

# Implantation of Autologous Selected Renal Cells in Diabetic Chronic Kidney Disease Stages 3 and 4—Clinical Experience of a “First in Human” Study



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**Introduction:** Animal models of chronic kidney disease demonstrate that a redundant population of therapeutically bioactive selected renal cells (SRCs) can be delivered to the kidney through intraparenchymal injection and arrest disease progression. Direct injection of SRCs has been shown to attenuate nuclear factor- $\kappa$ B, which is known to drive tissue inflammation, as well as the transforming growth factor- $\beta$ -mediated plasminogen activator inhibitor-1 response that drives tissue fibrosis.

**Methods:** We present experience from the first-in-human clinical study with SRCs. Seven male type 2 diabetic patients ( $63 \pm 2$  years of age) with chronic kidney disease stage 3 to 4 (estimated glomerular filtration rate  $25 \pm 2$  ml/min) were recruited. After blood and urine sampling, iohexol clearance, magnetic resonance imaging, and renal scintigraphy, patients underwent ultrasound-guided renal biopsy. Two cores of renal tissue were shipped to the manufacturing plant for cell isolation, culture, and product preparation. Formulated SRCs were transported back to study sites (range 59–87 days after biopsy) for intracortical injection using a retroperitoneoscopic technique.

**Results:** Laparoscopically assisted implantation of SRCs was uneventful in all patients. However, postoperative complications were common and suggest that other techniques of SRC delivery should be used. Kidney volume, split function, and glomerular filtration rate did not change during 12 months of follow-up. An extended 24-month follow-up in 5 of the patients showed a decline in estimated glomerular filtration rate (cystatin C).

**Discussion:** Postoperative complications following retroperitoneoscopic implantation of SRC in the kidney cortex seem to be related to the surgical procedure rather than to injection of the cell product. No changes in renal function were observed during the original 12-month protocol. Beyond the first 12 months after cell implantation, individual renal function began to deteriorate during further follow-up.

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KEYWORDS: autologous selected renal cells; chronic kidney disease; diabetes mellitus

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The incidence of chronic kidney disease (CKD) is rising worldwide and, because the prevalence is estimated to be 8% to 16%, CKD is considered a public health priority.<sup>1</sup> Worldwide, the incidence and prevalence of diabetes mellitus is expected to rise,<sup>2</sup> and

diabetes is today the most common cause of progressive loss of nephrons, end-stage renal disease, and need for renal replacement therapy in the western world.<sup>3</sup> Unfortunately, human kidneys have a limited ability to repair themselves, and renal progenitor cell activation during renal repair is low.<sup>4</sup> However, as CKD is characterized by low kidney cell proliferation and loss of regenerative processes,<sup>5</sup> maladaptive responses and fibrosis lead to progression of CKD. Although standard of care therapies, such as strict glycemic control and blockade of the renin–angiotensin–aldosterone axis,

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slow progression of diabetic nephropathy, they do not arrest or reverse it. A number of novel treatment strategies, such as antiproteinuric treatments, inhibitor of sodium–glucose co-transporter 2, antifibrotic agents, endothelin receptor antagonists, or transcription factors may slow or arrest the progression of diabetic kidney disease.<sup>6</sup> In addition, as regeneration of tissues and organs is now within the technological reach of modern medicine,<sup>7,8</sup> renal cell–based therapies have recently attracted interest for clinical use.<sup>4,7</sup>

Although human renal progenitor cells over the last decade have been shown to have a therapeutic effect in various animal models of CKD,<sup>9,10</sup> their use has been limited by their constrained availability and low nephrogenic potential. Their short lifespan and tendency to become senescent over time also limit their use.<sup>4</sup> Studies on regeneration following acute kidney injury suggest that tubular epithelial cells are central in the restoration of renal function.<sup>11</sup> As it has been documented that the kidney tubular epithelium is repaired by resident surviving epithelial cells<sup>12</sup> in the absence of progenitor cells,<sup>13</sup> the possibility of arresting progression by adult renal-derived cells may be an attractive alternative. Animal studies of experimental CKD show that implantation of intraparenchymal differentiated adult renal cells have regenerative capacity while reducing fibrosis and disease progression. In a rat model of CKD, human primary kidney cells enriched with erythropoietin (EPO)–positive cells improved chronic kidney injury.<sup>14</sup> As few of the injected cells were detected after 12 weeks, the nature of the beneficial effects observed were likely paracrine. In a study of 5/6 nephrectomized rats, Kelley *et al.*<sup>15</sup> demonstrated that a tubular cell–enriched subpopulation of primary renal cells improved survival and augmented kidney function. In a study of rats with progressive diabetic nephropathy, injection of a population of syngeneic selected renal cells (SRCs) enriched with tubular cells stabilized disease progression and improved survival.<sup>16</sup> Although the exact mechanism(s) for the observed favorable effects are not evident, activation of genes involved in regenerative processes, such as SOX2 and CD24, have been reported to be operative.<sup>17</sup> Moreover, recent data in rodents suggest that the beneficial effects of SRCs were transmitted via nuclear factor–κB and transforming growth factor–β1 pathways.<sup>18</sup> Based on the previous favorable experience with implantation of renal cells in diabetic ZSF1 rats with progressive diabetic nephropathy,<sup>16</sup> rodents undergoing 5/6 renal mass reduction<sup>15</sup> and normal mongrel dogs,<sup>19–21</sup> the primary objective of this first in-human, 2-center, open-label, single-group study was to assess the safety

