Induction Therapy With Autologous Mesenchymal Stem Cells in Living-Related Kidney Transplants

A Randomized Controlled Trial

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Context Antibody-based induction therapy plus calcineurin inhibitors (CNIs) reduce acute rejection rates in kidney recipients; however, opportunistic infections and toxic CNI effects remain challenging. Reportedly, mesenchymal stem cells (MSCs) have successfully treated graft-vs-host disease.

Objective To assess autologous MSCs as replacement of antibody induction for patients with end-stage renal disease who undergo ABO-compatible, cross-match–negative kidney transplants from a living-related donor.

Design, Setting, and Patients One hundred fifty-nine patients were enrolled in this single-site, prospective, open-label, randomized study from February 2008-May 2009, when recruitment was completed.

Intervention Patients were inoculated with marrow-derived autologous MSC ($1–2 \times 10^6$/kg) at kidney reperfusion and two weeks later. Fifty-three patients received standard-dose and 52 patients received low-dose CNIs (80% of standard); 51 patients in the control group received anti–IL-2 receptor antibody plus standard-dose CNIs.

Main Outcome Measures The primary measure was 1-year incidence of acute rejection and renal function (estimated glomerular filtration rate [eGFR]); the secondary measure was patient and graft survival and incidence of adverse events.

Results Patient and graft survival at 13 to 30 months was similar in all groups. After 6 months, 4 of 53 patients (7.5%) in the autologous MSC plus standard-dose CNI group (95% CI, 0.4%-14.7%; $P=.04$) and 4 of 52 patients (7.7%) in the low-dose group (95% CI, 0.5%-14.9%; $P=.046$) compared with 11 of 51 controls (21.6%; 95% CI, 10.5%-32.6%) had biopsy-confirmed acute rejection. None of the patients in either autologous MSC group had glucorticoid-resistant rejection, whereas 4 patients (7.8%) in the control group did (95% CI, 0.6%-15.1%; overall $P=.02$). Renal function recovered faster among both MSC groups showing increased eGFR levels during the first month after surgery than the control group. Patients receiving standard-dose CNI had a mean difference of 6.2 mL/min per 1.73 m^2 (95% CI, 0.4-11.9; P=.04) and those in the low-dose CNI of 10.0 mL/min per 1.73 m^2 (95% CI, 3.8-16.2; P=.002). Also, during the 1-year follow-up, combined analysis of MSC-treated groups revealed significantly decreased risk of opportunistic infections than the control group (hazard ratio, 0.42; 95% CI, 0.20-0.85, P=.02)

Conclusion Among patients undergoing renal transplant, the use of autologous MSCs compared with anti-IL-2 receptor antibody induction therapy resulted in lower incidence of acute rejection, decreased risk of opportunistic infection, and better estimated renal function at 1 year.

Trial Registration clinicaltrials.gov Identifier: NCT00658073

JAMA. 2012;307(11):1169-1177 www.jama.com

INDUCTION THERAPY, ROUTINELY implemented in organ transplant procedures, consists of biologic agents to block early immune activation.[1,2] For kidney transplants, lymphodepletion with antithymocyte globulin (ATG) or alemtuzumab has contributed to reducing acute rejection episodes and improving early graft function but remains associated with toxic effects, cytomegalovirus reactivation, and posttransplant lymphoproliferative disease.[3-5] Targeting interleukin 2-(IL-2) receptor α chain on activated T lymphocytes can reduce acute rejection episodes in kidney transplant when combined with standard immunosuppression.[2]

Novel induction immunosuppressive protocols with increased efficacy and minimal adverse effects are desirable. Appealing are the immunoregulatory properties of bone marrow–derived mesenchymal stem cells (MSCs), which represent a nonhematopoietic cell population that can differentiate into mesenchymal tissues (ie, bone, cartilage, or fat).[3] They inhibit T-cell proliferation, monocyte differentiation to dendritic cells, modulate B-cell functions, and suppress natural

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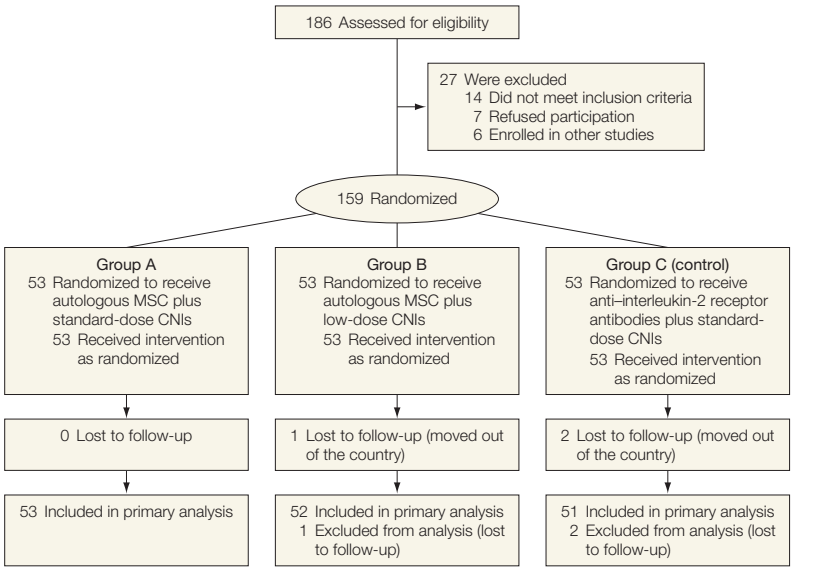
Corrected on March 21, 2012

