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Research Paper

Allogeneic Mesenchymal Precursor Cells (MPC) in Diabetic Nephropathy: A Randomized, Placebo-controlled, Dose Escalation Study

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ABSTRACT

Background: Diabetic nephropathy is the most common cause of end stage renal failure. We assessed the safety, tolerability, and explored therapeutic effects of adult allogeneic bone-marrow derived mesenchymal precursor cells (MPC) in patients with moderate to severe diabetic nephropathy.

Methods: Multicenter, randomized, double-blind, dose-escalating, sequential, placebo-controlled trial assessing a single intravenous (IV) infusion of allogeneic MPC (United States adopted name: rexlemestrocel-L) 150×10^6 ($n = 10$), 300×10^6 ($n = 10$) or placebo ($n = 10$) in adults with diabetic nephropathy with an estimated glomerular filtration rate (eGFR) 20–50 ml/min/1.73 m^2. Thirty patients at three Australian centers were enrolled between July 2013 and June 2014 and randomized 2:1, in two sequential dose cohorts, to receive rexlemestrocel-L or placebo. Study duration was 60 weeks. Primary endpoint was safety and tolerability. Primary exploratory efficacy endpoint was change from baseline in eGFR and directly measured GFR by ^{99}Tc-DTPA plasma clearance (mGFR) at 12 weeks post-infusion. The trial was registered on ClinicalTrials.gov (NCT01843387).

Findings: All patients completed the study and were included in analyses applied to the intention to treat population. There were no acute adverse events (AEs) associated with infusion and no treatment-related AEs or serious AEs were deemed treatment-related by investigators. No patients developed persistent donor specific anti-HLA antibodies. Relative to placebo, a single IV rexlemestrocel-L infusion showed trends of stabilizing or improving eGFR and mGFR at week 12. The adjusted least squares mean (LSM ± SE) differences from placebo in changes from baseline at 12 weeks in the rexlemestrocel-L groups were 4.4 ± 2.16 and 1.6 ± 2.15 ml/min/1.73 m^2 for eGFR and 4.1 ± 2.75 and 3.9 ± 2.75 for mGFR for the 150×10^6 and 300×10^6 cell groups, respectively.

Interpretation: This study demonstrates the safety of rexlemestrocel-L in diabetic nephropathy with suggestive effects on renal function to be confirmed in larger, appropriately powered trials.

1. Introduction

Diabetes is the most common underlying cause of chronic kidney disease leading to renal failure, accounting for about 40–50% of cases (Tuttle et al., 2014). Although inhibition of the renin-angiotensin aldosterone system can slow progression of diabetic kidney disease, the residual risk of progression to end stage renal failure is high (Lewis et al., 2001; Brenner et al., 2001). Appreciation of the multiple pathways by which progressive kidney injury occurs has led to a search for novel therapeutic approaches to slow, halt or reverse progression of renal disease in type 2 diabetic patients. Research has implicated inflammation as one contributing factor in the pathophysiology of diabetic nephropathy (Wada & Makino, 2013; Navarro-Gonzalez & Mora-Fernandez, 2011; Lim & Tesch, 2012). The anti-inflammatory properties of adult, bone-marrow derived mesenchymal lineage cells may have beneficial effects in diabetic nephropathy, as suggested by observed effects on renal function and histology in animal models of chronic kidney disease (Prockop & Oh, 2012; Singer & Caplan, 2011; Cantaluppi et al., 2013). Other properties such as tropism for damaged tissues and secretion of a broad range of bioactive molecules with subsequent paracrine effects contribute to the effects on renal function and histopathology in preclinical chronic and acute kidney injury models (Papazova et al., 2015; Meirelles Lda et al., 2009; Hickson et al., 2016). In addition, the capacity of this cell type to reprogram macrophages from a proinflammatory M1 phenotype to the alternatively activated or anti-inflammatory M2 phenotype may also promote tissue repair (Maggini et al., 2010; Kim & Hematti, 2009).

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This first in human study was designed to assess the overall safety of MPC and to explore its effects on renal function in patients with moderate to severe diabetic nephropathy as assessed by glomerular filtration rate measured directly by ^{99}Tc DTPA plasma clearance (mGFR) and estimated (eGFR) from serum creatinine using the Modification of Diet in Renal Disease (MDRD) equation (Levey et al., 1999).