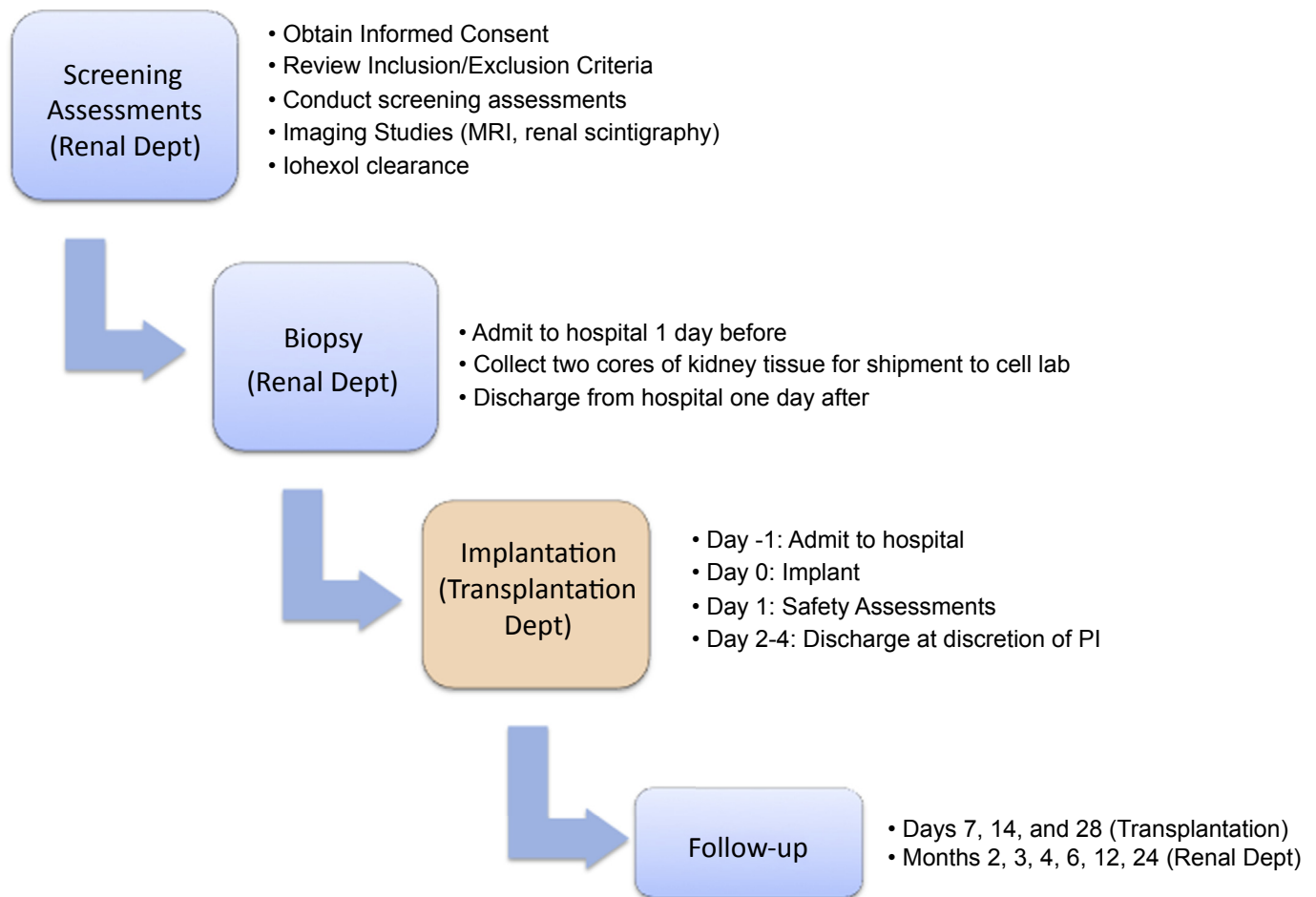
of implantation of expanded autologous SRCs ( $100 \times 10^6$  cells/ml) formulated in a gelatin-based hydrogel directly in the renal cortex. The secondary objective of the protocol was to assess the effects of SCR on renal function after a 12-month observation period. We also present data on 24-month follow-up in 5 of the patients.

