fibrosis score remained stable both within the MSC (week 4, 15.2 ± 6.6 and week 24, 16.2 ± 5.3, *p*=0.526) and control group (week 4, 17.0 ± 4.6 and week 24 17.3 ± 5.7, *p*=0.870) (*Figure 2*; *Figure S2*). Delta Banff scores from 4 to 24 weeks were similar in the two groups, in particular the delta ti-score (*p*=0.8), the delta interstitial fibrosis/tubular atrophy (IFTA) score (*p*=0.4), and the delta ah-score (*p* = 0.4) (*Figure S3*).

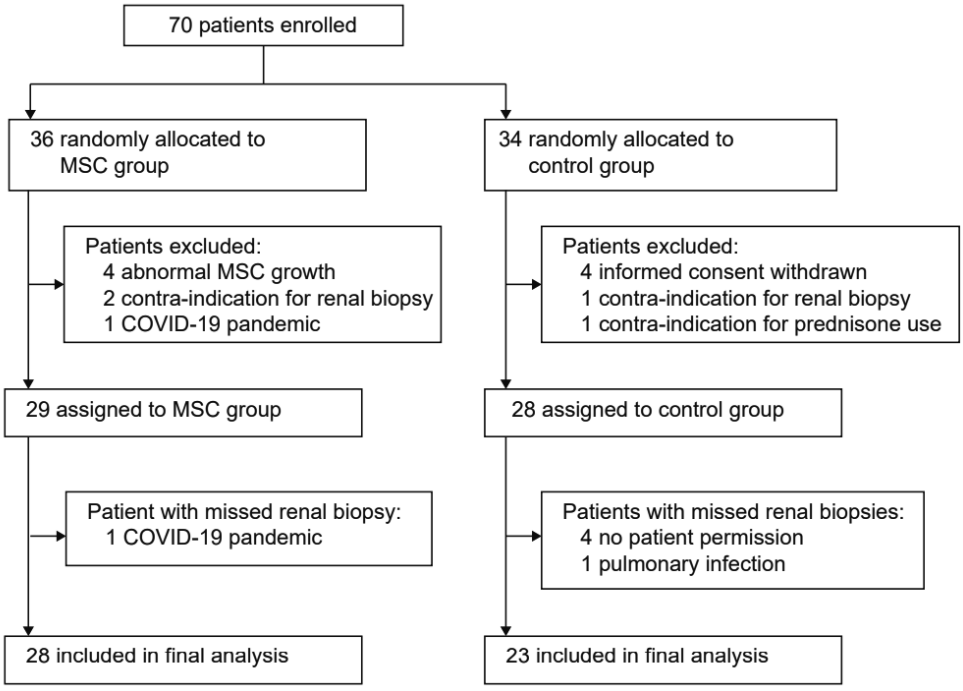

Figure 1. Trial profile. *MSC = mesenchymal stromal cell.*

Table 1. *Baseline Characteristics.*

Characteristic	MSC group (n=29)	Control group (n=28)
Recipient		
Age, mean (SD), yr	50 (14%)	50 (15%)
Male sex, no. (%)	26 (90%)	20 (71%)
Body weight, mean (SD), kg	81 (14%)	82 (14%)
Primary diagnosis, no. (%)		
IgA nephropathy	7 (24%)	3 (11%)
Hypertension	3 (10%)	9 (32%)
Polycystic kidney disease	9 (31%)	3 (11%)
Diabetes	5 (17%)	0
Reflux nephropathy	0	2 (7%)
Membranous nephropathy	1 (3%)	1 (4%)
Lupus nephritis	1 (3%)	0
Other	2 (7%)	3 (11%)
Unknown	1 (3%)	7 (25%)
Donor		
Age, mean (SD), yr	55 (13%)	51 (11%)
Male sex, no. (%)	14 (48%)	10 (36%)
eGFR (pre-donation), mean (SD)	109.7 (12.0)	109.3 (12.7)
Transplant		
Type, related, no. (%)	13 (45%)	15 (54%)
HLA A/B mismatch, mean (SD)	2.3 (1.3)	2.4 (0.9)
HLA DQ/DR mismatch, mean (SD)	1.2 (0.6)	1.3 (0.5)
Cold-ischemia time, mean (SD), hr	3.1 (0.6)	3.0 (0.5)
1st warm ischemia time, mean (SD), min	3.7 (2.1)	5.2 (4.3)
2nd warm ischemia time, mean (SD),	27.0 (3.7)	31.1 (14.4)
Cytomegalovirus IgG status, no. (%)		
D+ / R+	9 (31%)	6 (21%)
D+ / R-	7 (24%)	9 (32%)
D- / R+	1 (3%)	2 (7%)
D- / R-	12 (41%)	11 (39%)
Epstein-Barr virus IgG D+/R, no. (%)	1 (3%)	1 (4%)

GFR = glomerular filtration rate, HLA = Human Leukocyte Antigen. All data are described as mean (SD) or No. (%) (mentioned in every specific variable row).

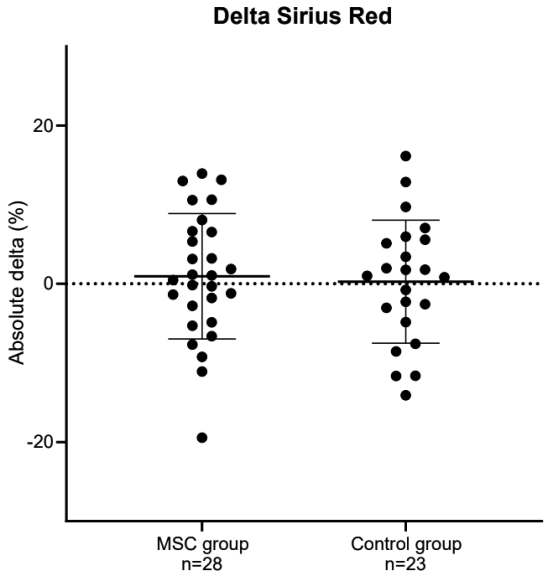

Figure 2. Interstitial fibrosis scores. *Quantitative progression of interstitial fibrosis (delta Sirius Red) between the 4 and 24-week renal biopsy (percentage). MSC = mesenchymal stromal cell.*

Table 2. *Secondary endpoints (graft loss, renal function, biopsy scores) during the study period of 24 weeks.*

Endpoint study period of 24 weeks	**MSC group (n=29)**	**Control group (n=28)**
Graft loss, No. (%)	0	0
eGFR <30ml/min/1.73m2, No. (%)	0	3 (12%)
Patients with for-cause biopsies, No. (%)	1 (3%)	4 (14%)
ABMR, TCMR II and BK nephropathy	1	
BK nephropathy		1
Acute tubular necrosis		1
Hyaline thickening		1
No abnormalities		1
4 wks	29 (100%)	28 (100%)
ABMR	1	0
No rejection	28	28
24 wks	28 (97%)	23 (82%)
TCMR IA	1	2
ABMR	2[a]	0
ABMR and TCMR IA	1	1
No rejection	24	20

MSC = mesenchymal stromal cell; eGFR = estimated glomerular filtration rate; ABMR = antibody mediated rejection; TCMR = T-cell mediated rejection; TIN = tubulointerstitial nephritis; IFTA = interstitial fibrosis and tubular atrophy. All data are described as No (%) (also mentioned in every specific variable row). [a] one patient demonstrated ABMR at 4 and 24 weeks.