2. Methods

2.1. Study Population

The study population was male and female patients ≥45 and ≤85 years old with type 2 diabetes and advanced diabetic nephropathy (e.g. eGFR 20–50 ml/min/1.73 m^2) (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013) who were receiving a stable, standard of care therapeutic regimen of the maximum tolerated recommended dose of an angiotensin converting enzyme inhibitor (ACEi) or a angiotensin 2 receptor blocker (ARB) for at least 3 months prior to screening. Because at the time that this study was initiated the potential for allosensitization from systemic infusion of cells from unrelated donors was unknown, only patients who, in the opinion of the investigator and, in accordance with the current consensus recommendations in Australia would be unlikely candidates for kidney transplant were included. Women of childbearing potential who were surgically sterile or agreed to use contraception were eligible to participate in the study. Exclusion criteria included: New York Heart Association Class III or IV heart failure and myocardial infarction or stroke within 6 months of screening. Complete eligibility criteria are provided in the Supplemental Study Protocol.

2.2. Study Procedures

This multicenter, randomized, double-blind, placebo-controlled, sequential, dose-escalation study assessed the safety, tolerability, and exploratory efficacy of a single intravenous infusion of rexlemestrocel-L. The study was conducted at 4 centers in Australia with patients enrolled at 3 clinical sites and all infusions conducted at the same phase 1 unit. The study consisted of an initial screening period not to exceed 4 weeks and a 60 week double-blind treatment and follow-up period including safety and renal function assessments, immune system responses and clinical laboratory parameters. The study, conducted between July 2013 and August 2015, was approved by the ethics committees of the participating centers and conducted in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonization - Good Clinical Practice guidelines. All participants provided written informed consent. The trial was registered on ClinicalTrials.gov (NCT01843387).

2.3. Randomization

An Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS) was accessed to randomize eligible patients. Patients were randomized to receive one of two rexlemestrocel-L doses or placebo in a 2:1 ratio using a sequential, escalating dose cohort paradigm: cohort 1: 150×10^6 [n = 10] or placebo [n = 5]; and cohort 2: 300×10^6 [n = 10] or placebo [n = 5]. The randomization within each cohort was balanced by permuted block stratification, based on screening eGFR ≤30 ml/min/1.73 m^2 or >30 ml/min/1.73 m^2.

2.4. Study Procedures

Treatment was administered by IV infusion on Day 0 following baseline assessments. Patients, investigators, and the sponsor were blinded to treatment allocation through the entire 60-week study. The investigational product is comprised of a STRO-3 immuno-selected, culture-expanded, immature subfraction of adult, bone marrow–derived mononuclear cells from healthy paid adult donors (U.S. adopted name rexlemestrocel-L) (Simmons & Torok-Storb, 1991; Gronthos et al., 2007; Skyler et al., 2015). Full details of rexlemestrocel-L source, donor screening, preparation, and investigational product administration are provided in the Supplementary Appendix. Importantly, however, these cells do not express human leukocyte antigen (HLA) Class II and CD80 and CD86 co-immunostimulatory molecules. Rexlemestrocel-L or saline placebo were suspended in 100 ml normal saline and infused with filtration over 45 min. All infusions were prepared by an unblinded pharmacist at the phase 1 unit who provided to the blinded clinical staff visually identical infusion products comprised of rexlemestrocel-L or saline suspended in 100 ml normal saline. Vital signs and oxygen saturation were monitored continuously during and for 6 hour post-infusion. All patients remained on their background medications and received standard of care management throughout the study.

2.5. Study Oversight

This study was sponsored by Mesoblast, Inc. and was designed by the sponsor with input from the authors and the contract research organization (CRO; Medpace, Inc., Cincinnati OH). The study database was held by the CRO and employees of Medpace performed the statistical analyses. All authors participated in manuscript preparation, made the decision to submit the manuscript for publication, and vouch for the completeness and accuracy of the data.

An independent Data Monitoring Committee (DMC) comprised of independent physicians with expertise in nephrology, diabetes, and cardiology, and the conduct of clinical trials, and an independent biostatistician ensured the safety of the patients enrolled in the study. The DMC reviewed safety data when all patients in the first dose cohort completed the Week 1 visit and issued a certificate of non-objection to advance to the higher dose cohort to the sponsor. Formal stopping rules were in place for the DMC to hold enrollment or recommend termination the study of any event of Common Terminology Criteria for Adverse Events (CTCAE) (United States Department of Health and Human Services, 2010) grade 4 or higher pulmonary/upper respiratory toxicity occurring at any time after study drug infusion or CTCAE grade 3 or higher immune system toxicity occurring within 7 days of study drug infusion.

2.6. Outcomes

Safety was assessed by adverse events, laboratory measurements (hematology, chemistry, and urinalysis), vital signs, 12-lead ECGs, physical examination findings, and review of antibody specificity testing for anti-HLA Class I and Class II antibodies and anti-murine and anti-bovine antibodies. Pre-specified safety parameters of special interest included any adverse event reported during the infusion or the 6-hour post-infusion period and any adverse events in the immune system or respiratory system organ classes. GFR was estimated from serum creatinine (eGFR) using the 4-variable Modification of Diet in Renal Disease (MDRD) equations at every visit and measured directly by ^{99}Tc DTPA plasma clearance (mGFR) at baseline and 12 weeks. Urinary albumin and protein, urinary albumin: creatinine and urinary protein: creatinine ratios, and creatinine clearance were assessed from a 24-h urine collection at baseline and week 12. Selected biomarkers were measured at the same timepoints.