## MATERIALS AND METHODS

Seven adult, male, type 2 diabetic, CKD stage 3 to 4 patients at the Department of Renal Medicine, Karolinska University Hospital, Stockholm, Sweden ( $n = 6$ ) and Department of Renal Medicine, University of North Carolina at Chapel Hill, North Carolina, USA ( $n = 1$ ) were recruited for the study. No female type 2 diabetic CKD stage 3 to 4 patients agreed to participate. The study was approved by the regional committees of ethics in Stockholm and Chapel Hill and adhered to the statutes of the Declaration of Helsinki. All patients provided written consent to participation. A detailed list of inclusion and exclusion criteria is listed in the [Supplementary Material](#) online. In short, adult (30–70 years of age), type 2 diabetic patients with GFR 15 to 50 ml/min and clinical course compatible with diabetic nephropathy, ongoing angiotensin-converting enzyme inhibitor/angiotensin receptor blocker treatment and a kidney size  $>10$  cm (cortical thickness  $>5$  mm) were eligible for inclusion. Patients who satisfied the eligibility criteria and provided signed informed consent entered a screening phase including full physical examination, electrocardiography and laboratory assessments (hematology, serum chemistry, and urinalysis). Magnetic resonance imaging (MRI) was performed to assess kidney volume and cortex thickness. Renal scintigraphy was performed to assess split kidney function and iohexol clearance to estimate GFR. Eligible patients underwent a kidney biopsy according to the standard clinical procedure (2 cores) to obtain autologous cells for implantation. The study protocol is depicted in [Figure 1](#). The protocol was approved by both the Food and Drug Administration and the Medical Products Agency (MPA) Uppsala, Sweden. The clinical trial ([clinicaltrials.gov](#)) number is NCT02008851.

## Cell Preparation

Neo-Kidney Augment (NKA) is an injectable product that is currently being investigated to treat CKD in clinical trials. NKA is a cell therapy composed of autologous SRCs formulated in a gelatin-based hydrogel. NKA is manufactured by first obtaining a kidney biopsy sample from the patient at the clinical site, which is shipped in cell culture medium (Dulbecco's



**Figure 1.** Simplified flow schedule of the protocol. MRI, magnetic resonance imaging.

modified Eagle's medium, obtained from Thermo Fisher (Waltham, MA), at 4°C to the manufacturing site). Because the whole kidney biopsy material is needed for cell preparation histology, molecular analysis of kidney specimen is not performed. Aseptic procedures are conducted in Class 100 Biological Safety Cabinets (BSC). The cell culture-processing core consists of 2 production suites and an adjacent incubator room validated to Class 10,000 (ISO 7) standards. One Class II Type A2 (Class 100, ISO 5) biological safety cabinet is located in each production suite for tissue processing and cell culture operations. All patient samples are handled in individual production suites to provide temporal segregation during processing. The production support areas (media/materials staging, clean staging, etc.) are designed to Class 100,000 (ISO 8) standards. All open tissue processing and cell culture operations are performed within Class II Type A2 (Class 100, ISO 5) BSC using an aseptic technique. The preparation of SRCs from patient biopsy samples has been previously described in detail<sup>15,16,22,23</sup> and is provided in the [Supplementary Material](#). A detailed description of markers of the injected cells is provided in the [Supplementary Material](#).

### Implantation Procedure

In the Swedish patients (patients 1–6), a Pfannenstiel incision was made and a hand-assist device was placed in the wound (Gelpoint, Applied Medical Resources Corporation). Moving the peritoneum medially by blunt manual dissection created a retroperitoneal space.<sup>24</sup> Gas pressure was kept at a maximum of 12 mm Hg. The medio-arterial portion of Gerota's fascia of the left kidney was opened to expose the perirenal fatty tissue, which was abundant in all cases. The fatty tissue was removed to expose the kidney capsule, essentially all of the medial and lateral aspects as well as almost the entire convex/lateral part of the kidney. The dissection allowed positioning of the kidney in alignment with the injecting needle and visualizing whether there was any penetration or leakage of the injected material. From the left iliac fossa, a guiding cannula was inserted transcutaneously to puncture the kidney capsule at the lower pole. An 18-gauge needle was thereafter inserted through the guiding cannula into the renal cortex along the convex longitudinal axis of the kidney. The SRC concentration per milliliter of delivered NKA is  $100 \times 10^6$  cells/ml, and the dosing volume is set at 3 ml for each 100 g of kidney weight,



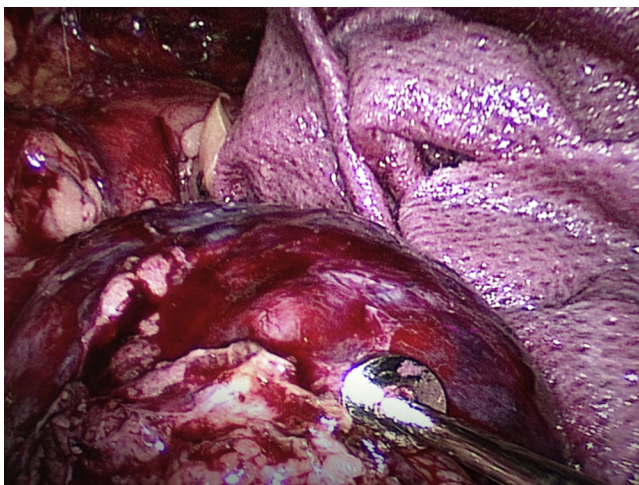
which was estimated from the results of the MRI study performed on or shortly after the screening visit. A 2-ml quantity of NKA was deposited at 4, 3, 2, and 1 cm from the puncture of the capsule over a 10- to 15-minute time period (total 8 ml) (Figure 2). The needle was kept in place for 5 minutes to promote hemostasis. No per-operative bleeding and only minimal amounts of NKA (<1 ml) was observed backing out of the puncture hole in any of the procedures. The ports were removed under direct vision to detect any bleeding from the port sites. The wound was not drained, and the abdominal wall was closed with a running suture. In the US patient, the surgical technique for implantation differed (Supplementary Material).

