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RESEARCH LETTER

Safety and Feasibility of a Novel Percutaneous Locoregional Injection Technique of Renal Cellular Therapy for Chronic Kidney Disease of Diabetes

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Early cell and gene therapies show promise to achieve positive treatment outcomes, primarily for cancer and hereditary conditions; however, there is a lack of therapeutic options for chronic organ failure, such as chronic kidney disease (CKD).[1,2] CKD represents a growing global health crisis, predicted to affect more than 250 million people with type 2 diabetes as the leading cause in greater than 40% of selected populations and the dominant cause for end-stage kidney disease.[3,4]

Most CKD therapies are small-molecule medications targeting a biochemical pathway or enzyme modulator in a damaged renal framework. In contrast, preclinical and early clinical CKD cell therapies offer the potential to restore and repair the glomerular-tubular unit to improve intrinsic renal function and reduce secondary comorbidities.[5,6] Most early-phase cell-based CKD trials use autologous mesenchymal stem cell lines delivered by intravenous injections, yet the probability of mechanistic effects is unpredictable secondary to systemic circulation dilution and blood trapping. Furthermore, in the diabetic kidney, intravenous cell delivery may also be attenuated by underlying fibrosis and microvascular disease.

An alternative method of cell delivery is a locoregional injection directly into the diseased kidney. Direct cell injections have been performed with chronic liver disease and, more recently, via transendocardial delivery in heart failure trials, whereas there are limited reports of direct cell injection in kidneys with CKD.[7–9,S1] We describe a novel technique and preliminary observations regarding injection safety and feasibility of precision delivery of homologous autologous renal progenitors into the renal cortex of patients with type 2 diabetic CKD.

METHODS

Between October 2017 and November 2020, 87 consecutive direct parenchymal homologous autologous cells injections using a 20/25-gauge coaxial needle system were analyzed in 51 enrolled patients from two phase II trials (RMCL-002 [NCT02836574] and REGEN-003 [NCT03270956]) with type 2 diabetic CKD stages 3-4 who met eligibility criteria (see Supplemental Material). Estimated glomerular filtration rates (eGFR) of enrolled patients were 20 to 50 ml/min per 1.73 m^2 (RMCL-002) and 14 to 20 ml/min

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per 1.73 m^2 (REGEN-003). The international normalized ratio for all enrolled patients' was less than 1.5. Following a percutaneous kidney biopsy from each patient and cell expansion by Good Manufacturing Practice, progenitor cells were formulated in a thermolabile hydrogel concentration of 100×10^6 cells/ml and reinjected into the biopsied kidney 6 weeks later.

Renal volume was measured on magnetic resonance imaging to determine cell dose (3–8 ml). Cell injection was performed using computed tomographic (CT) guidance. A coaxial 20-gauge outer guiding needle (COOK, Inc, Bloomington, Indiana) was inserted into the subcapsular parenchyma of the lower pole of the previously biopsied kidney and a 25-gauge inner injection needle (0.51 mm diameter, IMD Inc, Huntsville, Utah) into the renal cortex, within 5 mm from the capsule (Figure 1). For patients with two subsequent injections, the procedure was performed in the same kidney 6 months apart. All procedures were performed by experienced interventional radiologists receiving training and on-site proctoring. Patients underwent conscious sedation and were discharged from the day-surgery unit after recovery.

The protocol required hemoglobin, hematocrit, and renal chemistries before and after injection. Intra- and end-procedure intermittent CT scanning took place to guide the injections and assess for bleeding. Renal ultrasonography was performed during post-injection recovery and on day 1 postprocedure to assess for hematoma. Also, patients temporarily refrained from anticoagulation and antiplatelet medications. The primary endpoints were changes in eGFR and complications related to the injection and cell product. Major renal bleeds were defined as those requiring blood transfusion, extended hospitalization, or an interventional procedure. Statistical analyses were performed using R (4.0.5, R Core Team, 2021, Vienna, Austria).