2.7. Assay Procedures

Immune profiling consisted of Panel Reactive Antibodies (PRA) by flow cytometry to detect the presence of donor specific antibodies (DSA) assessed on Day 0 and weeks 4, 12, 36 and 60. Assays were performed using a Luminex platform by the Blood Center of Wisconsin. Human leukocyte antigen (HLA) status was reported as negative if Class I or Class II percent PRA (%PRA) of the total antigens tested was

<5%; positive status was defined as %PRA ≥5% and ≤20% and highly positive was defined as %PRA >20%. Positive DSA was identified when antibody specificities were directed to the MPC donor HLA antigens. IL-6 and TNF-a were measured by ELISA and hsCRP was determined by nephelometry.

2.8. Statistical Analyses

The primary objective of this study was to assess safety and tolerability of MPC therapy. Accordingly, there was no formal hypothesis testing or accompanying power analysis to indicate a sufficient sample size to identify significant treatment differences in renal function outcomes between rexlemestrocel-L and placebo. Efficacy analyses were primarily descriptive and hypothesis generating. *p*-Values for selected efficacy analyses were generated for exploratory purposes.

Demographic and baseline characteristics were summarized for all randomized patients by treatment group. Safety analyses were applied to the safety population, defined as all patients that received study treatment and had at least one follow-up safety evaluation. All efficacy analyses were applied to the intent-to-treat (ITT) population, defined as all randomized patients who received study treatment and had at least one evaluable post-baseline renal function assessment. In case of missing data the last evaluable assessment was carried forward to the endpoint (LOCF). The primary efficacy variables were changes from baseline in eGFR and mGFR at week 12. Treatment differences in efficacy endpoints were obtained using an analysis of covariance model with treatment and eGFR strata (≤30 or >30 ml/min/1.73 m^2) as factors and baseline value as covariate. The difference in least-squares means (LSM) and corresponding standard errors are presented. Sensitivity analysis was applied to compare eGFR values derived using the MDRD equation and the more recent, widely accepted Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Levey et al., 2009). This and an additional subgroup analysis are provided in the Supplemental Appendix. Data were analyzed using SAS Version 9.1. The statistical analysis plan for this study is available as a supplemental Statistical Analysis Plan.

2.9. Role of the Funding Source

The study was sponsored and funded by Mesoblast, Inc. The sponsor provided oversight to the contract research organization, Medpace, Inc., responsible for data collection, data review, statistical analysis and the clinical study report. The sponsor had no role in the data collection or analysis. The sponsor reviewed the manuscript before it was submitted for publication but did not control the interpretation of the results or the decision to submit the manuscript for publication. The corresponding author had full access to all the data and had full responsibility for the decision to submit the manuscript for publication.

3. Results

3.1. Study Participants

Thirty patients were enrolled at 3 centers in Melbourne, Australia between July 2013 and June 2014. The disposition of patients is shown in Fig. 1.

All randomized patients completed the study. Demographic and key baseline characteristics are shown in Table 1.

3.2. Safety

All patients received the full infusion. No adverse events were reported during infusion or the 6 hour post-infusion monitoring period. Over the entire 60 week study period 7 (70%), 8 (80%) and 9 (90%) of patients in the placebo and rexlemestrocel-L 150×10^6 and 300×10^6 groups experienced any adverse event (Table 2A). Adverse events were generally mild to moderate intensity, resolved without sequelae and none led to premature study discontinuation or were considered to be treatment-related by the investigators (Table 2A). The most commonly reported TEAEs were edema peripheral (5 reported events), lower respiratory tract infection (Wada & Makino, 2013), urinary tract infection (Brenner et al., 2001), cataract (Brenner et al., 2001), and anemia (Brenner et al., 2001) which were generally balanced across