### Magnetic Resonance Imaging

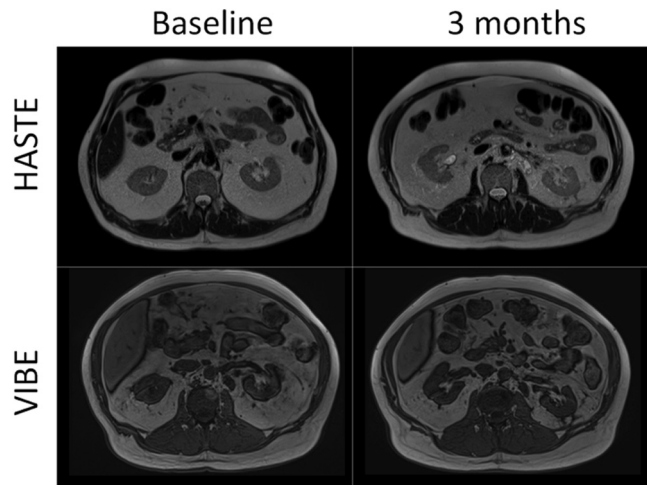
Both before implementation (<30 days) and 3 and 6 months postimplantation, MRI was performed using a 1.5-T MR unit (Siemens Magnetom Aera, Siemens AEG, Erlangen, Germany). Cortical thickness was measured in the dorsal part of the upper pole of the kidney (Figure 3) using a 4-mm-thick axial T2 Haste image. The kidney volume was quantified by manual segmentation using a dataset of breath hold 2.5-mm-thick VIBE images obtained without fat saturation.

### Renal Scintigraphy

Kidney function was evaluated in the supine position 3 hours after i.v. injection of 50 MBq <sup>99m</sup>Tc-DMSA (CIS bio international, Gif sur Yvette Cedex, France). An



**Figure 2.** Selected renal cell (SRC) implantation procedure. The extensive dissection allowed positioning of the kidney in alignment with the injecting needle and visualizing whether there was any penetration or leakage of the injected material. From the left iliac fossa, a guiding cannula was inserted transcutaneously to puncture the kidney capsule at the lower pole. An 18-gauge needle was thereafter inserted through the guiding cannula into the renal cortex along the convex longitudinal axis of the kidney. A 2-ml quantity of selected renal cells was deposited at 4, 3, 2, and 1 cm from the puncture of the capsule over a 10- to 15-minute time period (total 8 ml).



**Figure 3.** Changes in magnetic response imaging (MRI) after 3 months. MRI was conducted at screening and after 3, 6, and 12 months, respectively. Exclusion criteria for participation included major cysts, kidney size <10 cm, and cortical depth <0.5 cm at the implantation site (upper dorsal part of the kidney). (Upper panels) Size measurements were done on 4-mm-thick T2 weighted Haste images. (Lower panels) Volumetry was performed on 2.5-mm-thick T1 VIBE images. Note the difference in perirenal fat dorsal to the left kidney before and after surgery, caused by the extensive dissection to align the kidney with the needle at implantation. No scars or remaining hemorrhage were apparent following renal cell implantation.

anterior and posterior acquisition with a preset time of 20 minutes using a double-headed gamma camera (Symbia T16 SPECT/CT, Siemens, Erlangen, Germany) equipped with a low-energy, high-resolution collimator, 256 × 256 matrix. Differential renal function was assessed using region-of-interest drawings including the entire kidney, with a geometrical mean calculated from both projections.

### Biochemical and Other Analyses

Analyses of high-sensitivity C-reactive protein, creatinine, cystatin C, iohexol clearance, hemoglobin, albumin, Ca, PO<sub>4</sub>, and albumin–creatinine ratio (ACR) were performed with validated routine methods at the accredited Clinical Laboratory of Karolinska University Hospital, Stockholm, Sweden and University of North Carolina Hospitals, Chapel Hill, North Carolina, USA.

### Statistical Analyses

Data are expressed as mean ± SEM. Statistical significance was set at the level of  $P < 0.05$ . Comparisons over time were assessed by the Student *t* test.

## RESULTS

### Patient Characteristics

Demographic and clinical characteristics along with status at 24-month follow-up of 7 patients undergoing implantation with SRCs are shown in Tables 1 and 2.

**Table 1.** Clinical characteristics of patients undergoing selected renal cell implantation

Patient	NKA date	Age (yr)	BMI (kg/m <sup>2</sup> )	ESA (IU/kg/BW)	SBP (mm Hg)	DBT (mm Hg)
1	22 Aug 13	64	28.7	0	136	81
2	04 Sep 13	53	35.7	0	155	82
3	14 Nov 13	68	37.3	69	148	66
4	28 Nov 13	67	28.6	0	140	70
5	12 Dec 13	70	29.5	117	167	79
6	25 Sep 14	62	29.6	0	120	70
7	25 Apr 14	57	38.5	0	134	82
		63 ± 2	32.6 ± 1.7		143 ± 6	76 ± 3

Data are mean ± SEM.

BMI, body mass index; DBP, diastolic blood pressure; ESA, erythropoietin-stimulating agents; IU/kg/BW, international units per kilogram of body weight; NKA, neo-kidney augment; SBP, systolic blood pressure.