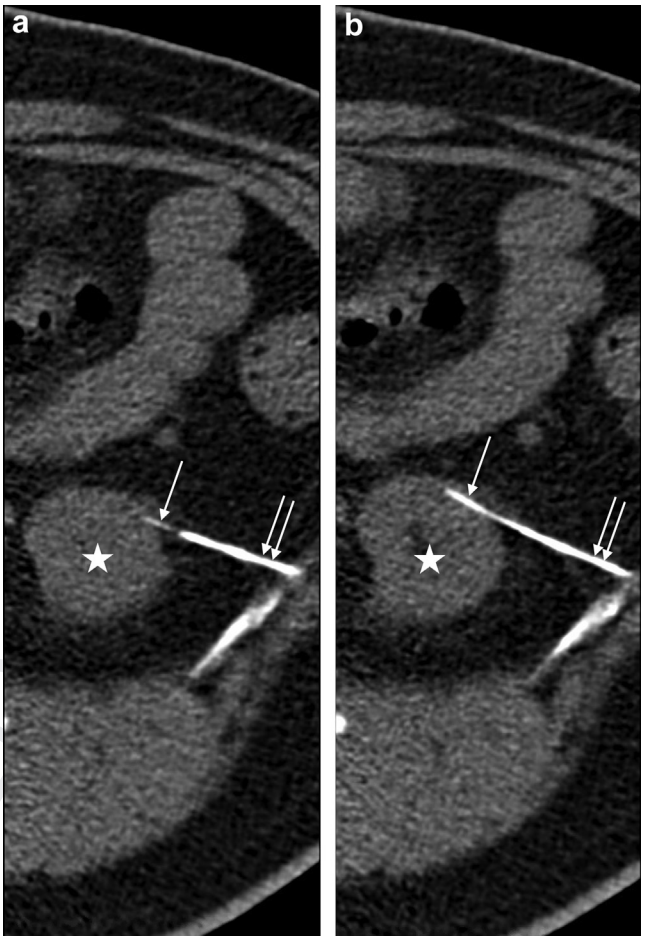

Figure 1. Computer tomography axial images with oblique maximum intensity projection show a 49-year-old male with type 2 diabetes and chronic kidney disease undergoing homologous autologous renal progenitor cells injection in the left renal cortex. (a) A coaxial 20-gauge outer guiding needle (double arrows) is positioned at the cortex of the left kidney (star), and a 25-gauge inner needle (single arrow) is advanced into the subcapsular parenchyma. (b) The 25-G inner needle (single arrow) is further advanced through the 20-G outer needle (double arrows) into the cortex, with the tip positioned approximately 5 mm from the capsule of the left kidney (star) and homologous autologous renal progenitor cells are injected.

RESULTS

This analysis included 87 cell injections in 51 patients (Table 1). Sixty-nine injections were completed in 41 patients and 18 in 10 patients with eGFRs 20 to 50 ml/min per 1.73 m^2 (RMCL-002) and 14 to 20 ml/min per 1.73 m^2 (REGEN-003), respectively. All patients remained hemodynamically stable throughout the procedure. All locoregional injections into the renal cortex were technically successful, and CT scans documented final needle locations in the subcapsular renal cortex (Figure 1). There were no significant differences in hemoglobin ($P = 0.3$ [RMCL-002, first injection], $P = 0.2$ [RMCL-002, second injection], $P = 0.2$ [REGEN-003, first injection], $P = 0.14$ [REGEN-003, second injection]) and hematocrit ($P = 0.2$ [RMCL-002, first injection], $P = 0.3$ [RMCL-002, second injection], $P = 0.3$ [REGEN-003, first injection], $P > 0.9$ [REGEN-003, second injection]) between pre- and post-cell injections (Table 2). Differences in creatinine, blood urea nitrogen, and eGFR were also not significant (Table 2, Figure 2). There were no procedure-related bleeds or cell extravasation documented during the CT-guided injections. However, a delayed subcapsular hematoma that required hospitalization without transfusion was present on a post-injection ultrasound in a female patient (1/1% [1 of 87 patients]) with eGFR of 15 ml/min per 1.73 m^2 (REGEN-003).

Table 1. Baseline characteristics of subjects and laboratory values before the injection of homologous autologous renal progenitor cells*

