RegenMed (Cayman) Ltd.

A PHASE II, OPEN-LABEL SAFETY AND EFFICACY STUDY OF AN AUTOLOGOUS NEO-KIDNEY AUGMENT (NKA) IN PATIENTS WITH TYPE 2 DIABETES AND CHRONIC KIDNEY DISEASE (RMCL-CL001)

Protocol Date and Version: **28 April 2016, Version 1.3**

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12 June 2015, Version 1.1
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Study Sponsor: RegenMed (Cayman) Ltd.	**Study References**
89 Nexus Way, Camana Bay Grand Cayman, Cayman Islands	NCT#: 02525263 EudraCT#: 2015-003156-49 IND #: 16482

1 PROTOCOL SIGNATURE PAGE

RMCL-CL001

Protocol Version: Version 1.3

The signature of the Investigator below constitutes his approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol as specified in both the clinical and administrative sections, including all statements regarding confidentiality.

This trial will be conducted in compliance with the protocol, in accordance with ICH GCP guidelines, in general in agreement with the most recent version of the Declaration of Helsinki, and in accordance with all applicable United States and European regulations.

Investigator Name

_____

_____ _____

Investigator Signature *Date*

_____ _____

Sponsor: RegenMed (Cayman) Ltd., *Date*

2 PROTOCOL SYNOPSIS

Title: A Phase II, Open-Label Safety and Efficacy Study of an Autologous Neo-Kidney Augment (NKA) in Patients With Type 2 Diabetes and Chronic Kidney Disease (RMCL-CL001)

Therapeutic Product: NKA is made from expanded autologous selected renal cell population (SRC) obtained from the patient's kidney biopsy. To manufacture NKA, kidney biopsy tissue from each enrolled patient will be sent to Twin City Bio, LLC, Winston Salem, North Carolina, where renal cells will be expanded and SRC selected. SRC will be formulated in a gelatin based hydrogel at a concentration of 100 x 10^6 cells/mL, packaged in a 10 mL syringe, and shipped to the clinical site for use.

Study Objectives:

Primary Objective: The primary objective of the study is to assess the safety and efficacy of NKA injected in one recipient kidney and determine if two injections of NKA provide stabilization of renal function.

- Primary Safety Outcome Measures: procedure and/or product related adverse events (AE's) through 12 months following the initial NKA injection.
- Primary Efficacy Outcome Measures: serial measurement of serum creatinine and estimation of GFR through 6 months following the second cell injection

Secondary Objective: The secondary objective of the study is to assess the safety and tolerability of NKA administration by assessing renal-specific adverse events over a 12 month period following a patient's first NKA injection.

- Secondary Safety and Tolerability Outcome Measures: renal-specific laboratory assessments through 12 months following the last NKA injection under this protocol, whether first or second.

Exploratory Objective: Exploratory objectives of the study are designed to assess the impact of NKA on renal function over a 12 month period following the initial NKA injection.

- Exploratory Outcome Measures: clinical diagnostic and laboratory assessments of renal structure and function (including eGFR, serum creatinine, and proteinuria) to assess changes in the rate of progression of renal disease; and effect of method of injection on these parameters.
- Exploratory quality of life outcome measure will be the Kidney Disease Quality of Life survey obtained at baseline and at 1, 3, 6, 7, 9, 12, 15, 18, 30, and 42 months after a patient's first NKA injection.

Study Design: Multi-center, prospective, open-label, single-group study. All enrolled subjects will be treated with up to two injections of NKA at least 6 months apart.

Randomization: Open-label, non-randomized.

Control Group: Each subject will serve as his or her own control; the patient's previous medical history, which must include a minimum 6 month period of observation of renal function, will serve as the comparator for rate of progression of renal insufficiency.

Sample Size: Up to 30 subjects will be injected with NKA. As this is a Phase II safety and efficacy study, robust statistical analysis will not be performed. Therefore, the sample size proposed for this study is a size typical for the active treatment group in Phase II studies, allowing for identification of safety outcomes and early efficacy in a limited population.