Patient survival, renal function, and biopsy scores

Patient survival during the study follow-up was 100% in both groups. All patients had a functioning kidney graft at the end of the 24-week study period (*Table 2*). eGFR was 56 ± 16 ml/min/1.73 m^2 in the MSC (*n*=29) and 42 ± 9 ml/min/1.73 m^2 in the control group (*n*=28) at the time of MSC infusion (*Figure 3A*). Mean eGFR and 24-h proteinuria (*Table S2*) in the MSC group were similar as compared with the control group, with a mean of 56 ± 15 ml/min/1.73 m^2 and 47 ± 16 ml/min/1.73 m^2, respectively, at week 24 (*Figure 3A*). The slope from 4 to 24 weeks in the MSC group (slope=−0.22; intercept=58.15) was not significantly different from the control group (slope=0.09; intercept=43.33) (*p*=0.08, *Figure 3B*). Only one acute rejection episode (combination of T cell [TCMR] and antibody-mediated rejection [ABMR]), documented by for-cause biopsy, was found during the study period in the MSC group (1/29 or 3.4%) (*Table 2*). In this patient, immune suppression had been further reduced due to persistent BK viremia/nephropathy. In the control group, four patients had an indication for a for-cause renal biopsy, without evidence of rejection (*Table 2*). The 24-week protocol biopsies showed SCAR in 14.3% and 13.0% of patients in the MSC (4/28) and control group (3/23), respectively. Protocol biopsies in the MSC group showed a chronic active TCMR Banff IA (*n*=1 patient), active ABMR (*n*=2, of which one also had active ABMR in the 4-week protocol biopsy; both having class I and II DSAs, C4d positive only at 6 months), and one mixed active ABMR and acute TCMR IA. Biopsies in the control group demonstrated acute TCMR Banff IA (*n*=2 patients) and a mixed active ABMR and acute TCMR IA (*n*=1 patient) (*Table 2*). All patients had a negative HLA antibody screening before and 4 weeks after transplantation. In the MSC group, seven patients developed dnDSA at week 24 (24%) (*Table 3*). Their protocol renal biopsies demonstrated no rejection (*n*=3), borderline suspicious for acute TCMR (*n*=1), ABMR (*n*=2, both C4d negative), and ABMR/TCMR IA (*n*=1, $C4d^+$). In the control group, two patients developed HLA class-II dnDSA without signs of rejection in their protocol biopsies.

Immunosuppressive drug levels and change of regime

Immunosuppressive drug levels were within or only slightly out of prespecified target ranges. EVL levels, however, were significantly lower at three time points in the control group (*Table S3*). All patients in the MSC group were on EVL at the end of the 24-week study period. In the MSC group, tacrolimus was reintroduced in one patient, because of acute rejection. In the control group, tacrolimus was discontinued in two patients because of BK nephropathy. EVL was switched to mycophenolate mofetil in four patients after a thrombovascular event and discontinued in two patients (CMV infection and infected lymphocele, respectively).

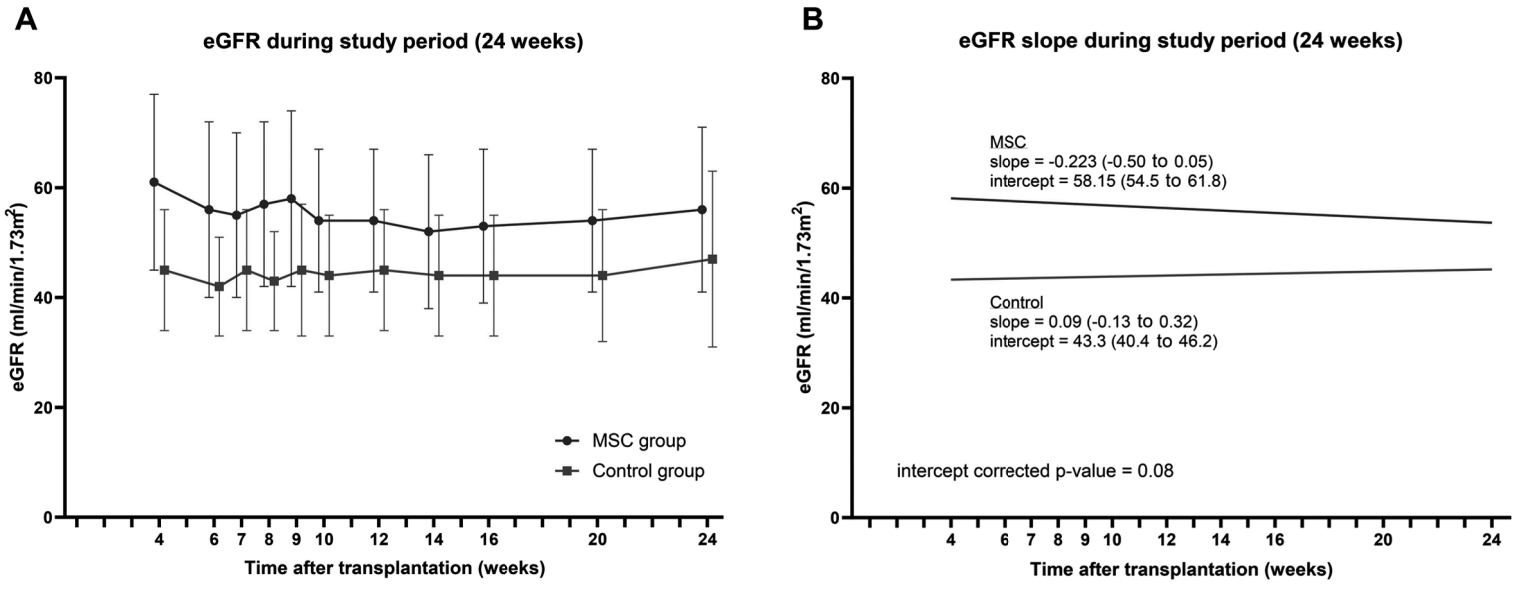

Figure 3. eGFR during the study period of 24 weeks. *(A) eGFR (ml/min/1.73 m²), calculated by the CKD-EPI formula and depicted per time point as mean ± SD, of patients in the MSC and control groups. (B) Slopes of the eGFR in the MSC group were not significantly different from the control group (p = 0.08). Slope and intercept data per group are described, including 95% confidence intervals.*

(Serious) adverse events

Forty-four SAEs were reported, of which 19 in the MSC and 25 in the control group. In total, 272 AEs were reported in the MSC and 301 in the control group (*Table 3*). There were no AEs directly related to the MSC infusions. In the control group, 15 viral infections (EBV, CMV, and BK viremia) developed and 14 in the MSC group (*Table 3*). BK nephropathy occurred in one patient in the MSC (3%) and in three patients in the control group (11%).