Figure 1. Study Flowchart



CNI indicates calcineurin inhibitors; MSC, mesenchymal stem cells.

killer cytotoxic effects.[5] Thus, MSCs offer new therapeutic opportunities to prevent transplant rejection.[6] Le Blanc et al[7] first reported the striking clinical response to haploidentical MSCs in a case of severe, treatment-resistant grade IV acute graft-vs-host disease. A multicenter phase 2 trial for steroid-resistant, severe acute graft-vs-host disease confirmed this observation, while showing no adverse effects after MSC infusion.[8] Mesenchymal stem cells obtained from either HLA-identical siblings, haploidentical, or HLA-mismatched third-party donors were similarly effective.[8] Our study aimed at examining the effect of autologous MSC infusion as an alternative to anti-IL-2 receptor antibody for induction therapy in adults undergoing living-related donor kidney transplants.

METHODS

Study Design

This, single-site prospective, randomized study aimed at comparing the risk-benefit profile of autologous MSC infusion vs anti-IL-2 receptor antibody (basiliximab; Novartis) induction therapy for living-related donor kidney transplants. Kidney donation followed the 2004 Amsterdam guidelines.[9] To prevent enrolling patients involved with organ trafficking,[10] all donors had a documented linear blood relationship with their respective recipient (eg, parent to children and siblings with the same parents). Donor renal function was assessed by technetium Tc 99m diethylene triamine pentaacetic acid plasma clearance. Transplants were performed according to ABO blood compatibility and negative HLA crossmatch results (OneLambda). Blood transfusions were never used before or after the transplants. Patients were considered ineligible for enrollment in the study if they had systemic infections, had prior treatment for cancer, were pregnant, were obese (body mass index [BMI] >28 calculated as weight in kilograms divided by height in meters squared), or had abnormal blood chemistries (ie, total cholesterol ≥300 mg/dL, triglycerides ≥400 mg/dL, white blood cell count ≤3000/μL, or platelets $\leq 75 \times 10^3$/μL). At our center, the mean time patients undergo dialysis before transplant is 6.8 or fewer months.

The Fuzhou General Hospital's institutional review board approved the study protocol. Candidate screening began in December 2007. All eligible patients were enrolled from February 2008 through May 2009 in accordance with the Declaration of Helsinki and adhering to the 2007 Chinese "Regulation on Human Organ Transplantation" (Order of the State Council No. 491).[40]

After providing written informed consent, enrolled participants were randomly assigned to 1 of 3 treatment groups, based on randomization numbers (STATA 9.0, StataCorp) assigned to each of the patients generated from the time and date of his/her first visit. No stratification and blocking were performed (FIGURE 1). Treatment groups were identified as groups A, B, or C to preserve blinding during statistical analysis. All groups received similar doses of mycophenolate mofetil (Roche) and corticosteroids. Additionally, group A received autologous MSCs plus standard-dose calcineurin inhibitors (CNIs; either cyclosporine [Neoral, Novartis] or tacrolimus [Astella]); group B received autologous MSC plus low-dose CNIs[39]; and group C, the control group, received anti–IL-2 receptor antibody plus the standard dose of CNIs.

Autologous MSC Cultures

Bone marrow cell aspirates (60-80 mL) were obtained while patients were under local anesthesia from the posterior iliac crest of the kidney recipient 1 month before the transplant. Following current good manufacturing practices, mononuclear bone marrow cells were isolated by Percoll (1.073 g/mL) centrifugation and allowed to adhere to a flask for 72 hours in low-glucose Dulbecco modified Eagle medium (Gibco-Invitrogen), followed by media change every 3 days. Cells' phenotype were assessed by flow cytometry, and their ability to differentiate into adipocytes and osteocytes in culture was confirmed in vitro following the 2006 International Society of Cellular Therapy's criteria.[11] At 70% to 80% confluence, cells were detached and replated at 1×10^6/175 cm^2 culture to process for infusion[12] and tested negative for endotoxin, hepatitis C virus, hepatitis B virus, HIV, syphilis, fungus, *Myco-*

plasma species, and *Chlamydia* before infusion. G-banding karyotype analysis was performed to confirm absence of chromosomal aberrations in the final cellular product.

Autologous MSC Infusion

Confluent autologous MSCs at passage 3 to 4 were collected in M199 culture media (Gibco-Invitrogen) containing 1% human serum albumin, and stored for up to 4 hours at 4°C. Mesenchymal stem cells suspensions of 1×10^6/mL were transferred into 20-mL syringes for intravenous infusion over 15 to 20 minutes. Each participant received autologous MSC infusion ($1–2 \times 10^6$/kg each) 10 minutes before the graft's vein and artery were unclamped, and 2 weeks posttransplantation.

Surgery

All grafts were from kinship-related donors. Kidneys were biopsied prior to the transplant procedure and placed into the right iliac fossa. Verapamil was injected into the renal artery before vascular anastomosis. Cold and warm ischemia times were recorded.