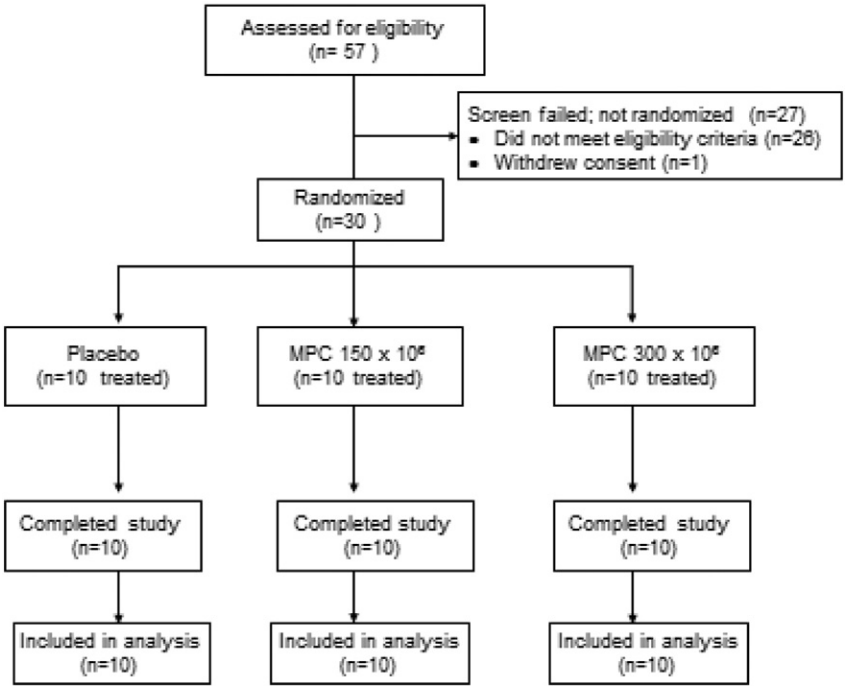

Fig. 1. Disposition of patients.

Table 1
Demographic and baseline characteristics.

Parameter		Placebo (N = 10)	Rexlemestrocel-L	
			150×10^6 (N = 10)	300×10^6 (N = 10)
Gender, n (%)	Male	8 (80.0%)	9 (90.0%)	7 (70.0%)
	Female	2 (20.0%)	1 (10.0%)	3 (30.0%)
Age, years		74.8 ± 7.9[a]	70.5 ± 7.4	64.8 ± 10.1
Race, n (%)	Caucasian	10 (100.0%)	9 (90.0%)	10 (100.0%)
	Asian	0 (0.0%)	1 (10.0%)	0 (0.0%)
Height, cm		166.3 ± 8.7	172.3 ± 8.4	171.2 ± 10.4
Baseline weight, kg		83.5 ± 20.2	97.4 ± 21.8	101.3 ± 25.0
Body mass index, kg/m^2		30.3 ± 6.4	32.7 ± 6.2	34.2 ± 5.9
Baseline eGFR (MDRD), ml/min/1.73 m^2		34.6 ± 9.22	35.7 ± 10.35	34.6 ± 12.38
≤30 ml/min/1.73 m^2, n (%)		3 (30%)	3 (30%)	5 (50%)
>30 ml/min/1.73 m^2, n (%)		7 (70%)	7 (70%)	5 (50%)
Baseline mGFR, ml/min/1.73 m^2		34.6 (9.7)	37.4 (12.11)	35.4 (11.44)
Baseline HbA1c (%)		6.8 ± 1.3	7.5 ± 1.2	7.9 ± 2.1
<8%[b]		8 (80%)	5 (50%)	6 (60%)
≥8%[b]		2 (20%)	5 (50%)	4 (40%)
Albumin-creatinine ratio (ACR, mg/g)		408 ± 552	391 ± 787	404 ± 736
ACR <30 mg/g, n (%)		0 (0%)	0 (0%)	3 (30%)
ACR 30–300 mg/g, n (%)		6 (60%)	8 (80%)	4 (40%)
ACR >300 mg/g, n (%)		4 (40%)	2 (20%)	3 (30%)
CKD regimen, n (%)[b]				
ACEi, n (%)		1 (10.0%)	3 (30.0%)	1 (10.0%)
ARB, n (%)		8 (80.0%)	7 (70.0%)	8 (80.0%)

[a] Values are Mean ± SD unless otherwise noted.
[b] One patient in the Placebo group was on a regimen of ACEi plus ARB therapy; one patient in the 300×10^6 group had documented ACEi and ARB intolerance.

treatment groups. A total of 15 treatment-emergent serious adverse events (SAEs) were reported by 2 (20%), 4 (40%), and 1 (10%) patients in the placebo and rexlemestrocel-L 150×10^6 and 300×10^6 groups, respectively (Table 2B). SAEs of diabetic foot ulcer/infection were reported in 3 rexlemestrocel-L-treated patients (two in the 150×10^6 and one in the 300×10^6 group). All of these patients had a history of diabetic neuropathy and/or prior diabetic foot ulcer including a prior hallux amputation in one patient. Serious cardiac disorders were reported in 2 patients: one placebo-treated patient experienced acute myocardial infarction and two hospitalisations for congestive heart failure and one patient in the rexlemestrocel-L 150×10^6 group was hospitalised for atrial fibrillation. No SAEs were judged by the investigators to be related to treatment. No acute allergic or immunologic adverse events were reported. AEs of dyspnea exertional, asthma, cough, pleural effusion, and wheezing were balanced across treatment groups and none occurred either during or immediately after infusion or were deemed related to treatment.