Immune monitoring

Immune monitoring studies demonstrated that absolute numbers of peripheral blood $CD45^+$ leukocytes and $CD14^+$ monocytes remain stable after transplantation between weeks 6 and 52 in the MSC and control groups (*Figure 4A,B*). $CD19^+$ B cells and $CD56^+$ NK cells decreased after alemtuzumab-based induction in both groups and re-appeared from week 12 onwards; however, no statistically significant change was measured between the groups (*Figure 4C,D*). $CD3^+CD8^+$ T cells, $CD3^+CD4^+$ T cells, as well as $CD4^+CD25^{hi}CD127^{lo}$ Tregs showed a decrease after alemtuzumab-based induction in both groups while still being suppressed at week 52 (*Figure 4E,G*). Total Treg numbers were significantly higher in the MSC group with tacrolimus withdrawal as compared to the control group at 24 and 52 weeks after transplantation (p=0.014 and p=0.047, respectively), due to the increase in absolute number of $CD4^+CD25^{hi}CD127^{lo}CD45RA^-$ memory Tregs (p=0.040 and p=0.047) (*Figure 4G,H*). Absolute numbers of naïve Tregs ($CD4^+CD25^{hi}CD127^{lo}CD45RA^+$) were similar in both groups (*Figure S4*). Percentages of total and naïve Tregs were not different between the two groups at any time points, whereas percentages of memory Tregs within the total CD4 population were elevated in the control group only at week 12, which normalized the weeks thereafter (*Figure S5*).

Post hoc analysis

In the post hoc longer (intermediate)-term follow-up analysis (up to 5 years), graft loss was observed in two patients in the control group (*Table 4*). Renal function in the MSC group was preserved with an eGFR between 47 and 57 ml/min/1.73 m^2 (*Table 4*). In the patients in the control group, eGFR gradually declined with a mean of 42 ml/min/1.73 m^2 at year 1 and 37 ml/min/1.73 m^2 at year 5, while seven patients dropped with their eGFR <30 ml/min/1.73 m^2. For-cause biopsies were indicated in one patient in the MSC and eight patients in the control group. In the for-cause biopsy in the MSC group, recurrence of IgA nephropathy was found (n=1). In the control group, acute TCMR IB (n=1), acute TCMR II (n=1), mixed active ABMR and acute TCMR IB (n=1), BK nephropathy (n=2), tubulointerstitial nephritis/pyelonephritis (n=1), IFTA grade III (n=1), and medullary inflammation NOS (sv negative) (n=1) were observed. In the post hoc analyses, none of the seven patients with de novo DSA needed a for-cause biopsy renal biopsy or developed an eGFR <30 ml/min/1.73 m^2. However, it is of importance to note that in three of these seven patients CNI was restarted by their treating nephrologist after the 24-week study period (*Table S4*).

Table 3. *Secondary endpoints (SAE, AE, viral infections, dnDSA) during the study period of 24 weeks.*

Endpoint study period of 24 weeks	MSC group (n=29)	Control group (n=28)
Serious adverse events, total, No.	19	25
Injury, poisoning and procedural complications	6	7
Infections and infestations	2	7
Gastrointestinal disorders	2	3
Renal and urinary disorders	2	2
Metabolism and nutrition disorders	2	2
Therapeutic and nontherapeutic responses	2	1
Investigations	1	1
Vascular disorders	0	1
Musculoskeletal and connective tissue disorders	0	1
Immune system disorders	1	0
Psychiatric disorders	1	0
Adverse events, total, No.	272	301
Investigations	51	46
Blood and lympathic system disorders	39	36
Infections and infestations	32	38
Vascular disorders	35	31
Metabolism and nutrition disorders	26	30
Gastrointestinal disorders	21	32
Renal and urinary disorders	5	17
Injury, poisoning and procedural complications	9	15
General disorders and administration site conditions	10	12
Nervous system disorders	6	10
Musculoskeletal and connective tissue disorders	9	7
Cardiac disorders	10	5
Respiratory, thoracic and mediastinal disorders	5	7
Skin and subcutaneous tissue disorders	8	4
Psychiatric disorders	2	4
Reproductive system and breast disorders	1	2
Neoplasm benign, malignant and unspecified	1	2
Eye disorders	1	2
Immune system disorders	0	1
Ear and labyrinth disorders	1	0
Viral infections, No. (%)		
EBV virus infection[a]	1 (3%)	2 (7%)
CMV virus infection[a]	2 (7%)	3 (11%)
BK virus infection[b]	11 (38%)	10 (36%)
BK nephropathy	1 (3%)	3 (11%)
dnDSA, No. (%)		
Yes	7 (24%)	2 (7%)
Anti-class I	0	0
Anti-class II	4 (14%)	2 (7%)
Anti-class I and II	3 (10%)	0
No	22 (76%)	26 (89%)

MSC = mesenchymal stromal cells, EBV = Epstein-Barr virus, CMV = cytomegalovirus, dnDSA = de novo donor specific antibodies measured at week 24. [a] Peak serum levels (logarithmic) of EBV and CMV range from 2.5 to 3.2 and from 2.7 to 4 respectively. [b] Peak serum levels of BK range from 5.1 to 6.9 in patients with BK nephropathy and from 2.6 to 6.9 in patients without signs of BK nephropathy. [c] dnDSA are considered positive in case of an MFI ≥ 500.