Immunosuppression Regimen

Only the control group received 20 mg of anti–IL-2 receptor antibody intravenously within 2 hours of surgery and 4 days after surgery. Those in the MSC standard-dose CNI group and the control group received immunosuppression with steroids, mycophenolate mofetil, and either cyclosporine or tacrolimus. Tacrolimus was initiated at 0.12 mg/kg, targeting trough levels of 8 to 12 mg/kg for the first trimester, 5 to 8 mg/kg for the second, and 3 to 7 ng/mL for the third trimester and beyond. Cyclosporine was initiated at 6.5 mg/kg with its target levels (concentration 2 hours after dose, C_2) of 1000 to 1200 ng/mL in the first trimester, 800 to 1000 ng/mL in the second trimester, and 600 to 800 ng/mL in the third trimester and beyond. Mycophenolate mofetil was given orally at either 2.0 g/d for patients who weighed 80 kg or more or 1.5 g/d for those who weighed less than 80 kg. Immediately after surgery and through day 3, patients received 6 mg/kg of methylprednisolone intravenously, 240 mg/d on day 4, 160 mg/d on day 5, and 80 mg/d on day 6. On days 7 through 14, patients received 30 mg/d of prednisone. Doses were tapered to 20 mg/d for the first trimester, 10 to 15 mg/d for the second trimester, and 5 to 10 mg/d for the third trimester and beyond. Those in MSC low-dose CNI group received reduced 80% of the standard CNI dose.[10] Acute rejection episodes were treated with methylprednisolone pulse therapy. Glucorticoid-resistant rejection was treated with ATG (Fresenius).

Follow-up

Passive participant follow-up was conducted weekly for the first trimester and monthly thereafter for 1 year. Acute rejection was defined as an increase of 0.3 mg/dL of serum creatinine (nadir creatinine), confirmed by renal biopsy within 24 hours of initiation of antirejection therapy. Biopsies were read and the severity of lesions was scored by a blinded pathologist. Acute rejection episodes were classified according to Banff 97 criteria.[13] Acute humoral rejection was confirmed by complement C4d immune staining in peritubular capillaries.

End Points

The primary outcome was the incidence of biopsy-confirmed acute rejection and estimated glomerular filtration rate (eGFR) within the first year. The eGFR was calculated from the adapted Modification of Diet in Renal Disease formula (eGFR [mL/min per 1.73 m^2] = $186 \times$ [serum creatinine]$^{-1.154} \times$ [age]$^{-0.203} \times$ [0.742 if female] $\times$ [1.227 for Chinese]).[14]

The secondary outcome was 1-year patient and graft survival and the incidence of adverse events, including opportunistic infections.

Safety

Incidence of adverse events, vital signs, laboratory parameters (hematology, clinical chemistry, and urine analyses), infections, failure to achieve primary closure of the surgical wounds at 2 weeks, and formation of lymphocele requiring intervention within 6 months after surgery were recorded.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (v.10.1) and SAS 9.1 (SAS Institute Inc). Results are expressed as means and 95% confidence intervals. Power and sample size considerations assume a 30% incidence of acute rejection within the first year after kidney transplant in China[15] and a reduction to 7.5% with MSC therapy based on the preliminary pilot study performed at our center (data unpublished) and to published reports.[16] χ^2 Tests of independence considering 3 independent proportions demonstrated adequate power to detect this assumed difference with 53 patients per group (type I error, 0.05; 80% power). In considering early renal function recovery measured by eGFR, a mean (SD) difference in eGFR levels between those treated with MSCs and those treated with anti–IL-2 receptor antibodies of more than 10 mL/min (11 mL/min) would be adequately powered with approximately 20 patients per group (type I error). Thus, we elected to enroll 53 patients per group, which is adequately powered for our hypotheses. Incidence of biopsy-proven acute rejection and other categorical outcomes were analyzed and compared among groups using χ^2 tests. Continuous pretransplant measures were compared using 1-way analysis of variance with 3 levels of a group. Repeated measures observed for eGFR were analyzed by linear mixed-model regression, and parameter estimates were adjusted for age, BMI, sex, and CNI type. Only P values $<.05$ with a 2-sided test were considered statistically significant. For multiple comparisons, P values reported correspond to overall F tests for contrasts for each factor (eg, group or postoperative surgery day). Subgroup comparisons were based on tests of interaction and were considered for differential effects of group and postoperative surgery by age, sex, BMI, and CNI type. Risk of oppor-

tunistic infection was evaluated with χ^2 tests of association among treatment groups, as well as with Cox models, considering time to first occurrence of opportunistic infection. For this end point, there were enough observed events to make meaningful estimations and comparisons of hazards and corresponding survival curves. Kaplan-Meier estimates of survival curves were evaluated to verify the proportional hazards assumption, as well as potential stratification by other factors such as sex and CNI type (which would allow the shape of the hazard to depend on the level of these other factors but still assume proportionality among groups).

To convert cholesterol from mg/dL to mmol/L, multiply by 0.259; triglycerides from mg/dL to mmol/L, by 0.0113; and creatinine from mg/dL to μmol/L, by 88.4.