The patients' basal renal functions are shown in Table 3. The longitudinal individual changes in S-creatinine, estimated glomerular filtration rate (eGFR) (cystatin C), glomerular filtration rate (GFR) (iohexol clearance), and ACR following SRC implantation are shown in Figure 4.

### Changes in Renal Function

Although no significant differences between screening S-creatinine (235 ± 18 μmol/l) versus S-creatinine at 6 months (271 ± 89 μmol/l; *P* = 0.349), 12 months (307 ± 41 μmol/l; *P* = 0.127), and 18 months (297 ± 27 μmol/l; *P* = 0.120) were observed, a significantly higher S-creatinine level (339 ± 35 μmol/l; *P* = 0.048) was observed 24 months after implantation in 5 patients. Compared to screening eGFR (25 ± 2 ml/min/

**Table 2.** Comorbidity at screening, serious adverse events (SAEs), and clinical status at 24-month follow-up

Patient	Comorbidity prior to implantation	SAEs during observation period	Relationship to NKA	Status at 24-month follow-up
1	Stroke, lymphoma	Pneumonia and fatigue	Possible	Myocardial ischemia and heart failure <sup>a</sup>
2	None	Fatigue and postoperative wound infection	Not related	Chronic obstructive pulmonary disease
3	BPH, peripheral arterial insufficiency	Urinary tract infection and fluid retention	Not related	Urinary tract infections, TUR-P
4	Stroke, TIA	Fatigue	Not related	BPH, inguinal hernia
5	Cox arthrosis, stroke, cardiac arrhythmia	Cecal volvulus and anastomotic hemorrhage	Not related	Erosive gastritis, constipation
6	Foot ulcer	No SAE reported		Doing well <sup>b</sup>
7	Sleep apnea, hyperlipidemia	No SAE reported		Myocardial ischemia <sup>c</sup>

BPH, benign prostatic hyperplasia; NKA, neo-kidney augment; TIA, transient ischemic attack; TUR-P, transurethral resection of prostate.

<sup>a</sup>Death due to cardiac failure 30 months after implantation.

<sup>b</sup>12-Month control.

<sup>c</sup>18-Month control.

1.73 m<sup>2</sup>), eGFR tended (20 ± 2 ml/min/1.73m<sup>2</sup>; *P* = 0.08) to be lower at 12 months of follow-up. In 5 patients who completed 24 months of follow-up, eGFR was significantly lower (13 ± 5 ml/min/1.73 m<sup>2</sup>; *P* = 0.006) than in the screening phase. Iohexol clearance at screening (26 ± 3 ml/min/1.73 m<sup>2</sup>) did not differ from iohexol clearance at 6 months (25 ± 3 ml/min/1.73 m<sup>2</sup>; *P* = 0.981) or 12 months (23 ± 4 ml/min/1.73 m<sup>2</sup>; *P* = 0.658) after cell implantation. Compared to ACR at screening (198 ± 46 mmol/mol), no significant differences were observed at 6 months (322 ± 117 mmol/mol; *P* = 0.344), 12 months (340 ± 124 mmol/mol; *P* = 0.250), or 24 months (284 ± 119 mmol/mol; *P* = 0.466; *n* = 5) after SRC implantation.

### MRI and Renal Scintigraphy

No significant changes in renal volume (by MRI) in the left (216 ± 23 to 207 ± 24 ml) or right (237 ± 28 to 243 ± 32 ml) kidney were observed 12 months after NKA. No changes in either cortical thickness (by MRI) or renal split function (by renal scintigraphy) were observed 12 months after NKA (Figure 5). The evolution of S-creatinine and eGFR (cystatin-C) including historical analyses of S-creatinine and eGFR (cystatin-C) in the 6 Swedish patients before and after NKA are shown in Supplementary Figure 1.

### Postoperative Complications

Nine serious adverse events (SAEs) were reported after the laparoscopic surgical procedure (Table 2). The 24-hour period after kidney cell implantation was unremarkable in all patients. The postoperative SAEs during the observation period included fatigue (*n* = 3), wound infection (*n* = 1), pneumonia (*n* = 1), urinary tract infection (*n* = 1), overhydration (*n* = 1), cecal volvulus (*n* = 1), and anastomotic hemorrhage (*n* = 1). Although the SAEs were deemed not to be related to the NKA product in 6 cases, a relation could not be ruled out in the first patient, who experienced fatigue and pneumonia of unknown etiology in the postoperative period. However, as bronchioalveolar lavage did not reveal any renal epithelial cells, a relation to NKA seems less likely. Detailed descriptions of the medical history and clinical course following retroperitoneoscopic renal cell implantation in each of the 7 patients are presented in the Supplementary Material.