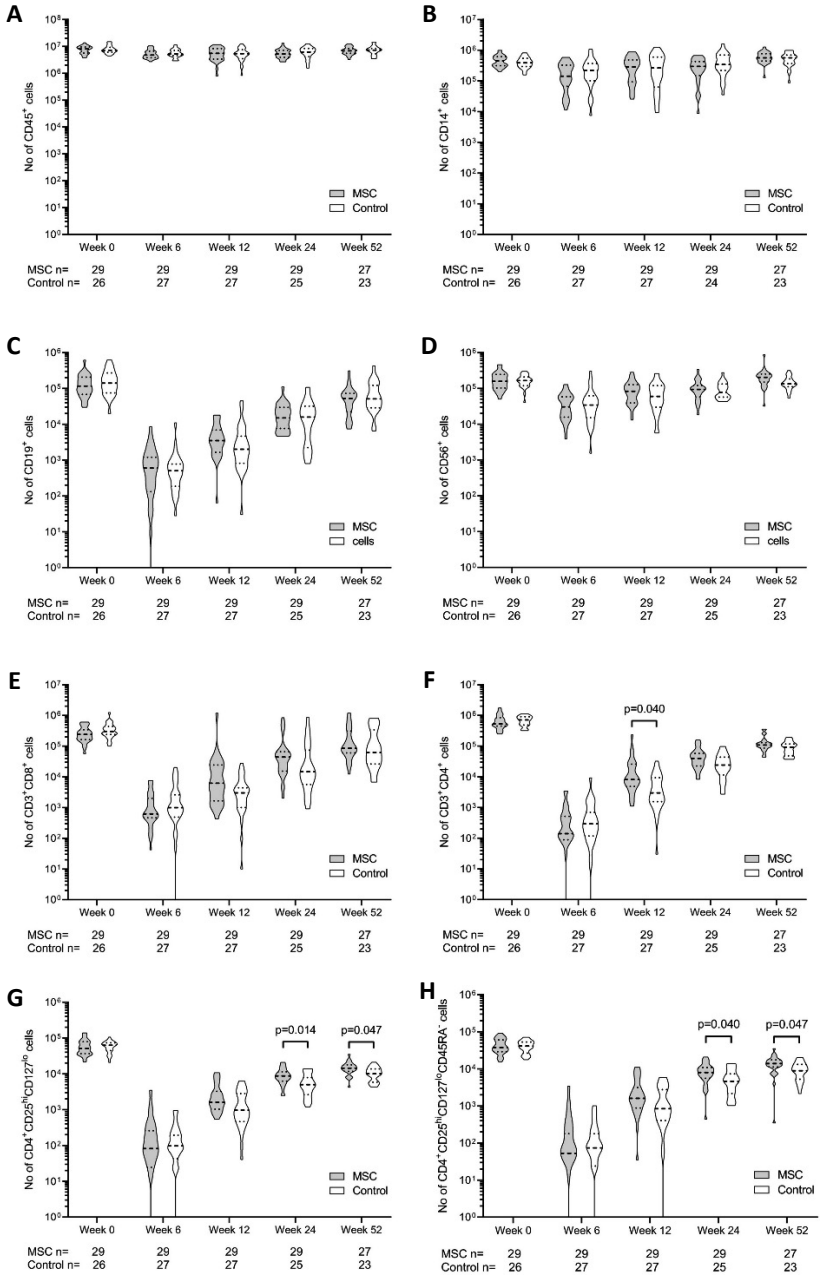

Figure 4. Peripheral blood immune cell composition before and after MSC infusion. *Absolute numbers of (A) CD45⁺ leucocytes, (B) CD14⁺ monocytes, (C) CD19⁺ B cells, (D) CD56⁺ NK cells, (E) CD8⁺ T cells, (F) CD4⁺ T cells, (G) CD4⁺CD25hiCD127lo Tregs, and (H) CD4⁺CD25hiCD127loCD45RA- memory Tregs per mL of blood are shown at baseline before transplantation, before the first MSC infusion (week 6), and time points after both infusions (weeks 12, 24, and 52). Violin plots are given for every time point with the number of individuals studied at each time point below the x-axis. p values are given for the differences between MSC and control groups when <0.05 after Bonferroni correction for multiple testing. MSC = mesenchymal stromal cell; NK = natural killer; Treg = regulatory T cell*

Table 4. *Post-hoc analysis (1-5 years) of endpoints (graft loss, renal function, biopsy scores).*

Endpoint post hoc analysis	MSC group	Control group
1 year	n=26	n=26
2 year	n = 20	n = 20
3 year	n = 10	n = 14
4 year	n = 7	n = 10
5 year	n = 6	n = 7
Graft loss, No.	0	2[a]
Time after Tx, yr		3.8 and 4.5
eGFR, mean (SD) [n], ml/min/1.73m^2		
1 yr	57 (15%) [n=26]	42 (11%) [n=26]
2 yr	55 (15%) [n=20]	39 (12%) [n=20]
3 yr	53 (14%) [n=10]	34 (14%) [n=14]
4 yr	47 (10%) [n=7]	36 (12%) [n=9]
5 yr	50 (20%) [n=6]	37 (15%) [n=5]
eGFR <30ml/min/1.73m2, No.	0	7
Time after Tx, median (IQR), yr		3 (1-3)
Patients with for-cause biopsies, No. (%)	1 (3%)	8 (29%)
Recurrence IgA nephropathy	1	
TCMR IB		1
TCMR II		1
ABMR and TCMR IB		1
BK nephropathy		2
InvestigationsTIN/pyelonephritis		1
IFTA grade III		1
Medullary inflammation		1

MSC = mesenchymal stromal cell, eGFR = estimated glomerular filtration rate, ABMR = antibody mediated rejection, TCMR = T-cell mediated rejection, TIN = tubulointerstitial nephritis, IFTA = interstitial fibrosis and tubular atrophy. All data are described as the total count. Numbers between parenthesis are percentages (also mentioned in the specific variable row).
[a] 1 patient TCMR and recurrence membranous nephropathy; 1 patient chronic transplant dysfunction.

Discussion

In this randomized clinical study, we found that quantitative fibrosis scores and renal function remained stable in patients with MSC therapy and concomitant early tacrolimus withdrawal within the study period of 24 weeks. Only one acute rejection episode was documented in the MSC group after further reduction of clinical immunosuppression in the context of persistent BK viremia/nephropathy. Of interest, there were significantly higher numbers of Tregs in the MSC group with tacrolimus withdrawal compared to the controls. In addition, post hoc analyses demonstrated preserved renal function in the MSC group without evidence of late rejection. Clinical studies with MSCs in kidney transplantation, mainly phase 1 trials with still limited numbers of patients, have demonstrated that MSC treatment after kidney transplantation is safe and feasible.[9-12,20,21] In most studies, MSCs were administered at an early time point against the background of regular immune suppression with the aim to induce immunologic tolerance. The current strategy with MSCs and complete withdrawal of CNI have not been studied before in a randomized trial. Minimization of CNIs is a well-established strategy to limit structural long-term damage to the graft and minimize the side effects associated with clinical immunosuppression.[5,22] A number of trials have demonstrated the efficacy of EVL in conjunction with reduced exposure to CNIs in preventing organ loss or dysfunction in kidney transplant recipients.[23] Of importance, complete avoidance and replacement of a CNI by EVL in de novo transplant recipients are not justified, since unacceptable high acute rejection rates were observed with this strategy.[24] The capability of MSCs to allow reduction of 50% CNI was demonstrated in a previous study with third-party MSCs in 16 living kidney transplant recipients.[21] The combination of an mTOR inhibitor and MSCs was chosen in the current study since experimental evidence demonstrated tolerogenic properties and an increase in regulatory immune cell subsets.[14] In our study, fibrosis scores were similar in both the MSC group and the controls, thereby failing to meet the primary end point, and the incidence of acute rejection 24 weeks after implantation was low. One explanation might be the use of alemtuzumab,[13] which was chosen as we anticipated a higher immunological risk due to the early CNI withdrawal. Indeed, given the potency of the immunosuppression regimen used in our study, seeing differences in fibrosis scores and rejection with the short study duration is unlikely. Of interest, however, the post hoc analysis with follow-up up to 5 years showed a higher incidence of for-cause biopsies in the control group, with findings of both BPAR and BK nephropathy, suggesting that the effect of MSC infusion in combination with CNI withdrawal carried through way beyond the period that alemtuzumab is effective. Future studies with a sufficient number of patients and duration of follow-up are needed to be able to draw more definite conclusions. Several studies have reported an increased incidence of dnDSA in renal transplant recipients receiving EVL, especially when converted early after transplantation, and it was also suggested that the use of alemtuzumab-based induction could aggravate this.[25,26] In general, dnDSA has been shown to be associated with poor graft