RESULTS

Donor and Recipient Characteristics

One hundred eighty-six consecutive patients with end-stage renal disease (ESRD) were screened. All 159 eligible patients eventually enrolled after 27 were excluded (Figure 1). Recipients had a mean BMI of 21 (range, 14-31) and were a mean age of 38 years (range, 18-61 years). Less than 3% tested positive for cytomegalovirus. All characteristics were consistent with the southern Chinese population undergoing renal transplantation for ESRD, mainly for glomerulonephritis (~80%).[15] Age, sex, HLA compatibility, cytomegalovirus donor-recipient status, basal panel

Table 1. Primary and Selected Secondary End Points (1-Year Follow-Up)[a]

End Point	Autologous Mesenchymal Stem Cell Treatment		Control (n = 51)	*P* Value Overall Type 3[b]
	Standard-Dose CNI (n = 53)	Low-Dose CNI (n = 52)		
Primary end point				
eGFR, mean (95% CI), mL/min per 1.73 m^2,[c]				
Posttransplant				
0 d	6.8 (4.7-8.8)	5.3 (3.1-7.6)	5.8 (3.0-8.6)	.56
7 d	77.0 (67.4-86.6)[d]	74.9 (66.3-83.6)[d]	52.6 (44.5-60.7)	<.001
14 d	84.9 (75.2-94.6)[e]	77.8 (69.0-86.6)	69.6 (61.0-78.3)	.07
1 mo	91.1 (83.7-98.4)[f]	81.4 (73.8-89.0)	79.0 (69.9-88.1)	.08
2 mo	90.1 (84.3-96.0)	85.6 (79.9-91.3)	82.3 (74.1-90.5)	.28
3 mo	88.9 (82.8-95.0)	87.9 (80.5-95.3)	85.8 (78.8-92.9)	.81
6 mo	90.6 (84.2-97.1)	82.7 (76.6-88.8)	89.4 (83.0-95.9)	.62
12 mo	93.2 (86.2-100.2)	86.7 (79.0-94.3)	85.5 (78.2-92.9)	.49
Acute rejection, No. (%) [95% CI]				
At 6 mo				
Biopsy-confirmed	4 (7.5) [0.4-14.7][g]	4 (7.7) [0.5-14.9][h]	11 (21.6) [10.5-32.6]	.02
Corticosteroid-resistant	0	0	4 (7.8) [0.6-15.1]	
Histological severity				
Banff I/II	4 (7.5) [0.4-14.7]	4 (7.7) [0.5-14.9]	7 (13.7) [4.5-23.0]	.007
Banff III	0	0	4 (7.8) [0.6-15.1]	
At 12 mo				
Biopsy-confirmed	8 (15.1) [5.5-24.7]	9 (17.3) [7.1-27.5]	13 (25.5) [13.8-37.2]	.37
Corticosteroid-resistant	0	1 (1.9) [0-5.6]	4 (7.8) [0.6-15.1]	.06
Histological severity				
Banff I/ II	8 (15.1) [5.5-24.7]	8 (15.4) [5.7-25.1]	7 (13.7) [4.5-23.0]	.07
Banff III	0	1 (1.9) [0-5.6]	4 (7.8) [0.6-15.1]	
Secondary, No. (%) [95% CI]				
Delayed graft function	5 (9.4) [1.6-17.3]	4 (7.7) [0.5-14.9]	4 (7.8) [0.6-15.1]	.94
Duration of dialysis, mean (range), d	17.4 (10.5-24.3)	15.3 (7.9-23.1)	16.3 (10.0-22.5)	.28
Graft loss	1 (1.9) [0-5.5]	2 (3.8) [0-9.0]	1 (2.0) [0-5.7]	.85
Acute rejection	0	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.85
Chronic rejection	1 (1.9) [0-5.5]	1 (1.9) [0-5.6]	0	
Death	0	0	0	

Abbreviations: CNI, calcineurin inhibitors; eGFR, estimated glomerular filtration rate.
[a] The χ^2 test was used to compare the difference in acute rejection among the groups. Repeated eGFR analyses were estimated with mixed-linear regression and were adjusted for age, body mass index, and sex.
[b] *P* values for comparisons between autologous mesenchymal stem cell–treated groups and the control group for eGFR were calculated with the use of linear mixed-model regression analysis.
[c] eGFR calculation was based on a modified Modification of Diet in Renal Disease equation adjusted specifically for Chinese.
[d] $P < .001$.
[e] $P = .02$.
[f] $P = .045$.
[g] $P = .04$.
[h] $P = .046$.

reactive antibody, time undergoing dialysis, cause of ESRD, operation time, and warm and cold ischemia times were comparable among the 3 groups (eTable 1 available at http://www.jama.com). The 105 patients in the 2 autologous MSC groups displayed classical MSC phenotypes (>96% of cells were positive for CD29, CD73, CD90, CD105, and <2% of cells were positive for CD34 and CD45 by flow cytometry analysis) and could differentiate into osteoblasts and adipocytes in vitro. All autologous MSC preparations displayed normal karyotypes (data not shown). Donor demographics were comparable among groups (eTable 1). Three participants (2 in the control group and 1 in the autologous MSC low-dose CNI group) were lost to follow-up after emigrating from China. They were excluded from analysis (Figure 1). All participants remained in the study group to which they were assigned, and there were no missing values from the 12-month follow-up.