	Total (N = 51)	RMCL-002 trial† (n = 41)	REGEN-003 trial‡ (n = 10)	P
Age, yrs	64.5 ± 10.3 (37.0-80.0)	66.0 ± 10.6 (37.0-80.0)	58.3 ± 5.3 (48.0-65.0)	0.007
Sex				0.14
Women	15 (29)	10 (24)	5 (50)	
Men	36 (71)	31 (76)	5 (50)	
BMI, kg/m^2	33.5 ± 6.3 (20.4-52.5)	33.1 ± 5.6 (20.4-44.9)	35.1 ± 8.5 (23.8-52.5)	0.7
Number of injections				0.7
1	15 (29)	13 (32)	2 (20)	
2	36 (71)	28 (68)	8 (80)	
Hemoglobin, g/dl	12.4 ± 1.8 (8.6-16.6)	12.7 ± 1.8 (8.6-16.6)	10.9 ± 1.2 (9.7-12.5)	0.003
Hematocrit, %	36.9 ± 5.5 (25.0-52.0)	37.8 ± 5.5 (25.0-52.0)	33.3 ± 3.8 (27.0-38.0)	0.013
Platelet count, ×10^3/μl	264.1 ± 95.4 (121.0-509.0)	248.9 ± 88.8 (121.0-509.0)	326.6 ± 100.8 (167.0-484.0)	0.020
Total bilirubin, mg/dl	0.4 ± 0.2 (0.2-1.1)	0.4 ± 0.2 (0.2-1.1)	0.3 ± 0.1 (0.2-0.4)	0.014
ALT, U/l	20.0 ± 9.0 (7.0-46.0)	21.5 ± 9.1 (10.0-46.0)	13.7 ± 5.8 (7.0-23.0)	0.011
AST, U/l	19.8 ± 7.6 (8.0-43.0)	21.0 ± 7.6 (8.0-43.0)	14.7 ± 5.3 (10.0-27.0)	0.006
BUN, mg/dl	42.2 ± 16.3 (19.0-80.0)	38.0 ± 14.3 (19.0-80.0)	59.5 ± 12.7 (39.0-78.0)	<0.001
Creatinine, mg/dl	2.2 ± 0.7 (1.2-3.9)	2.0 ± 0.6 (1.2-3.5)	3.2 ± 0.4 (2.7-3.9)	<0.001
eGFR, ml/min per 1.73 m^2	31.7 ± 10.8 (13.0-53.0)	35.2 ± 9.1 (21.0-53.0)	17.5 ± 2.4 (13.0-21.0)	<0.001
INR	1.0 ± 0.1 (0.9-1.3)	1.0 ± 0.1 (0.9-1.3)	1.0 ± 0.1 (0.9-1.1)	0.13
PTT, s	12.4 ± 12.8 (9.4-102.0)	12.8 ± 14.3 (9.4-102.0)	10.4 ± 0.5 (9.7-11.3)	0.7
APTT, s	25.2 ± 2.3 (20.5-36.3)	25.3 ± 2.5 (20.5-36.3)	24.6 ± 0.9 (23.1-25.9)	0.4

ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; INR, international normalization ratio; PTT, partial thromboplastin time.
*Values are presented as mean ± standard variation (range) or as n (%).
†RMCL-002 trial includes patients with eGFR of 20 to 50 ml/min per 1.73 m^2.
‡REGEN-003 trial includes patients with eGFR of 14 to 20 ml/min per 1.73 m^2.

DISCUSSION

We present results on the safety and feasibility of a novel locoregional precision delivery method for a cell-based therapy currently in phase II clinical trials. Cellular-based therapies are a rapidly growing classification of Advanced Therapy Medicinal Products, and these therapies have shown encouraging clinical outcomes, most notably treating hematologic cancers. The most common cell product delivery method is via intravenous injections with the emerging use of direct tissue deposit techniques in benign conditions (eg, orthopedic, dermal, and cardiac indications).[S2]

Early phase trials are underway using mesenchymal stem cell intravascular injection therapy for CKD regeneration, although the reliability of cell secretory effects on target cells is unknown. Diabetic microvascular disease, systemic recirculation dilution, and lung trapping attenuate the cell quantity to the glomerular-tubule environment, reducing the efficacy and dose accuracy with intravenous injections.

Alternately, locoregional injections into the recipient's renal cortex allow renal progenitor cells to impart direct restorative effects in the microenvironment of effete CKD tubules and glomeruli, primarily. Preclinical trials of progenitor cell injections in multiple CKD animal models showed local and distant nephron repair and improved kidney function[6,S3,S4] In a phase I, first-in-human trial, six patients underwent laparoscopic renal subcapsular injection of selected renal cells (SRCs).[S1] All included patients had type 2 diabetic CKD with a glomerular filtration rate of 15 to 50 ml/min. Post–SRC injection, iohexol clearance and albumin-creatinine ratio remained stable to 12 and 24 months, respectively. Although laparoscopically assisted implantation of SRCs was uneventful, it resulted in surgery-related complications and extended hospital admissions. The procedure was converted to a percutaneous image-guided technique for the phase II trials with a smaller needle platform and moderate sedation to mitigate adverse events and maintain efficacy by cell delivery into the renal cortex. Before needle selection, *in vitro* testing was performed to determine shear and stress effects on renal epithelial cells injected through smaller bore needles. No harmful effects on cell viability or potency were identified during autologous cell injections through the 25-gauge needle design.