Table 2
Safety.

	Placebo (N = 10)	Rexlemestrocel-L	
		150×10^6 (N = 10)	300×10^6 (N = 10)
A. Summary of Treatment-Emergent Adverse Events (TEAEs)[a]			
Patients with any TEAEs	7 (70.0%)	8 (80.0%)	9 (90.0%)
Patients with any treatment-related TEAEs[a]	0 (0.0%)	0 (0.0%)	0 (0.0%)
TEAEs leading to discontinuation	0 (0.0%)	0 (0.0%)	0 (0.0%)
Patients with any serious TEAEs	2 (20.0%)	4 (40.0%)	1 (10.0%)

B. Listing of serious adverse events

Group	Patient	Serious adverse event
Placebo	A	Fall
Placebo	B	Acute myocardial infarction
		Anemia
		Asthma
		Cardiac failure congestive
		Cardiac failure congestive
		Syncope
		Upper gastrointestinal hemorrhage
150×10^6	C	Gangrene
150×10^6	D	Infected skin ulcer
150×10^6	E	Atrial fibrillation
		Renal failure chronic
150×10^6	F	Benign prostatic hyperplasia
300×10^6	G	Diabetic ulcer
		Diverticulitis

[a] Treatment-emergent adverse events are those with an onset during or after treatment infusion.

One active-treated patient developed antibodies specific to the donor HLA (antibody specificity to donor antigen B40; mean fluorescence intensity 530) at week 4 that were undetectable at week 12; donor specific anti-HLA panel reactive antibodies (DSA) present at baseline in one patient persisted throughout the entire study with no associated adverse events; and one placebo-treated patient developed panel reactive antibodies specific to the donor HLA at week 60 with antibody specificity to donor antigen CW6 and mean fluorescence intensity 4779. The reason for an isolated observation of DSA in a placebo treated patient is unknown. Possible explanations for a non-exposed patient developing DSA include some sensitizing event such as a blood transfusion, vaccination, infection or exposure to some other unidentified antigen. Infection could upregulate the immune system resulting in expression of these antibodies. This patient did not receive a transfusion between week 36 and 60. There were no clinically significant increases in either Class I or Class II %PRA at any timepoint.

3.3. Exploratory Efficacy

The primary exploratory efficacy parameter was the effect of a single IV administration of rexlemestrocel-L on renal function over 12 weeks as assessed by isotopically measured and estimated GFR based on serum creatinine. Relative to placebo, the LSM (SE) change from baseline in mGFR at week 12 (Fig. 2A) was 4.1 ± 2.75 for the 150×10^6 group ($p = 0.15$) and for the 3.9 ± 2.8 ml/min/1.73 m^2 in the 300×10^6 group ($p = 0.17$). The placebo-adjusted LSM change in eGFR at week 12 (Fig. 2B) was 4.4 ± 2.2 ($p = 0.05$) and 1.6 ± 2.2 ml/min/1.73 m^2 ($p = 0.47$) for the 150×10^6 and 300×10^6 groups, respectively.

Additional selected efficacy parameters are shown in Table 3. There were no effects of treatment on urinary albumin, protein, albumin-creatinine, protein-creatinine ratios, creatinine clearance, lipid profile, HbA1c or blood pressure. There was a statistically significant decrease in the median IL-6 values for the 300×10^6 group compared to placebo at week 12 ($p = 0.01$; Table 3). There were no significant changes or differences among groups in any other biomarkers which included TNF-α, adiponectin, TGF-β, uric acid and FGF23. Changes in mGFR and eGFR at 12 weeks analyzed by the primary pre-specified subgroup of baseline eGFR ≤30 or >30 ml/min/1.73 m^2 are provided in the Supplemental Appendix, Figs. S1 and S2. There was a suggestion of a more pronounced treatment effect in patients with a baseline eGFR >30 ml/min/1.73 m^2: within the subgroup with higher baseline eGFR, the eGFR change from baseline at 12 weeks was significantly different for the rexlemestrocel-L 150×10^6 group compared to placebo (($p = 0.04$); Supplemental Fig. S2C).

The effects of a single infusion of rexlemestrocel-L or placebo on eGFR change from baseline over the entire 60 week post-infusion study period are shown in Fig. 3. Relative to placebo there was a suggestion of stabilization of eGFR in the rexlemestrocel-L 150×10^6 group, most notably at the 12-week primary endpoint.