Renal Graft Function

Delayed graft function rates were similar among groups (TABLE 1). Pretransplant eGFR levels were also comparable. After transplantation, eGFR quickly increased (Table 1 and TABLE 2). Estimates obtained from linear mixed-model regression comparing overall eGFR values among groups demonstrated significantly higher levels among those in the MSC standard-dose CNI group than those in the control group over the entire 1-year posttransplant follow-up with a mean difference of 9.1 mL/min per 1.73 m^2 (95% CI, 1.6-16.5; P=.02; Table 2). Both MSC groups had a greater increase in eGFR levels during the first month after surgery than the control group, with the standard-dose CNI group having a mean difference of 6.2 mL/min per 1.73 m^2 (95% CI, 0.4-11.9; P=.04) and the low-dose CNI group having a mean difference of 10.0 mL/min per 1.73 m^2 (95% CI, 3.8-16.2; P=.002; Table 2). At each time point measured, eGFR levels were significantly higher in the standard-dose group than in the control group with a mean of 24.4 mL/min per 1.73 m^2 (95% CI, 11.9-37.0; P<.001) 7 days after surgery, 15.3 mL/min per 1.73 m^2 (95% CI, 2.3-28.3; P=.02) 14 days after surgery, and 12.1 mL/min per 1.73 m^2 (95% CI, 0.3-23.8; P=.045) 30 days after surgery (Table 2). The low-dose group had significantly higher increased eGFR levels than the control group 7 days after surgery with a mean difference of 22.4 mL/min per 1.73 m^2 (95% CI, 10.8-34.0; P<.001; Table 2). Sixty days after surgery, the control group's eGFR levels had become comparable with those in both MSC groups and then remained stable up to a year (Table 1 and Table 2). The CNI type was not found to have any significant main effect on the eGFR values nor was it found to have any confounding effect on the other factors considered in this analysis, specifically by group or days after surgery.

Acute Rejection

Six months after surgery, 4 of 53 patients (7.5%) in the MSC standard-dose CNI group (95% CI, 0.4%-14.7%; P=.04) and 4 of 52 patients (7.7%; 95% CI, 0.5%-14.9%; P=.046) in low-dose CNI group experienced biopsy-proven acute rejection compared with 11 of 51 patients (21.6%) in the control group (95% CI, 10.5%-32.6%; Table 1). Patients in neither MSC group experienced corticosteroid-resistant acute rejection, whereas 4 patients (7.8%) in the control group did (95% CI, 0.6%-15.1%; P=.02). Four patients (7.5%) in the MSC standard-dose group (95% CI, 0.4%-14.7%) and 4 (7.7%) in the low-dose group (95% CI, 0.5%-14.9%) had Banff grade I or II histological changes compared with 7 (13.7%) in the control group (95% CI, 4.5%-23.0%). No one in either MSC group had grade III changes compared with 4 (7.8%) in the control group (95% CI, 0.6%-15.1%; overall P=.007; Table 1). During the study period, no patients in the MSC groups and 4 in the control group required ATG treatment for steroid-resistant acute rejection. No significant difference in acute rejection incidence was found among groups at study end (Table 1).

Table 2. Estimated eGFR Differences Between Groups

Time Point, d	eGFR Difference (95% CI), mL/min per 1.73 m^2	*P* Value[a]
Autologous MSC + Standard-Dose CNI vs Control Group		
0	1.0 (–2.0 to 4.0)	.51
7	24.4 (11.9 to 37.0)	<.001
14	15.3 (2.3 to 28.3)	.02
30	12.1 (0.3 to 23.8)	.045
60	7.8 (–2.2 to 17.8)	.13
90	3.1 (–6.3 to 12.4)	.52
180	1.2 (–7.9 to 10.3)	.80
360	7.7 (–2.4 to 17.8)	.14
7-30[b]	6.2 (0.4 to 11.9)	.04
0 – 360[b]	9.1 (1.6 to 16.5)	.02
Autologous MSC + Low-Dose CNI vs Control Group		
0	–0.5 (–3.6 to 2.7)	.78
7	22.4 (10.8 to 34.0)	<.001
14	8.2 (–3.9 to 20.3)	.18
30	2.4 (–9.3 to 14.1)	.69
60	3.3 (–6.5 to 13.0)	.51
90	2.1 (–8.0 to 12.1)	.69
180	–6.7 (–15.4 to 2.0)	.13
360	1.1 (–9.3 to 11.6)	.83
7-30[b]	10.0 (3.8 to 16.2)	.002
0-360[b]	4.0 (–2.9 to 10.9)	.25
Autologous MSC + Standard-Dose vs Low-Dose CNI		
0	1.5 (–1.3 to 4.2)	.30
7	2.1 (–10.7 to 14.8)	.75
14	7.1 (–5.8 to 20.0)	.28
30	9.7 (–0.7 to 20.1)	.07
60	4.6 (–3.4 to 12.6)	.26
90	1.0 (–8.5 to 10.5)	.84
180	7.9 (–0.7 to 16.5)	.07
360	6.5 (–3.7 to 16.7)	.21
7-30[b]	–3.8 (–9.4 to 1.8)	.19
0-360[b]	5.0 (–1.8 to 11.9)	.15

Abbreviations: eGFR, estimated glomerular filtration rate; CNI, calcineurin inhibitor; MSC, mesenchymal stem cell
[a] Repeated measure analysis by linear mixed model regression.
[b] Averaged over time points indicator.