The process of cell biodistribution in renal tissues was shown by injecting labeled SRCs into animal kidneys, and cellular movement was established using immunofluorescence and magnetic resonance imaging.[S3,S5] The migration of SRCs from the injected renal cortex is mediated by cytokines, which elicit a chemotactic response. The nephron restoration

Table 2. Comparison of laboratory test results between before and after injection of homologous autologous renal progenitor cells*

	Pre-injection	Post-injection	*P*
RMCL-002† trial			
First injection (n = 41)			
Hemoglobin, g/dl	12.0 ± 1.8	11.9 ± 1.6	0.3
Hematocrit, %	36.5 ± 5.5	36.0 ± 4.5	0.2
BUN, mg/dl	39.5 ± 16.0	38.9 ± 13.6	0.7
Creatinine, mg/dl	2.1 ± 0.7	2.2 ± 0.9	0.4
eGFR, ml/min per1.73 m^2	32.6 ± 10.5	32.0 ± 10.9	0.9
Second injection (n = 28)			
Hemoglobin, g/dl	12.3 ± 1.7	12.5 ± 1.3	0.2
Hematocrit, %	38.1 ± 4.7	37.6 ± 3.9	0.3
BUN, mg/dl	36.2 ± 10.0	35.1 ± 13.0	0.4
Creatinine, mg/dl	1.9 ± 0.6	2.1 ± 0.7	0.065
eGFR, ml/min per 1.73 m^2	35.1 ± 9.4	32.8 ± 9.5	0.031
REGEN-003‡ trial			
First injection (n = 10)			
Hemoglobin, g/dl	10.4 ± 1.1	10.7 ± 1.1	0.2
Hematocrit, %	31.4 ± 3.2	32.1 ± 4.3	0.3
BUN, mg/dl	59.4 ± 15.8	57.9 ± 15.0	0.7
Creatinine, mg/dl	3.5 ± 0.7	3.5 ± 0.7	0.6
eGFR, ml/min per 1.73 m^2	16.3 ± 3.1	15.8 ± 2.7	0.6
Second injection (n = 8)			
Hemoglobin, g/dl	10.4 ± 1.2	10.0 ± 1.3	0.14
Hematocrit, %	30.2 ± 2.9	30.2 ± 2.9	>0.9
BUN, mg/dl	60.6 ± 14.5	60.0 ± 16.6	0.9
Creatinine, mg/dl	3.8 ± 0.6	4.0 ± 0.6	0.4
eGFR, ml/min per 1.73 m^2	14.1 ± 2.7	13.5 ± 3.2	0.4

BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.
*Values are presented as mean ± standard variation (n).
†RMCL-002 trial included patients with eGFR of 20 to 50 ml/min per 1.73 m^2.
‡REGEN-003 trial included patients with eGFR of 14 to 20 ml/min per 1.73 m^2.

following SRC injection is associated with the integration of cells into areas of damage, inflammation, and fibrosis with local paracrine effects resulting in anti-fibrotic, anti-inflammatory, and improved renal function.[S6]

CT-guided percutaneous procedures have proven feasibility and safety for many indications but remain undescribed in the local delivery of cell therapies to treat CKD.[S7] In our trials, thin-section CT scans were performed through the kidneys to identify a safe pathway and target in the renal cortex of the lower pole. Despite the small injection needle caliber, CT image reconstructions at 1 to 5 mm allow verification of the needle tip within the thinned renal cortex during each injection while allowing real-time safety surveillance for hematoma development.

Renal pathophysiology and related comorbidities of stage 3/4 type 2 diabetic CKD, principally anemia, higher eGFR stage, and females, are risk factors for increased bleeding during percutaneous interventions.[S8,S9] Postrenal biopsy bleeding risks have been well-described in previous studies, with wide ranges due to heterogeneity of the disease, biopsy techniques, and hospital status.[S9,S10]

Although no complications have been published specific to our trial's intervention, percutaneous biopsy for medical renal disease and tumor masses can furnish a benchmark for risk approximation. Poggio *et al.*[S10] reported an 11% (range, 9% to 16%) rate of hematoma as the most common complication for biopsy of intrinsic renal diseases in a systematic review and meta-analysis. At the same time, other complication-related treatments have been noted, such as transfusion rates (1% to 5.7%), angiographic interventions (0.2% to 0.6%), nephrectomy (0 to 0.1%) and death (0 to 0.06%).[S8,S9,S10,S11] Among renal tumor mass biopsy specimens, median overall complication rates were 8.1% (interquartile range, 2.7% to 11.1%), with perirenal hematoma being the most common and a few reporting treatments meeting Clavien Surgical Complication grades greater than IIIa.[S7,S12]

The accuracy of procedure-related bleeding in our study was verified by pre- and post-injection, and 24-hour follow-up ultrasonography with comparative hemoglobin/hematocrit levels and intraprocedural CT scans to ascertain acute or concealed subclinical hematomas. The low bleeding complication rate of 1.1% in a high-risk CKD population is ascribed to the small-needle platform and CT guidance compared to larger gauge cutting renal biopsy needles with multiple passes.