survival and increased acute rejection in kidney transplant recipients.[27] In the large ELEVATE Trial, however, conversion to EVL at 10–14 weeks posttransplant was associated with renal function parameters similar to that observed with standard therapy. In this study, the dnDSA data, available in a subset of patients, suggested more frequent anti-HLA Class-I DSA under EVL. Differences in propensity to develop dnDSA, however, did not appear to have resulted in ABMR within the 2-year observation frame of the study.[28] In our study, we also found an increased incidence of dnDSA in patients where tacrolimus was withdrawn. This was associated with (asymptomatic) signs of ABMR in the protocol biopsies of three of these patients of which one, in retrospect, already had subclinical ABMR in the 4-week biopsy. There were no signs of deteriorating graft function in these patients. Furthermore, the post hoc analyses showed no graft losses, no need for additional for-cause biopsies, and stable renal function in these patients as well as the MSC group as a whole. Nevertheless, given the epidemiological association with graft loss (which is, however, based on for-cause DSA measurements), the nephrologists taking care of these patients restarted the CNI in three patients after the study period. Longer follow-up in all patients is warranted to draw more definite conclusions here. Variable outcomes on renal function after MSC therapy have been described and it has been suggested that timing of MSC administration is of major importance. Indeed, early clinical trials have demonstrated an engraftment syndrome with infiltration of immune cells and C3 deposits when MSCs were administered 7 days after renal transplantation, which was not observed when MSCs were given before implantation.[29] In the study by Erpicum et al., eGFR values at day 7 were higher in the MSC-treated patients.[12] In our study, patients in the MSC group started with a higher eGFR, as compared to controls, which was preserved throughout the study period and the post hoc follow-up period. This unequal randomization was, to the best of our knowledge, found by chance and could have influenced our results. In the control group, there was increased graft loss as well as a higher number of patients with inferior renal function (i.e., eGFR <30 ml/min/1.73 m^2), possibly due to an increase in BPAR and BK nephropathy in these patients.

So far, hardly any safety issues have been reported after systemic infusion of MSCs in humans, except for a transient fever and one cardiac event with an unclear causal relationship to the intervention.[12] In our study, there were no side effects directly related to the MSC infusion. We found that (S)AEs (including viral infections) were similar in the two groups. This is in contrast to our previous study where an increased incidence of viral infections was observed after MSC therapy.[10] Possibly this is due to the fact that MSCs were given on top of regular immune suppression in our previous study. This observation is of particular relevance with the ongoing COVID-19 pandemic. Recent observational studies have shown that kidney transplant recipients are at increased risk for severe morbidity due to their systemic immune suppression and often reduced renal function.[30]

MSCs have shown to condition the immune system, by releasing extracellular vesicles or membrane particles or by undergoing apoptosis. This may actively engage recipient monocytes/phagocytes and eventually Tregs, enabling long-term tolerogenic activity that becomes self-sustained even after disappearance of the infused MSCs themselves.[8,31] Of interest, in our current study, we found an increase in the absolute number of Tregs in the MSC group with tacrolimus withdrawal versus control, which has not been reported before in a randomized clinical trial with MSCs in transplant recipients. However, since there was a difference in tacrolimus use between both groups and a difference in total $CD4^+$ T cell counts at week 12, it is not possible to deduce the results solely to the MSC treatment. Concomitantly, the percentage of memory Tregs within total CD4 T cells showed an increase in the control group compared to the MSC group at 12 weeks (*Figure S5*), after which the percentages in total and Treg subsets remained similar, indicating that the increase in absolute Treg numbers in the MSC group is at least partially due to changes in the total $CD4^+$ T cell number.

At present, randomized trials with MSCs are still very limited and the field is only slowly advancing also due to stringent regulatory requirements, the need for clinical grade cell production facilities, and the associated costs. However, we recently also reported the feasibility of administration of third-party "off-the-shelf" MSCs in kidney transplant recipients.[11] This option makes manufacturing and regulation easier and the use of MSC suitable for a wider spectrum of clinical application and much more feasible. We believe that the results of our current trial set the stage for the next steps and use of MSCs in the field of kidney transplantation to reduce the need for excessive use of clinical immunosuppressants.

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References

1. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. Am J Transplant 2011;11(3):450-462.

2. Coemans M, Susal C, Dohler B, Anglicheau D, Giral M, Bestard O et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. Kidney Int 2018;94(5):964-973.
3. Wekerle T, Segev D, Lechler R, Oberbauer R. Strategies for long-term preservation of kidney graft function. Lancet 2017;389(10084):2152-2162.
4. Rostaing L, Kamar N. mTOR inhibitor/proliferation signal inhibitors: entering or leaving the field? J Nephrol 2010;23(2):133-142.
5. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. Transplantation 2004;78(4):557-565.
6. Sawinski D, Trofe-Clark J, Leas B, Uhl S, Tuteja S, Kaczmarek JL et al. Calcineurin Inhibitor Minimization, Conversion, Withdrawal, and Avoidance Strategies in Renal Transplantation: A Systematic Review and Meta-Analysis. Am J Transplant 2016;16(7):2117-2138.
7. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105(4):1815-1822.
8. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med 2017;9(416).
9. Perico N, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavinato RA et al. Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. Clin J Am Soc Nephrol 2011;6(2):412-422.
10. Reinders ME, de Fijter JW, Roelofs H, Bajema IM, de Vries DK, Schaapherder AF et al. Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. Stem Cells Transl Med 2013;2(2):107-111.
11. Dreyer GJ, Groeneweg KE, Heidt S, Roelen DL, van Pel M, Roelofs H et al. Human leukocyte antigen selected allogeneic mesenchymal stromal cell therapy in renal transplantation: The Neptune study, a phase I single-center study. Am J Transplant 2020.
12. Erpicum P, Weekers L, Detry O, Bonvoisin C, Delbouille MH, Gregoire C et al. Infusion of third-party mesenchymal stromal cells after kidney transplantation: a phase I-II, open-label, clinical study. Kidney Int 2019;95(3):693-707.
13. Group CSC, Haynes R, Harden P, Judge P, Blackwell L, Emberson J et al. Alemtuzumab-based induction treatment versus basiliximab-based induction treatment in kidney transplantation (the 3C Study): a randomised trial. Lancet 2014;384(9955):1684-1690.
14. Ge W, Jiang J, Baroja ML, Arp J, Zassoko R, Liu W et al. Infusion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. Am J Transplant 2009;9(8):1760-1772.
15. Reinders ME, Bank JR, Dreyer GJ, Roelofs H, Heidt S, Roelen DL et al. Autologous bone marrow derived mesenchymal stromal cell therapy in combination with everolimus to preserve renal structure and function in renal transplant recipients. J Transl Med 2014;12:331.
16. Grimm PC, Nickerson P, Gough J, McKenna R, Stern E, Jeffery J et al. Computerized image analysis of Sirius Red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. J Am Soc Nephrol 2003;14(6):1662-1668.
17. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M et al. The Banff 2019 Kidney Meeting Report (I): Updates on and clarification of criteria for T cell- and antibody-mediated rejection. Am J Transplant 2020.
18. Moes DJ, van der Bent SA, Swen JJ, van der Straaten T, Inderson A, Olofsen E et al. Population pharmacokinetics and pharmacogenetics of once daily tacrolimus formulation in stable liver transplant recipients. Eur J Clin Pharmacol 2016;72(2):163-174.
19. Streitz M, Miloud T, Kapinsky M, Reed MR, Magari R, Geissler EK et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. Transplant Res 2013;2(1):17.