Patient and Graft Survival

The 1-year patient survival rate was 100% in all groups (Table 1). One participant in the control group died of pneumonia at 399 days after surgery. The 12-month graft loss was comparable among groups: 1 patient (1.9%) in the MSC standard-dose CNI group (95% CI, 0%-

5.5%), 2 (3.8%) in the low-dose CNI (95% CI, 0%-9.0%), and 1 (2.0%) in the control group (95% CI, 0%-5.7%).

Immunosuppression

Dose and target trough levels of CNIs are summarized in eTables 2-3 (available at http://www.jama.com). For most time points assessed during the 1-year follow-up, CNI trough levels in the MSC low-dose CNI group were significantly lower than in both groups that had received the standard dose of CNI (eTable 3).

Adverse Events

Lower adverse events were seen in both autologous MSC groups than in the control group (TABLE 3). The incidence of opportunistic infection was significantly lower in the MSC low-dose CNI group than in the control group but was not lower in the standard-dose group compared with the control group (Table 3). Initial results indicated comparable risk between MSC-treated groups but significantly decreased risk of opportunistic infection in the low-dose CNI group than in the control group (hazard ratio, 0.28; 95% CI, 0.10-0.76; $P = .01$; Table 3; FIGURE 2A). During the 1-year follow-up, a significant decreased risk of op-

Table 3. Adverse Events (1-Year Follow-Up)[a]

Events	No. (%) of Patients [95% CI]			*P* Value Overall Type 3
	Autologous Mesenchymal Stem Cell Treatment			
	Standard-Dose CNI (n = 53)	Low-Dose CNI (n = 52)	Control Group (n = 51)	
Total adverse events	35 (66.0) [53.3-78.8][b]	32 (61.5) [48.4-74.6][c]	43 (84.3) [74.5-94.1]	.01
Leukopenia				
7 d	6 (11.3) [2.8-19.9]	5 (9.6) [1.7-17.6]	4 [0.6-15.1]	.80
14 d	5 (9.4) [1.6-17.3]	6 (11.5) [2.9-20.1]	3 (5.9) [0-12.2]	.60
1 mo	3 (5.7) [0-11.9]	4 (7.7) [0.5-14.9]	2 (3.9) [0-9.1]	.71
3 mo	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	2 (3.9) [0-9.1]	.81
12 mo	0	0	1 (2.0) [0-5.7]	.36
Lymphopenia				
7 d	5 (9.4) [1.6-17.3]	5 (9.6) [1.7-17.6]	3 (5.9) [0-12.2]	.74
14 d	8 (15.1) [5.5-24.7]	7 (13.5) [4.3-22.7]	5 (9.8) [1.8-17.8]	.71
1 mo	4 (7.5) [0.4-14.7]	6 (11.5) [2.9-20.1]	4 (7.8) [0.6-15.1]	.73
3 mo	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.79
12 mo	0	0	0	>.99
All infections	28 (52.8) [39.4-66.3]	20 (38.5) [25.4-51.6]	31 (60.8) [47.6-73.9]	.07
Opportunistic infection	10 (18.9) [8.3-29.4][d,e]	5 (9.6) [1.7-17.6][f]	15 (29.4) [17.1-41.7]	.03
Candida	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	
Cytomegalovirus	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	
EB virus	3 (5.7) [0-11.9]	1 (1.9) [0-5.6]	5 (9.8) [1.8-17.8]	
Herpes simplex virus	3 (5.7) [0-11.9]	2 (3.8) [0-9.0]	4 (7.8) [0.6-15.1]	
Time to first opportunistic infection, HR vs control group[h]	0.6 (0.25-1.24)[g]	0.28 (0.10-0.76)		.04
Other infections	18 (34.0) [21.2-46.7]	15 (28.8) [16.6-41.0]	16 (31.4) [18.9-43.9]	.85
Nasopharyngitis	6 (11.3) [2.9-19.9]	4 (7.7) [0.5-14.9]	6 (11.8) [3.1-20.4]	
Pneumonia	4 (7.5) [0.4-14.7]	2 (3.8) [0-9.0]	4 (7.8) [0.6-15.1]	
Urinary tract infection	5 (9.4) [1.6-17.3]	6 (11.5) [2.9-20.1]	4 (7.8) [0.6-15.1]	
Phlebitis	3 (5.7) [0-11.9]	3 (5.8) [0-12.0]	2 (3.9) [0-9.1]	
Hematuria	2 (3.8) [0-8.9]	3 (5.8) [0-12.0]	4 (7.8) [0.6-15.1]	.67
Proteinuria	2 (3.8) [0-8.9]	2 (3.8) [0-9.0]	3 (5.9) [0-12.2]	.84
Complications of transplanted kidney	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.79
Delayed wound healing at 2 wk	1 (1.9) [0-5.5]	0	2 (3.9) [0-9.1]	.35
Lymphocele	1 (1.9) [0-5.5]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	.42

Abbreviations: CNI, calcineurin inhibitors; EB, Epstein-Barr; HR, hazard ratio.
[a] *P* values for comparisons between indicated experimental groups for total events. Infection and the times to the first opportunistic infection (OI) were calculated with the use of the χ^2 test.
[b] $P = .03$ vs Control group.
[c] $P = .009$ vs Control group.
[d] $P = .20$ vs Control group.
[e] $P = .18$ vs Mesenchymal stem cell low-dose CNI.
[f] $P = .01$ vs Control group.
[g] $P = .01$ vs Control group.
[h] Hazard ratio, 0.42 (95% CI, 0.20-0.85; P .02) when the 2 autologous mesenchymal stem cell groups were combined and compared against the control group.