## DISCUSSION

The primary aim of this first-in-human, phase 1, open-label study of NKA was to investigate the safety of laparoscopic implantation of SRCs in the cortex of the recipient kidney. Although all cell implantations were uneventful, the rate of postoperative complications was significant in this patient group. Beside fatigue and

**Table 3.** Baseline data on renal functions in study patients

Patient	Renal volume R versus L (ml) <sup>a</sup>	Cortical thickness R versus L (mm) <sup>a</sup>	Split function R versus L (%) <sup>b</sup>	GFR <sup>c</sup> (ml/min)	eGFR <sup>d</sup> (ml/min)	S-creatinine (μmol/l)	Urea (mmol/l)	ACR (mmol/mol)
1	169 versus 144	13 versus 13	55 versus 45	27	34	215	18.5	40
2	237 versus 257	10 versus 10	46 versus 54	39	22	168	17.5	218
3	369 versus 297	27 versus 19	60 versus 40	26	19	261	17.0	248
4	214 versus 231	18 versus 13	47 versus 53	nd	28	192	24.5	409
5	223 versus 175	21 versus 16	60 versus 40	27	25	227	21.8	74
6	208 versus 194	16 versus 17	46 versus 54	15	19	283	22.4	227
7	238 versus 259	nd	51 versus 49	19	23	291	19.3	169
	237±69 versus 216±56	18±6 versus 15±3	52±2 versus 48±6	26 ± 3	25 ± 2	235 ± 18	20.1 ± 2.8	198 ± 46

ACR, albumin–creatinine ratio; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; L, left; ND, not determined; R, right.

Data are mean ± SEM.

<sup>a</sup>By magnetic resonance imaging.

<sup>b</sup>By renal scintigraphy.

<sup>c</sup>By iohexol.

<sup>d</sup>By cystatin C.

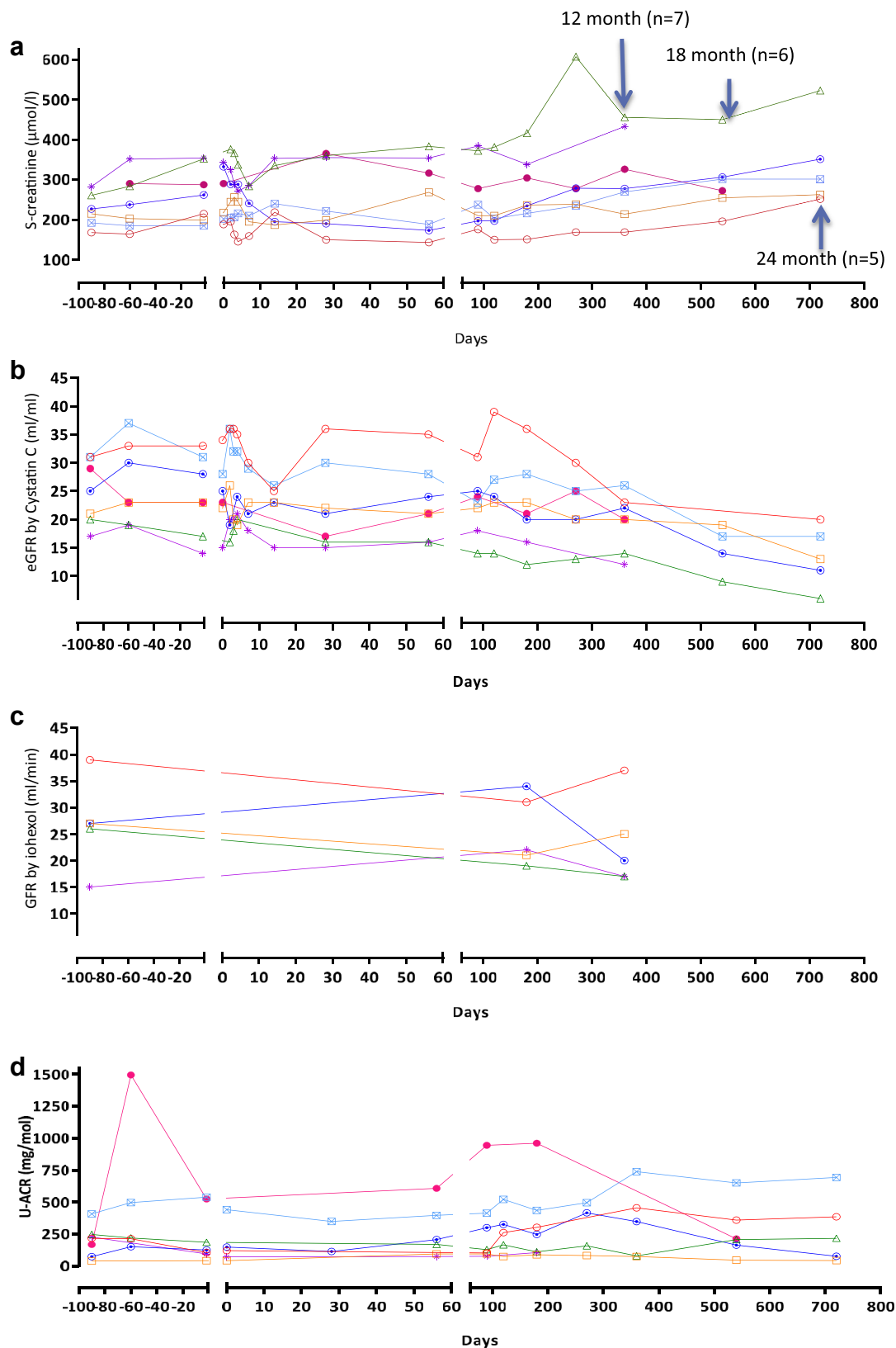
infectious complications, a serious case of postoperative volvulus and subsequent hemicolectomy occurred. There was no direct correlation with the cell implantation procedure and the development of a volvulus. Future trials of renal cell implantation should incorporate alternative delivery methods, such as percutaneous image-guided neo-kidney augmentation, of autologous SCR into the renal cortex as potentially safer alternatives.