20. Perico N, Casiraghi F, Todeschini M, Cortinovis M, Gotti E, Portalupi V et al. Long-Term Clinical and Immunological Profile of Kidney Transplant Patients Given Mesenchymal Stromal Cell Immunotherapy. Front Immunol 2018;9:1359.
21. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, Ma JJ et al. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. Oncotarget 2016;7(11):12089-12101.
22. Nankivell BJ, Kuypers DR. Diagnosis and prevention of chronic kidney allograft loss. Lancet 2011;378(9800):1428-1437.
23. Pascual J, Berger SP, Witzke O, Tedesco H, Mulgaonkar S, Qazi Y et al. Everolimus with Reduced Calcineurin Inhibitor Exposure in Renal Transplantation. J Am Soc Nephrol 2018;29(7):1979-1991.
24. Vincenti F, Ramos E, Brattstrom C, Cho S, Ekberg H, Grinyo J et al. Multicenter trial exploring calcineurin inhibitors avoidance in renal transplantation. Transplantation 2001;71(9):1282-1287.
25. Liefeldt L, Brakemeier S, Glander P, Waiser J, Lachmann N, Schonemann C et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. Am J Transplant 2012;12(5):1192-1198.
26. Todeschini M, Cortinovis M, Perico N, Poli F, Innocente A, Cavinato RA et al. In kidney transplant patients, alemtuzumab but not basiliximab/low-dose rabbit anti-thymocyte globulin induces B cell depletion and regeneration, which associates with a high incidence of de novo donor-specific anti-HLA antibody development. J Immunol 2013;191(5):2818-2828.
27. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant 2012;12(5):1157-1167.
28. de Fijter JW, Holdaas H, Oyen O, Sanders JS, Sundar S, Bemelman FJ et al. Early Conversion From Calcineurin Inhibitor- to Everolimus-Based Therapy Following Kidney Transplantation: Results of the Randomized ELEVATE Trial. Am J Transplant 2017;17(7):1853-1867.
29. Perico N, Casiraghi F, Gotti E, Introna M, Todeschini M, Cavinato RA et al. Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. Transpl Int 2013;26(9):867-878.
30. Elias M, Pievani D, Randoux C, Louis K, Denis B, Delion A et al. COVID-19 Infection in Kidney Transplant Recipients: Disease Incidence and Clinical Outcomes. J Am Soc Nephrol 2020.
31. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS et al. Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells. Stem Cells 2018;36(4):602-615.

Supplementary information

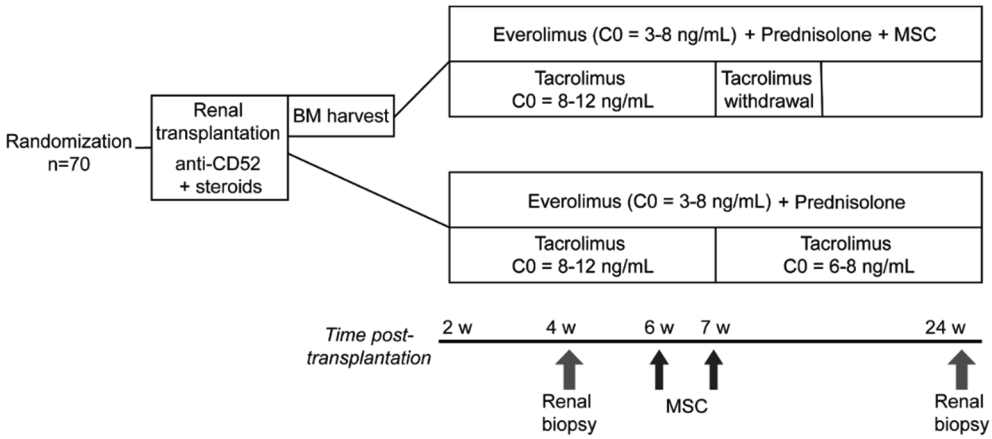


Figure S1. Schematic presentation of study interventions and immunosuppressive regimen. *All patients received prednisolone the entire period. As described in the study protocol, patients received prednisone dose of 100mg (day 1-3), 50mg (day 4), 20mg (day 5-14), 15mg (day 15-21) and 10mg (from day 22). Directly after the 2nd MSC infusion, the MSC group received a higher dose of prednisolone (15mg) for two weeks. In addition patients received alemtuzumab-based induction at day 0 and 1 (15 mg subcutaneously) after transplantation. Target trough level of everolimus was 3-8 ng/mL in both groups. The tacrolimus target was 8-10 ng/mL the first 6 weeks post transplantation and lowered to 6-8 ng/mL in the control group 7 weeks post transplantation. Patients received 2 doses of 1-2.0x10^6 million autologous BM MSC per kg body weight IV, 7 days apart, 6 and 7 weeks after transplantation. Tacrolimus was halved at the time of the second MSC infusion and stopped 1 week later. At that time point patients received 2 weeks 15 mg prednisolone (instead of 10mg).*
MSC = mesenchymal stromal cells, BM = bone marrow, IV = intravenous.

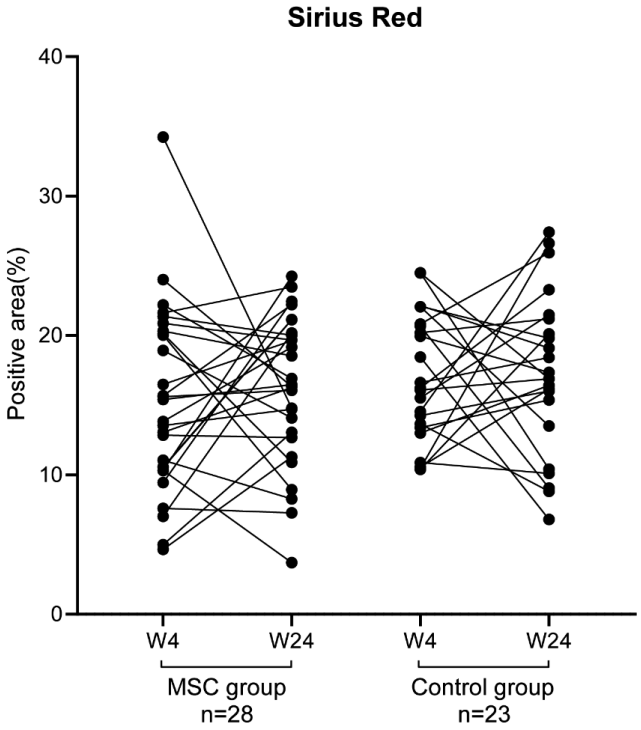
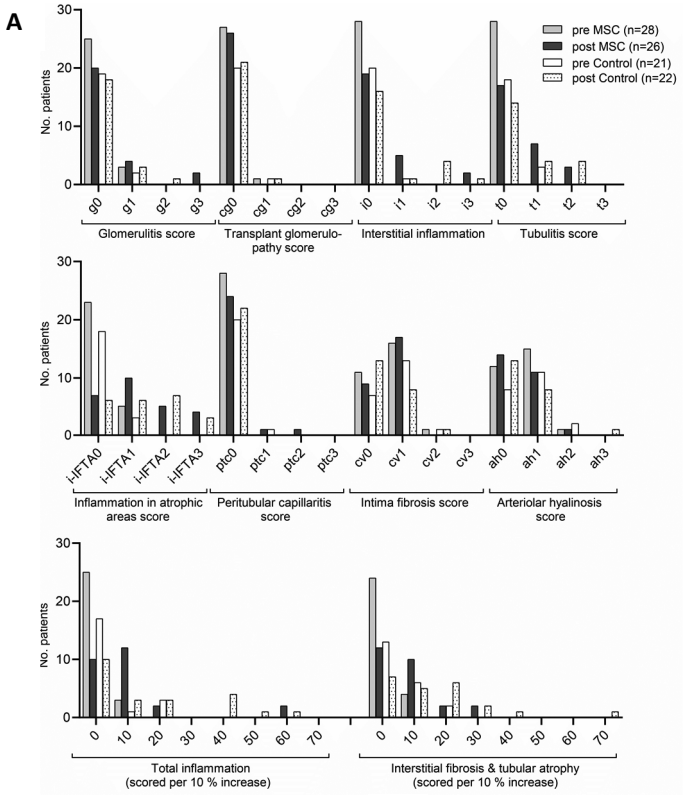