portunistic infections was observed in MSC-treated groups with 10 patients (18.9%) in the standard-dose CNI (95% CI, 8.3-29.4) and 5 patients (9.6%) in the low-dose CNI group (95% CI, 1.7-17.6) vs 15 patients (29.4%) in the control group (95% CI, 17.1-41.7; overall $P = .03$; Table 3). Combined analysis of MSC-treated groups revealed significantly decreased risk of opportunistic infection than the control group (hazard ratio, 0.42; 95% CI, 0.20-0.85; $P = .02$; Figure 2B). Sex and CNI type were not found to have any significant association with risk of opportunistic infection. Incidence of other adverse events was comparable among groups (Table 3).

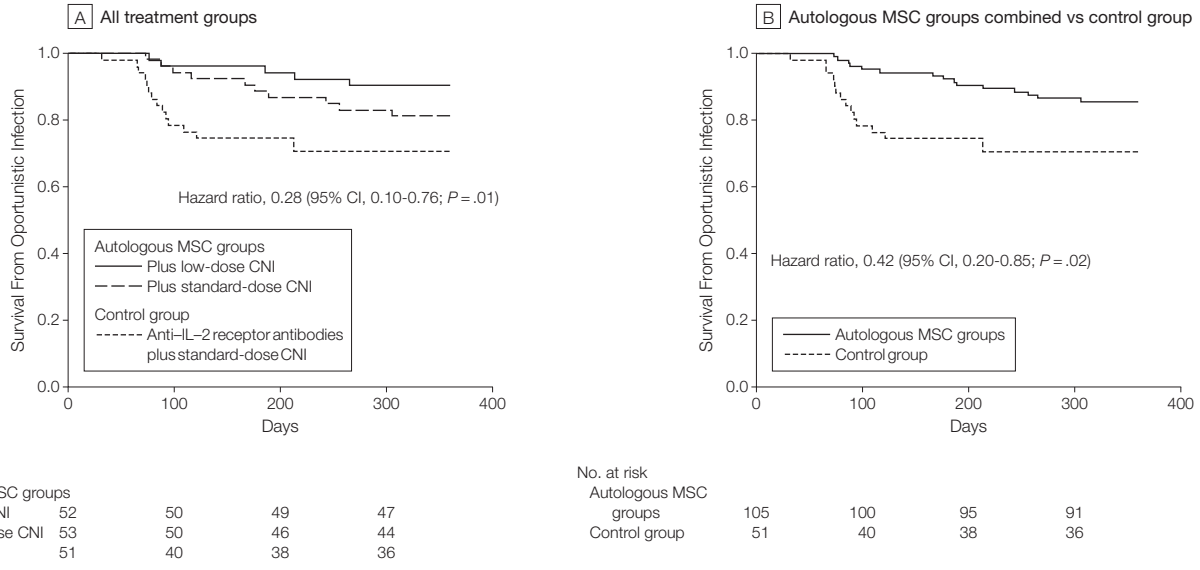
COMMENT

Over the past 2 decades, significant improvement in kidney transplant and patient survival has resulted from the introduction of new antirejection agent combinations.[17] Attention has turned recently toward novel interventions to further improve graft and patient survival.[18-20] The recognized immunomodulatory properties of MSCs[5,21] provided the rationale for exploring their use in the induction phase of transplant immunosuppression. In a preclinical model, MSC infusion prolonged the time to rejection of histoincompatible skin grafts.[22] Several reports demonstrated that MSCs, whether from the donor or a third party, from bone marrow or adipose tissue, can treat steroid-resistant acute graft-vs-host disease.[7,8]

There is no consensus on the best source of MSCs. Le Blanc et al[7,8] used third-party MSCs to treat graft-vs-host disease in recipients of autologous bone marrow cell transplants. For solid organ transplants, practical reasons support autologous MSCs, considering the need to isolate and expand cells in adequate numbers prior to transplant, which would be cumbersome to achieve from allogeneic cadaveric organ donors.

Clinically, nonrandomized trials[23,24] have shown sustained safety (≤5 years) of autologous MSCs for amyotrophic lateral sclerosis (ALS),[25,26] multiple sclerosis (MS),[26] and stroke.[27] A pilot study showed increased proportion of regulatory T cells, decreased lymphocyte proliferative responses, lower costimulation and HLA-DR expression on myeloid dendritic cells after MSC inoculation of patients with MS and ALS.[26] Autologous MSCs were shown to improve Crohn disease lesions refractory to other therapies.[28,29] A pilot study involving 2 patients showed lack of adverse events and no negative effect of autologous MSC inoculum on 1-year patient and graft survival following living-related donor kidney transplants under conventional immunosuppression.[30] In our prospective randomized trial on a large patient population, autologous MSCs could replace anti-IL-2 receptor–induction therapy in living-related donor kidney transplants. Recipients of autologous MSCs showed lower frequency of biopsy-confirmed acute rejection in the first 6 months than the control group. None of au-