4. Discussion

Therapies that delay or prevent progression of diabetic nephropathy to end stage renal failure would be of immense clinical and economic value. Because of the ability of allogeneic mesenchymal lineage cells to track to injured tissues (Togel & Westenfelder, 2011) and potentially exert anti-inflammatory, immunomodulatory and other paracrine

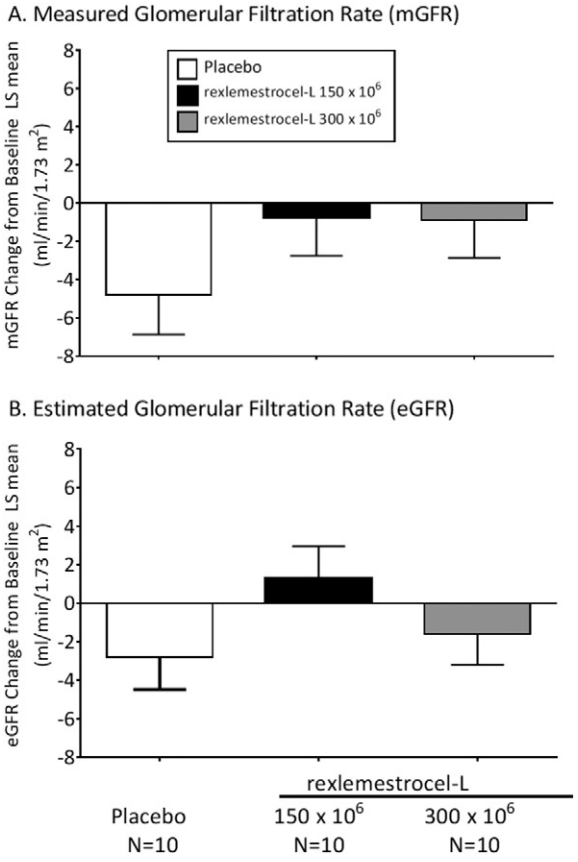

Fig. 2. mGFR least squares mean change (SE) from baseline at 12 weeks by group (A). eGFR least squares mean change (SE) from baseline at 12 weeks by group (B). Values are least squares mean ± SE derived from ANCOVA model using treatment and eGFR strata as factors and baseline value as covariate.

effects these cells may represent such a candidate therapy. Paracrine effects of these cells in vivo are likely to explain their clinical potential (Psaltis et al., 2010; See et al., 2011) as there is little evidence of engraftment of systemically administered cells (von Bahr et al., 2012).

This study represents the first double-blind, placebo-controlled trial of an allogeneic, bone-marrow derived mesenchymal lineage cell product in patients with chronic kidney disease due to type 2 diabetes. The study was designed to assess acute and chronic safety and immunological sensitization potential following a single infusion of rexlemestrocel-L. In addition, although the study was not powered for efficacy, a hypothesis-generating signal was observed with consistency between isotopically measured and creatinine-based estimations of renal function at the prespecified primary endpoint 12 weeks post-infusion. The 12 week timepoint was selected as the primary exploratory endpoint based on similar early phase clinical development studies in diabetic nephropathy (Pergola et al., 2011; Ruilope et al., 2014) and a previous study in subjects with type 2 diabetes that showed a trend for improved glycemic control at 8 weeks which dissipated thereafter (Skyler et al., 2015).

No observed changes in glycemic parameters (HbA1c, fasting plasma glucose or insulin) were observed. While this study was conducted in patients with nephropathy consequent to type 2 diabetes, the most common cause of end-stage renal disease, the subjects in our study varied widely with respect to degree of hyperglycemia (HbA1c range: 5.1% to 11.2%) and concomitant diabetes medications: 23.3% were on a background regimen of a single oral agent, most commonly sulfonylurea or biguanide; 20% were on insulin; 20% were on multiple oral agents, 20% on insulin plus oral; and 17% were managed by diet alone. Moreover, per protocol, investigators were allowed to make any changes in type 2 diabetes therapy over the entire study period as deemed appropriate for individual patients.

The infusions were well-tolerated and the safety profile we report is comparable across all treatment groups. Theoretical risks of an allogeneic cell therapy including allergic risks due to excipients such as fetal calf serum or immunogenic responses to human antigens (donor HLA) were not observed. Cross matching between donor cells and recipient was not performed prior to infusion. In addition, subjects were not

Table 3
Renal and metabolic parameters at baseline and changes from baseline at week 12 endpoint.