Figure S2. Renal biopsies stained with Sirius Red. *Positive area (Sirius Red Staining) in % in protocol renal biopsies at 4 weeks (W4) and 24 weeks (W24) in the mesenchymal stromal cell (MSC) and control group.*



B

Delta Banff scores between 4 and 24 week protocol biopsy

Characteristic	MSC (n=28)	Control (n=23)	P Value
Glomeruli present – no.	-3 (-32, 25)	1 (-22, 30)	0.2
Globally sclerosed glomeruli - %	0 (-11, 33)	0 (-8, 19)	0.5
g score	0 (-1, 3)	0 (-1, 2)	0.7
cg score	0 (-1, 0)	0 (-1, 1)	0.6
mm score	0 (-1, 0)	0 (-1, 1)	0.2
i score	0 (0, 3)	0 (-1, 2)	0.8
t score	0 (0, 2)	0 (-1, 2)	0.4
ti score	10 (0, 60)	0 (-20, 50)	0.8
IFTA score	0 (-10, 30)	10 (-10, 50)	0.4
iIFTA score	1 (-1, 3)	1 (-1, 3)	0.6
ptc score	0 (0, 2)	0 (-1, 0)	0.1
v score	0 (0, 0)	0 (0, 1)	0.3
cv score	0 (-1, 1)	0 (-1, 2)	0.1
ah score	0 (-1, 1)	0 (-1, 2)	0.4

Data are median (min, max). Statistical test: Wilcoxon rank-sum test

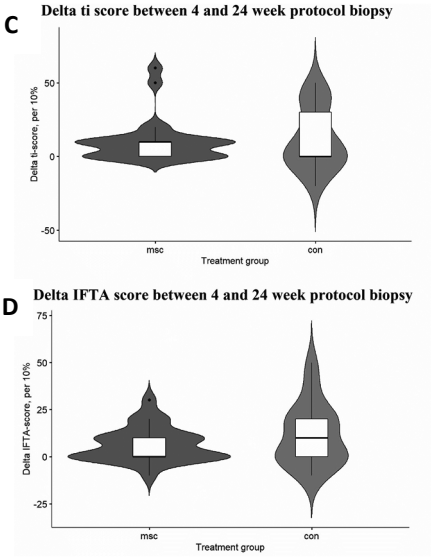


Figure S3. Banff scores of renal biopsies before (4 weeks) and after (24 weeks) transplantation in the mesenchymal stromal cell (MSC) and control group. *Banff scores (from representative biopsies (≥7 glomeruli and 2 vessels)) are depicted as absolute scores (A). Delta Banff scores between 4 and 24 weeks did not differ significantly (B), in particular ti score (C) and IFTA score (D) were comparable.*

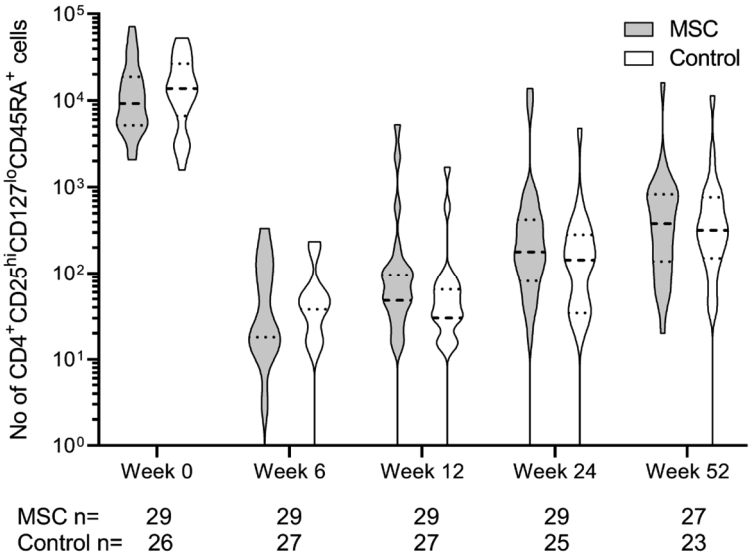


Figure S4. Absolute counts of naive regulatory T cells. *Naive regulatory T cells depicted as absolute counts before transplantation (Week 0) and 6, 12, 24, and 52 weeks after transplantation in the mesenchymal stromal cell (MSC) and control group.*

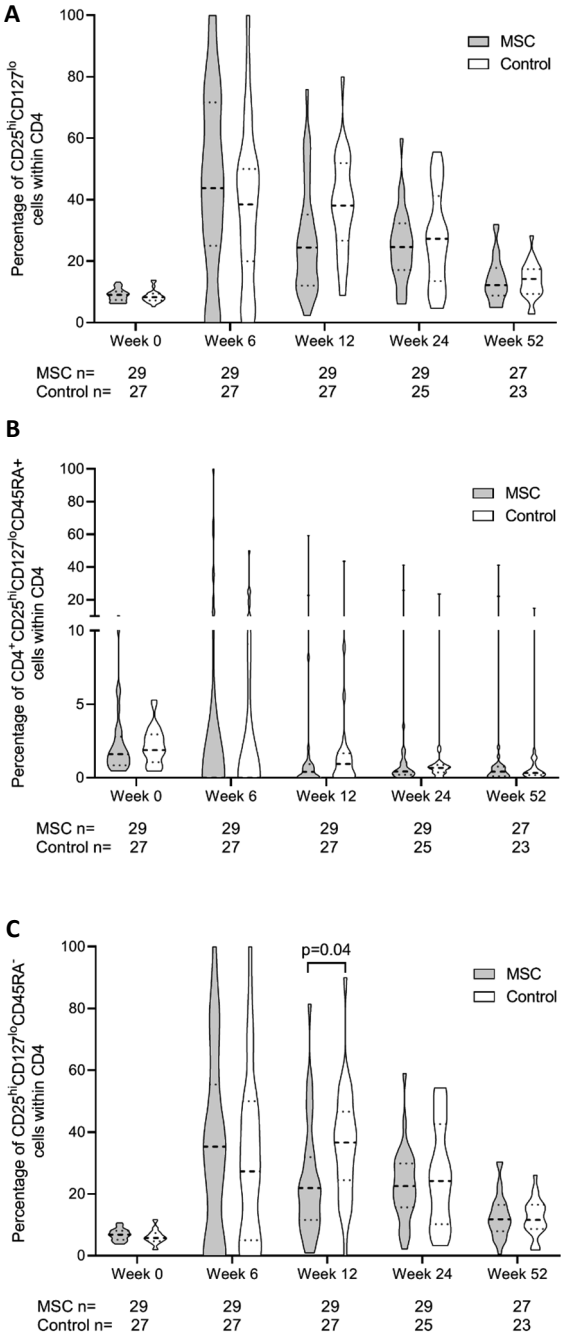


Figure S5. Percentages of total, naïve and memory regulatory T cells within CD4. *Total (A), naïve (B) and memory (C) regulatory T cells depicted as a percentage of the total CD4 count before transplantation (Week 0) and 6, 12, 24, and 52 weeks after transplantation in the mesenchymal stromal cell (MSC) and control group.*