Although the study was neither designed nor powered to study the effects of renal cell implantation on renal functions, a secondary objective of the protocol was to study renal functions 12 months after the intervention. We report no statistically significant effects of renal cell implantation on renal volume (MRI), split renal function (renal scintigraphy), or cortical thickness (MRI) 12 months after renal cell implantation (Figure 5). No significant changes in iohexol clearance, eGFR (cystatin C), ACR, or S-creatinine were observed at 12 months after implantation (Figure 4). However, extended follow-up to 24 months after SRC implantation demonstrated a decrease in eGFR (cystatin C), implying that a single cortical injection of SRCs does not confer long-term preservation of renal function. None of the patients had initiated renal replacement therapy at 24 months of follow-up. Our clinical observation are in agreement with animal data showing that SCR engraftment in the treated kidney last up to 6 months postimplantation.<sup>15,16</sup> One patient died of ischemic heart disease and heart failure about 30 months after implantation. It has been reported that direct renal injection of autologous stem cells resulted in hematuria and masses at the site of injection due to angiomyeloproliferative lesions in a case of lupus nephritis.<sup>25</sup> However, repeat MRIs of the kidney after the implantation procedure did not reveal any renal masses at the site of injection or any evidence of scarring or hemorrhage.

This safety study was not designed to study mechanistic pathways for a putative therapeutic bioactivity of

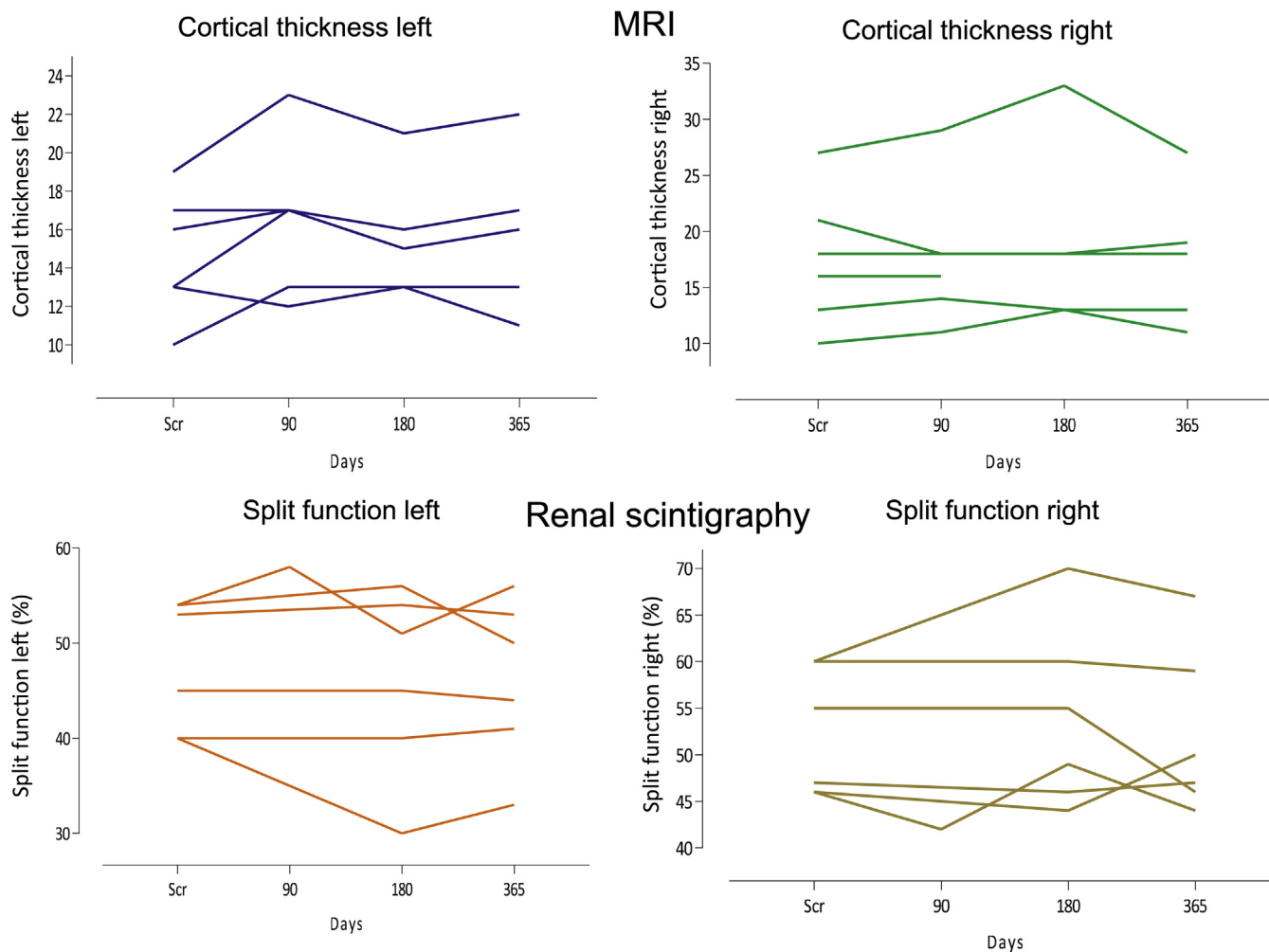
SRCs. Because SCRs consist of autologous, homologous cells that are naturally involved in renal repair and regeneration, it is possible that multiple mechanisms are active. Diverse paracrine mechanisms may catalyze the benefits documented in animal models using this cell population.<sup>15,16</sup> Detailed characterization show that the cell populations are composed of E-cadherin, pancadherin, cytokeratin 8/18/19, γ-glutamyl transpeptidase, and oxygen-responsive subpopulations.<sup>15,16,18</sup> Progressive fibrosis is a hallmark of diabetic kidney disease,<sup>26</sup> and transforming growth factor–β1, plasminogen activator inhibitor–1, and fibronectin may trigger pathologies that promote tissue inflammation and deposition as well as inadequate degradation of epithelial–mesenchymal transition of tubular epithelial cells.<sup>27</sup> Thus, the reduction in profibrotic markers up to 6 months after implantation of SRCs<sup>15,16,18</sup> may, at least in part, explain the beneficial effects observed in animal studies. *In vitro* studies demonstrate that SRCs are highly chemotactic to factors released by damaged tubules and that the cells migrate to and localize in tubules, peritubular space, and damaged glomeruli.<sup>15</sup> Tissue inflammation is another prominent feature of diabetic nephropathy that promotes disease progression. As SCRs modulated urine CCL/MCP1 and CCL5/RANTES protein levels, which indicates attenuated nuclear factor–κB response, SCRs may provide additional immune-modulatory cues to diseased renal tissue.<sup>18</sup>