	Placebo (N = 10)	Rexlemestrocel-L	
		150×10^6 (N = 10)	300×106 (N = 10)
Serum creatinine (mg/dl)[a]	1.95 ± 0.58	1.97 ± 0.58	2.03 ± 0.71
Change from baseline (mg/dl)	0.064 ± 0.0912	0.003 ± 0.0917	0.047 ± 0.0888
	n = 10	n = 10	n = 10
Creatinine clearance (ml/min)	46.4 ± 13.8	55.2 ± 24.1	54.0 ± 29.0
Change from baseline (ml/min)	3.1 ± 8.89	6.5 ± 7.96	−8.6 ± 7.48
	n = 8	n = 9	n = 9
Albumin-creatinine ratio (mg/g)[b]	205.2 (270.80)	142.0 (176.60)	158.8 (359.10)
Change from baseline (mg/g)	−9.6 (127.40)	21.0 (93.50)	18.0 (169.70)
	n = 9	n = 10	n = 10
Protein-creatinine ratio (mg/g)[b]	412.7 (317.00)	302.6 (347.60)	297.3 (350.00)
Change from baseline (mg/g)	−7.55 (149.050)	1.15 (175.650)	13.95 (225.150)
	n = 9	n = 10	n = 10
Cystatin-C (mg/l)	1.49 ± 0.561	1.61 ± 0.408	1.31 ± 0.450
Change from baseline (mg/l)	0.43 ± 0.131	0.30 ± 0.143	0.52 ± 0.128
	n = 10	n = 9	n = 10
HbA1c (%)	6.75 ± 1.34	7.53 ± 1.21	7.92 ± 2.13
Change from baseline (%)	−0.07 ± 0.240	−0.03 ± 0.218	−0.36 ± 0.216
	n = 9	n = 10	n = 10
Triglycerides (mmol/l)[b,c]	1.45 (1.40)	2.05 (0.50)	1.80 (1.00)
Change from baseline (mmol/l)	0.05 (0.60)	0.10 (0.50)	0.15 (0.45)
% Change from baseline (%)	6.32 (28.8)	5.44 (25.63)	10.53 (24.11)
	n = 10	n = 10	n = 10
Systolic blood pressure (mm Hg)	132.7 ± 14.85	135.0 ± 16.99	136.3 ± 20.41
Change from baseline (mm Hg)	8.0 ± 5.50	6.3 ± 5.51	6.1 ± 5.40
	n = 10	n = 10	n = 10
Diastolic blood pressure (mm Hg)	68.1 ± 11.12	72.0 ± 11.24	74.7 ± 12.18
Change from baseline (mm Hg)	4.2 ± 3.02	−2.6 ± 3.00	5.6 ± 2.98
	n = 10	n = 10	n = 10
hs-CRP (mg/l)[b]	1.00 (0.60)	3.50 (2.20)	0.95 (3.90)
Change from baseline (mg/l)	1.00 (3.90)	7.85 (16.90)	0.30 (1.25)
	n = 10	n = 9	n = 10
IL-6 (pg/ml)[b]	3.44 (1.90)	4.29 (2.30)	2.76 (2.00)
Change from baseline (pg/ml)	2.47 (2.85)	1.39 (5.46)	−0.15 (0.910)[d]
	n = 10	n = 9	n = 10
TNF-α (pg/ml)	2.98 ± 0.64	3.13 ± 0.99	2.98 ± 1.55
Change from baseline (pg/ml)	0.11 ± 0.19	0.34 ± 0.20	0.13 ± 0.18
	n = 10	n = 9	n = 10

[a] Baseline values are mean ± SD or median (IQR). Change from baseline values are least squares means ± SE obtained from an ANCOVA model with treatment and screening eGFR stratum (≤30 or >30 ml/min/1.73 m^2) as factors, and baseline values as covariate.
[b] Values are median (IQR). Treatment differences estimated using Hodges-Lehmann estimator and Moses method. Analysis by Cochran-Mantel-Haenszel test in a nonparametric ANCOVA model with treatment as factor adjusting for screening eGFR stratum and baseline value.
[c] Both change and percent change from baseline are shown for triglycerides.
[d] $p = 0.01$ versus Placebo.