Table S1. *Information and characteristics of processing/expansion of MSC cultures and infusion process.*

Characteristics	n=29
Patient Characteristics	
Age in years, mean (SD)	50 (14)
Male sex, no. (%)	26 (90)
Body weight, kg, mean (SD)	79.4 (14.4)
Culture expansion Characteristics	
Netto volume bone marrow, mL, mean (SD)	119 (11)
MNC in bone marrow ($x10^6$), mean (SD)	440 (273)
Time in culture, days, mean (SD)	22.3 (4.8)
Population doubling level, mean (SD)	3.2 (1.0)
Population doubling time, days, mean (SD)	5.9 (3.6)
Infusion Characteristics	
No. of MSCs administered / kg ($x10^6$), mean (SD)	1.45 (0.13), 1.46 (0.15)
Vitals before administration[a]	143/85, 77, 36.7
Vitals 30 min after administration[a]	141/85, 76, 36.6
Duration 1st (n=29) and 2nd infusion (n=28) mean, min (SD)	21 (12), 20 (10)
Infusion related adverse events	None

From patients allocated to the MSC group, autologous bone marrow mononuclear cells (BM-MNC) were harvested and obtained using ficoll density separation and plated at a density of 160,000 cells per cm^2 in DMEM low-glucose supplemented with 10% fetal bovine serum and antibiotics. At >70% confluency, cells were harvested using TripleSelect, expanded for 1 or 2 subsequent passages to obtain sufficient cells for infusion. After expansion, the final autologous MSC product was frozen and cryopreserved until administration to the patient in 2% human albumin / 0.9% NaCl solution containing 10% DMSO. Characteristics of the MSC infusions are shown. Dosage is given in cells ($x10^6$) per kilogram bodyweight for the first and second infusion. Blood pressure (mm Hg), heart rate (per min) and temperature (^oC) were given as vital signs monitored by MEWS scores (score <2: temp 35-38.5 ^oC, systolic blood pressure 80-200 mm Hg, heart rate 40-110 / minute). As indicated 28 patients received two MSC infusions and 1 patient received one infusion. All data are described as mean and standard deviation (SD). MNC = mononuclear cells, min = minutes. [a] Blood pressure, heart rate, temperature (^oC).

Table S2. *24-hour urine protein measures.*

Visit (weeks)	MSC (n=29)		Control (n=28)		P value[a]
	Total protein (g/24u)	n =	Total protein (g/24u)	n =	
Pre	2·71 ± 2·67	23	1·84 ± 2·19	24	0.12
W4	0·44 ± 0·52	27	0·45 ± 0·37	25	0.35
W6	0·34 ± 0·36	26	0·41 ± 0·28	26	0.14
W7	0·37 ± 0·35	27	0·44 ± 0·29	23	0.20
W8	0·37 ± 0·29	26	0·43 ± 0·35	23	0.30
W9	0·52 ± 0·46	27	0·38 ± 0·27	24	0.38
W10	0·52 ± 0·42	26	0·37 ± 0·33	24	0.11
W12	0·43 ± 0·34	29	0·31 ± 0·2	25	0.23
W14	0·42 ± 0·38	27	0·36 ± 0·26	25	0.87
W16	0·42 ± 0·41	29	0·35 ± 0·2	24	0.72
W20	0·46 ± 0·49	26	0·34 ± 0·2	25	0.89
W24	0·49 ± 0·63	25	0·35 ± 0·32	24	0.42

24 hour proteinuria in mesenchymal stromal cell (MSC) and control group at study visits. All data are described as mean and standard deviation. [#] *24-hour urine protein collected in the year before transplantation.* [a] *Unpaired t test*

Table S4. *Overview of patients on non-protocol immunosuppressive regime at end of study period of 24 weeks.*

	Reason for protocol deviation	n=
MSC group[#]		
Tacrolimus reintroduced	Acute rejection	1
Control group		
Tacrolimus discontinued	BK viremia	2
Everolimus switched to Mycophenolate Mofetil	Thrombo vascular event	4
Everolimus discontinued	CMV infection	1
Everolimus discontinued	Infection of urinoma	1

[#] *In 3 of the 7 patients in the MSC group CNI was reintroduced by their treating nephrologist after the 24 week study period because of dnDSAs developed at week 24.*

Table S3. *Everolimus and Tacrolimus trough blood levels.*

Visit (weeks)	Everolimus (ng/mL)					Tacrolimus (ng/mL)				
	MSC (n=29)		Control (n=28)		P value[a]	MSC (n=29)		Control (n=28)		P value[a]
	Total protein (g/24u)	n =	Total protein (g/24u)	n =		Total protein (g/24u)	n =	Total protein (g/24u)	n =	
W4	5·13 ± 1.5	29	5.47 ± 1.63	26	0.30	8·71 ± 1·97	28	8·51 ± 2·40	28	0·56
W6	5.26 ± 1.27	29	5.13 ± 1.57	23	0.40	7·53 ± 1·93	29	7·23 ± 1·91	27	0·36
W7	5.25 ± 0.9	28	5.34 ± 1.42	23	0.81	7·25 ± 1·50	27	6·44 ± 1·82	25	0·04
W8	5.38 ± 1.51	28	5.75 ± 1.49	24	0.23	3·25 ± 1·00	28	6·42 ± 1·57	26	<0·0001
W10	6.52 ± 1.70	25	5.32 ± 0.97	22	0.01	N.A.		5·38 ± 1·37	26	
W12	6.22 ± 1.64	26	4.90 ± 1.34	22	0.01	N.A.		5·07 ± 1·65	26	
W14	5.87 ± 1.38	26	5.24 ± 1.36	21	0.17	N.A.		5·37 ± 2·12	25	
W16	5.43 ± 1.44	29	5.36 ± 2.31	21	1.00	N.A.		4·85 ± 1·16	25	
W20	5.83 ± 1.35	28	5.17 ± 1.83	19	0.17	N.A.		5·20 ± 1·38	23	
W24	6.18 ± 1.73	28	4.72 ± 1.57	19	0.007	N.A.		4·77 ± 1·66	23	

Trough levels of everolimus and tacrolimus in mesenchymal stromal cell (MSC) and Control group at study visits. Target trough level of everolimus was 3-8 ng/mL. The tacrolimus target was 8-10 ng/mL the first 6 weeks post transplantation and lowered to 6-8 ng/mL in the control group 7 weeks post transplantation. All patients received prednisolone as described in Figure S1. In three patients in the MSC group (10%) and nine patients in the control group (32%) the prednisolone dose was lowered during the 24 week study period to 7.5mg (MSC: none, control: n=3) or 5mg (MSC: n=3, control: n=6) because of a (viral) infection. All data are described as mean and standard deviation. [1] *Unpaired t test*