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.1,4

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,02 whereas there are limited reports of direct cell injection in kidneys with CKD.7,9,51 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 m² (RMCL-002) and 14 to 20 ml/min.
Major renal bleeds were complications related to the injection and cell product. The primary endpoints were changes in eGFR and antiplatelet and anticoagulation medications. The hematoma. Also, patients temporarily refrained from recovery and on day 1 postprocedure to assess for ultrasonography was performed during post-injection guide the injections and assess for bleeding. Renal CT-guided injections. However, a delayed subcapsular hematoma that required hospitalization without trans- renal cortex 6 weeks later. There were no significant differences in hemoglobin (P = 0.3 [RMCL-002, second injection], P = 0.2 [RMCL-002, first injection], P = 0.2 [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 trans-fusion 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 m² (REGEN-003).
patients underwent laparoscopic renal subcapsular injection of selected renal cells (SRCs). 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. The migration of SRCs from the injected renal tissue deposit techniques in benign conditions (eg, orthopedic, dermal, and cardiac indications). Cellular-based therapies are a rapidly growing class 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).

Early phase trials are underway using mesenchymal stem cell intravenous 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. In a phase I, first-in-human trial, six

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (N = 51)</th>
<th>RMCL-002 trial (n = 41)</th>
<th>REGEN-003 trial (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>64.5 ± 10.3 (37.0-80.0)</td>
<td>66.0 ± 10.6 (37.0-80.0)</td>
<td>58.3 ± 5.3 (48.0-65.0)</td>
<td>0.007</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.14</td>
</tr>
<tr>
<td>Women</td>
<td>15 (29)</td>
<td>10 (24)</td>
<td>5 (60)</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>36 (71)</td>
<td>31 (76)</td>
<td>5 (60)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.5 ± 6.3 (20.4-52.5)</td>
<td>33.1 ± 5.6 (20.4-44.9)</td>
<td>35.1 ± 8.5 (23.8-52.5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Number of injections</td>
<td></td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Total bilirubin, mg/dl</td>
<td>0.4 ± 0.2 (0.2-1.1)</td>
<td>0.4 ± 0.2 (0.2-1.1)</td>
<td>0.3 ± 0.1 (0.2-0.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>20.0 ± 9.0 (7.0-46.0)</td>
<td>21.5 ± 9.1 (10.0-46.0)</td>
<td>13.7 ± 5.8 (7.0-23.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>19.8 ± 7.6 (8.0-43.0)</td>
<td>21.0 ± 7.6 (8.0-43.0)</td>
<td>14.7 ± 5.3 (10.0-27.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>42.2 ± 16.3 (19.0-80.0)</td>
<td>38.0 ± 14.3 (19.0-80.0)</td>
<td>59.5 ± 12.7 (39.0-78.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>2.2 ± 0.7 (1.2-3.9)</td>
<td>2.0 ± 0.6 (1.2-3.5)</td>
<td>3.2 ± 0.4 (2.7-3.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>31.7 ± 10.8 (13.0-53.0)</td>
<td>35.2 ± 9.1 (21.0-53.0)</td>
<td>17.5 ± 2.4 (13.0-21.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR</td>
<td>1.0 ± 0.1 (0.9-1.3)</td>
<td>1.0 ± 0.1 (0.9-1.3)</td>
<td>1.0 ± 0.1 (0.9-1.1)</td>
<td>0.13</td>
</tr>
<tr>
<td>PTT, s</td>
<td>12.4 ± 12.8 (9.4-102.0)</td>
<td>12.8 ± 14.3 (9.4-102.0)</td>
<td>10.4 ± 0.5 (9.7-11.3)</td>
<td>0.7</td>
</tr>
<tr>
<td>APTT, s</td>
<td>25.2 ± 2.3 (20.5-36.3)</td>
<td>25.3 ± 2.5 (20.5-36.3)</td>
<td>24.6 ± 0.9 (23.1-29.9)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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 (%).

1RMCL-002 trial includes patients with eGFR of 20 to 50 ml/min per 1.73 m².

2REGEN-003 trial includes patients with eGFR of 14 to 20 ml/min per 1.73 m².

**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).

Early phase trials are underway using mesenchymal stem cell intravenous 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. In a phase I, first-in-human trial, six
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 transcendocardial mechanical mapping injections of mesenchymal-line cells. S5,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.

ACKNOWLEDGMENTS

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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

REFERENCES

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.