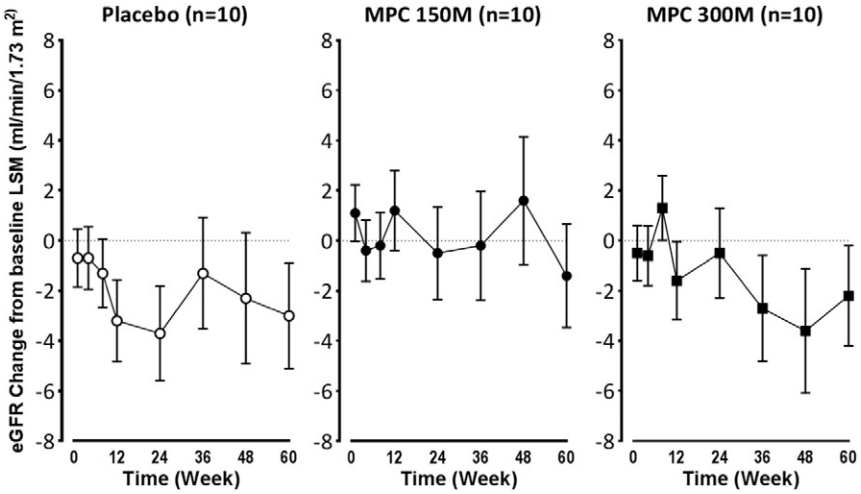

Fig. 3. eGFR least squares mean change (SE) from baseline over 60 week study by group. Values are least squares mean ± SE derived from ANCOVA model using treatment and eGFR strata as factors and baseline value as covariate. MPC150M = rexlemestrocel-L 150×10^6; MPC300M = rexlemestrocel-L 300×10^6.

excluded from participation based on assessment of antibodies to the donor HLA. Importantly in this patient population, no patients developed sustained antibodies specific to the donor HLA or showed clinically relevant sustained increases in Class I or Class II %PRA, consistent with the immune tolerant profile of this cell type and a previous study showing no evidence of rexlemestrocel-L induced antibodies or immune system events (Skyler et al., 2015). These cells are negative for HLA Class II and CD80 and CD86 co-immunostimulatory molecules, and exert potential immunomodulatory effects including inhibition of T-cell proliferation (Togel & Westenfelder, 2011). The observed lack of acute immunological responses to unmatched allogeneic MPC is particularly important in patients who may eventually require kidney transplantation. Possibly, repeated administration of this product may enhance the observed modest signal of GFR preservation, relative to placebo. Lack of any evidence of sustained sensitization and development of antibodies specific to the donor HLA suggests that repeat administration of this therapy may be a feasible option in this patient population. The design of future studies may include assessment of safety, tolerability and efficacy of single and repeated administration of the product.

There are limitations to our study. First, the sample size was too small to demonstrate statistically significant effects on renal function. Moreover, the possibility of type 1 error cannot be excluded based on multiple exploratory statistical analyses performed without adjustment for multiplicity. In addition, the small sample size (N = 30) while appropriate for a first in human investigation cannot exclude rarer safety events than would be detected over 60 weeks following a single infusion. Second, the study duration (12 weeks) to assess acute effects of a single administration with a 48 week follow-up is too brief to evaluate a chronic disease with variable and frequently slow progression. Selection of patients with documented recent rapid progression of their chronic kidney disease may more likely show treatment effects, particularly over a brief study duration. Third, the wide range of baseline albuminuria and proteinuria (ACR 21 to 3000 mg/g) as well as serum biomarkers in a small number of patients complicated the assessment of changes within and between groups in these parameters. Selection of subjects within a narrow range of baseline proteinuria may provide more useful information. With respect to serum biomarkers of inflammation, owing to within subject variability as well as sensitivity of the available assays, systemically measured inflammatory cytokines probably require substantially larger numbers of subjects per group to identify meaningful changes and treatment differences over time. Lastly, repeated isotopically measured GFR assessments beyond 12 weeks to confirm eGFR findings were not performed because of radioisotope exposure. Serum-creatinine based eGFR equations may underestimate or overestimate GFR.

In future studies, selection of patients with prognostic indicators of rapid progression to end stage renal failure would support exploration of a treatment paradigm whereby repeat infusions over a prolonged time course are demonstrated to be tolerated and result in durable and clinically meaningful responses. Repeat dosing, if demonstrated to be safe and well-tolerated, may provide greater clinical efficacy and may be appropriate in assessing the long-term effects on renal function. Although there were no immune related adverse events, we have not reinfused subjects with cells from the same donor to confirm lack of sensitization.

In conclusion, the safety, apparent immune tolerance of allogeneic MPC and a potential efficacy signal of rexlemestrocel-L relative to placebo, and the medical need to develop new therapies to preserve or enhance renal function in this population support further investigation in diabetic nephropathy in appropriately sized and powered studies of longer duration, including periodic dosing to assess the durability of effect and optimal dose and frequency of repeat administration.

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Contributors

DP, IF, and PK enrolled study patients. All authors had full access to the study data, interpreted the study results and wrote and revised the manuscript. KS served as study director for the trial and drafted the protocol and manuscript with input from DP. Statistical analyses were performed at Medpace, Inc. by Mei Chen, PhD. All authors had final responsibility for the decision to submit the manuscript for publication, revised the manuscript, reviewed the final manuscript and approved the manuscript for submission. Dr. David K. Packham is the guarantor of this work and, as such, had full access to all study data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of Interests

David K. Packham: has served as a consultant to Mesoblast, Inc. Karen R. Segal: is an employee of Mesoblast, Inc. Ian Fraser and Peter Kerr report no competing interests.

Prior Presentation

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2016.09.011.

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