The safety and feasibility of cell delivery are critical factors that maximize the efficacy of cell-based nephron-restorative therapies. Cell product loss during delivery may impact accurate dosing determination and end-organ effects. Few regulatory-approved cell treatments for chronic diseases use direct tissue injection, although late phase III trials are underway in chronic heart failure with transendocardial mechanical mapping injections of mesenchymal-line cells.[7, S13] CT-guided needle insertion and cell injection into thinned renal cortices are feasible with low, acceptable rates of bleeding risks. In addition, the percutaneous, minimally invasive nature of the procedure with conscious sedation adds to the safety of cell delivery in high-risk CKD stage 3-4 populations with trajectories toward end-stage kidney disease. Locoregional image-guided delivery of autologous cell therapies in CKD offers the potential for kidney function stabilization or improvement and delay of renal replacement therapies. Future trials are underway.

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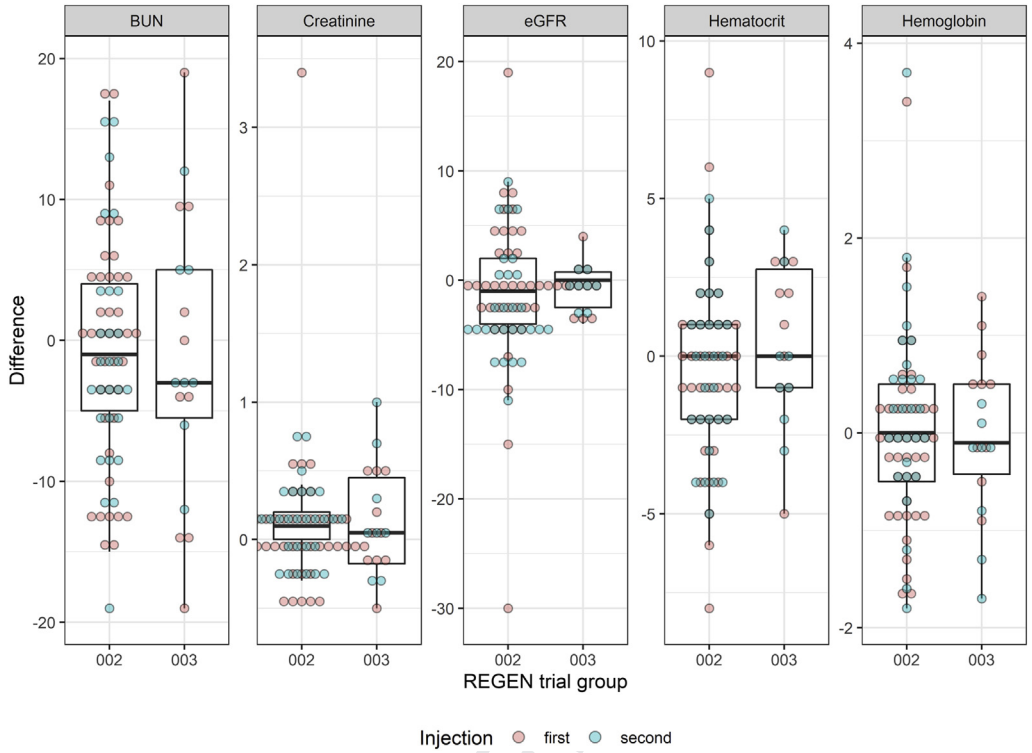

Figure 2. Boxplots of laboratory test values in RMCL-002 and REGEN-003 trial groups. There are no significant differences in creatinine, estimated glomerular filtration rate (eGFR), hematocrit, hemoglobin, and blood urea nitrogen (BUN) between the two groups.

STATEMENT OF ETHICS

The trials have been censured by Institutional Review Board approval and participant informed consent.

AUTHOR CONTRIBUTIONS

HY and JMS have made substantial contributions to the conception of the manuscript, drafting, and revisions of content, and agreed to be accountable for the accuracy and interpretation of the results data and approved the final version for publication. PDS, PRB, MPL, FSN, GJW, CTH, RDM, RN, EMC, ACE, and BST reviewed and agreed with manuscript content and approved the final version for publication.

DISCLOSURE

JMS is employed by ProKidney and an Executive Committee member. All the other authors have declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Inclusion and Exclusion Criteria

Supplementary References

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