Several limitations of this phase I safety study should be considered. As the diagnosis of diabetic nephropathy was made based on clinical history and was confirmed by renal biopsy in only 1 case (patient 6), we cannot exclude other or superimposed kidney diseases, such as nephrosclerosis. We also acknowledge that potential differences in rat models of progressive diabetic nephropathy and human diabetic nephropathy, especially with regard to disease duration, may play a role when results from NKA in animal models<sup>16</sup> are compared to results of this “first-in-human” study.



**Figure 4.** Changes in (a) S-creatinine, (b) estimated glomerular filtration rate (cystatin-C), (c) glomerular filtration rate (iothexol), and (d) albumin–creatinine ratio during the 24-month observation period following implantation of selected renal cells in the kidney cortex.





**Figure 5.** Changes in cortical thickness (by magnetic resonance imaging [MRI]) and split function (by renal scintigraphy) in the 6 Swedish patients in the study.

Although the data show no significant progression of eGFR during the first 12 months after kidney cell implantation, the limited number of patients and the substantial heterogeneity in patterns of kidney function decline reported in the 2 years before the start of renal replacement therapy,<sup>28</sup> we can draw no firm conclusions regarding the effects of NKA on kidney disease progression during the first 12 months. As we included older, obese, type 2 diabetic CKD patients (eGFR range 19–34 ml/min), additional studies are needed to determine whether implantation of SRCs in younger, non-obese, type 2 diabetic patients without significant comorbidity and better renal function would result in better outcome. Release of manufacturing product requires SRCs to be at least 70% viable at time of release, as well as to retain viability and both phenotypic and functional characteristics for 3 days at 3°C. All products implanted in this study had >90% cell viability at time of release. No data on renal cell viability after the trans-Atlantic airplane transport before implantation are available.

Other limitations include lack of data on the extent of loss of SRCs via the medulla to urine and/or loss to the bloodstream after implantation. It would have been of interest to perform a re-biopsy and to evaluate tissue expression of pro-fibrotic and pro-inflammatory markers after kidney cell implantation, as has been reported in preclinical studies.<sup>18–20</sup> However, because this was a safety study, postimplantation kidney biopsies were not performed.

Taken together, in this phase 1, first-in-human, 2-center SRC implantation study in obese, type 2 diabetic, CKD 3 to 4 patients, we report the 12-month follow-up and extended data to 24 months after the procedure. Based on the observation of multiple SAE after implantation, safer techniques for delivery of SRC should be considered in future studies in this vulnerable patient group. As extended 24-month follow-up in 5 patients showed a decline of eGFR, a single implantation of SRCs does not appear to provide beneficial effects on kidney function beyond 1 year.



## DISCLOSURE

TB is currently employed by RegenMed (Cayman) Ltd. All the other authors declared no competing interests.

## ACKNOWLEDGMENTS

We thank the patients who participated in the study. We are grateful to all those who carried out the extensive clinical and laboratory work.

## SUPPLEMENTARY MATERIAL

1. Preparation of selected renal cells
  2. Implantation procedure in Chapel Hill, North Carolina, USA
  3. Characterization of injected cells
  4. Detailed description of each patient's postprocedural clinical course
  5. Detailed protocol synopsis
  6. Evolution of S-creatinine using historical data in the 6 Swedish CKD patients (Supplementary Figure S1)
- Supplementary material is linked to the online version of the paper at [www.kireports.org](http://www.kireports.org).

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