Figure 2. First Occurrence of Opportunistic Infection



Kaplan-Meier estimates of survival curves were evaluated to verify proportional hazards assumption, as well as potential stratification by other factors such as sex and calcineurin inhibitor (CNI) types, which would allow shape of hazard to depend on level of these other factors but still assume proportionality among groups. Cox models were fit to model and compare risk of opportunistic infection among the groups. Patients with no observed opportunistic infection were considered to have censored data at the study end point. A, shows that the initial results indicate comparable risk between groups treated with autologous mesenchymal stem cells (MSCs) but significantly decreased risk of opportunistic infection in the MSC group receiving low-dose CNI compared with the control group. B, Because the autologous MSC treated groups were comparable, an overall comparison of autologous MSC-treated groups vs control was performed. Results of this comparison suggest autologous MSC-treated groups have significantly decreased risk of opportunistic infection compared with the control group.

tologous the MSC recipients required ATG for steroid-resistant acute rejection, whereas 7.8% of those in the control group did. Importantly, when acute rejection occurred, less severe renal lesions were present in patients receiving autologous MSCs than in patients in the control group. The incidence of acute rejection in the control group was slightly higher than previously reported in a trial that used a similar protocol,[4] despite the putative low risk of participants mostly lacking prior sensitization. Ethnicity differences between the 2 studies might have contributed to this observation.

We found that autologous MSC recipients had faster renal function recovery during the first month, displayed fewer adverse events and had reduced opportunistic infections than controls. Thus, autologous MSCs may replace anti-IL-2 receptor antibodies and may allow for using lower CNIs maintenance doses without compromising patient safety and graft outcome. Beneficial effects on cadaveric or living-related renal graft function allowing lowering immunosuppressive drug levels were reported following donor-specific, unfractioned bone marrow cell transplant.[31] In the absence of concomitant cellular therapy, improved renal allograft outcome—to a degree somewhat comparable with what was observed in the autologous MSC groups in our study—was matched only by potent lymphodepletion (alemtuzumab) but with the toll of severe infections in low-risk recipients.[31] Thus, should long-term safety of autologous MSC transplants be ascertained, cellular-based therapies may become a viable therapeutic option to improve graft and patient outcomes while reducing transplant immunosuppression toxic effects.

The risk for chromosomal aberrations, neoplastic transformation, increased telomerase activity, or both has been reported for human MSCs, generally following several passages in culture in the experimental setting.[32] We used early culture passages (P3-4) that displayed normal karyotypes. To the best of our knowledge, there are no clinical data supporting the development of neoplasms directly related to autologous MSC inoculum.[33] Another hypothetical risk is autologous MSC–enhanced immunosuppression and associated untoward adverse events (eg, de novo or reactivation of viral infections, lymphoproliferative disease, or progressive multifocal leukoencephalopathy). Careful assessment of the long-term safety of autologous MSC transplants is paramount, particularly in chronically immunosuppressed individuals who are more vulnerable to develop tumors and infections.

Additional potential benefits of autologous MSCs include improved recovery from ischemia or reperfusion injury,[34-36] a recognized important risk factor for graft failure and acute rejection.[1] Early function is a favorable prognostic factor for long-term graft survival.[37] We observed significantly faster eGFR increase in autologous MSC groups than in controls, indicating quicker recovery of graft function. When comparing overall graft function during the 1-year follow-up in the 2 groups receiving standard CNIs, the autologous MSC group showed significantly higher eGFR values than those who received the anti-IL-2 receptor. This is encouraging, considering that renal allograft function at 1 year is a good predictor of long-term outcome.[38]

A recent multicenter study demonstrated that a reduction in CNIs of about 50% alone may improve renal allograft function.[10] We targeted 20% lower CNI dose in the comparative autologous MSC group, which resulted in a greater trough-level reduction for cyclosporine (4%-15%) and tacrolimus (8%-19%) than those in the anti-IL-2 receptor group. This may explain why no further improvement in renal function was observed in the low-dose autologous MSC group. Nevertheless, the number of patients experiencing adverse events was significantly lower in autologous MSC recipients than in controls. Specifically, autologous MSC inoculum was associated with fewer opportunistic infections in the combined analysis, even more significantly in the low-dose CNI group. This may have important implications because opportunistic infection's occurring mostly during the first 3 to 6 months after transplant procedures are associated with the highest mortality rate in kidney transplant recipients in China.[15]

We report on a large-scale clinical application of autologous MSCs in allogeneic organ transplantation. Among patients undergoing living-related kidney transplants, the use of autologous MSCs compared with anti-IL-2 receptor–induction therapy resulted in a lower incidence of acute rejection, decreased risk of opportunistic infection, and better estimated renal function at 1 year. Importantly, autologous MSC inoculum was not associated with adverse events nor did it compromise kidney transplant survival. Extended monitoring of study participants will allow assessment of the long-term effects of autologous MSCs on renal allograft function, survival, and safety.

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Obtained funding: Tan.
Administrative, technical, or material support: Tan, Wu, Liao, Sun, Chen, Yang, Cai, Gao.
Study supervision: Tan, Ricordi.
Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.
Funding/Support: This study was supported in part by grants 2009Y4001 from the Key Science Research Project and 2008J1006 from the Key Laboratory, both of Fujian Province, China.

Role of the Sponsor: The funding agencies had no involvement in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
Online-Only Material: Three eTables are available at http://www.jama.com.
Additional Contributions: We thank all the patients whose participation made this study possible.

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