Study Population:

Male or female patients 30 to 70 years of age with Type 2 diabetes mellitus and CKD with eGFR between 20 and 50 mL/min/1.73m^2. An enrolled patient should have sufficient historical clinical data to determine his or her individual rate of CKD disease progression.

Inclusion Criteria: Unless otherwise noted, inclusion criteria must be met at Screening and prior to injection.

1. Male and female subjects, age 30 to 70 years on the date of informed consent.
2. Patients with type 2 diabetes mellitus (T2DM).
3. Patients with a well-established diagnosis of diabetic nephropathy as the underlying cause of their renal disease.
4. At screening, patients not previously injected with NKA with CKD defined as a GFR of 20 – 50 mL/min/1.73m^2 inclusive. Patients previously treated with a single NKA injection with eGFR 15 to 60 mL/min may also enroll in this clinical trial.
5. Microalbuminuria that cannot be explained by an alternative diagnosis. Microalbuminuria is defined as urinary albumin-creatinine ratio (UACR) ≥ 30 mg/g or urine albumin excretion ≥ 30 mg/day on 24 hour urine collection.
6. Prior to biopsy, systolic blood pressure between 105 and 140 mmHg (inclusive) and diastolic blood pressure ≤90 mmHg.
7. Ongoing and stable treatment with ACEI or ARB initiated at least 8 weeks prior to enrollment. Treatment must be stable for the 6 weeks immediately prior to injection. Stable treatment is defined as dose adjustment to no less than ½ of the current dosage and no more than 2X the current dosage over the 6 week period immediately prior to injection; dose interruptions of up to 7 days due to medical necessity are allowed. Patients who are intolerant to ACEI or ARBs may be included as long as they have stable BP within the acceptable limits.
8. Minimum of 2 measurements of eGFR or sCr taken at least 3 months apart (prior to screening) and within the previous 12 months to define the rate of progression of CKD. The patient should have sufficient historical data to provide a reasonable estimate of the rate of progression of CKD as determined following consultation with the Medical Monitor (to insure sufficient data is available). In addition, the rate of progression of CKD must be consistent over time. There is no defined rate of progression that is required to qualify for inclusion.
9. Willing and able to refrain from use of NSAIDs (including aspirin) and clopidogrel, prasugrel, or other platelet inhibitors peri-procedure (i.e., before and after both the biopsy and injection). The wash-out period before and after each procedure should be 7 days. Willing and able to refrain from use of fish oil and dipryridamole for 7 days before and 7 days after each procedure.
10. Willing and able to cooperate with all aspects of the study.
11. Willing and able to give signed informed consent.

Exclusion Criteria: Patients may not be enrolled if they meet any of the exclusion criteria listed below. Criteria should be assessed at Screening and before injection unless noted otherwise.

1. Type 1 diabetes mellitus (DM).
2. History of a renal transplant.
3. HbA1c > 10% at Screening. Patients with HbA1c > 8% at the time of screening should be offered diabetic teaching and advised to consult their primary physicians for further diabetic management.
4. Hemoglobin levels < 9 g/dL prior to injection. Hemoglobin levels should be measured within 48 hours before the procedure or per site standard practice.
5. Known allergy to kanamycin or structurally similar aminoglycoside antibiotics (as kanamycin is used during manufacture of NKA).
6. Abnormal coagulation status as measured by APTT, INR, and/or platelet count at Screening.
7. Not a good candidate for the injection procedure (based on the assessment of the surgeon who will be performing the injection) including patients who are morbidly obese, have excessive fat surrounding the kidney, have BMI > 45, or who are otherwise at excessive risk for serious complications.
8. Clinically significant infection requiring parenteral antibiotics within 6 weeks of injection.
9. Patients with small kidneys (average size < 9 cm) or only one kidney, as assessed by MRI or renal US at screening or if previously done within 1 year of screening.
10. Patients with a rapid decline in renal function over the last 3 months prior to injection or acute kidney injury.
11. Patients with any of the following conditions prior to injection: renal tumors, polycystic kidney disease, renal cysts or other anatomic abnormalities that would interfere with injection procedure (e.g., cysts in the pathway of the injection), hydronephrosis, skin infection over proposed injection sites, or evidence of a urinary tract infection.
12. Female subjects who are pregnant, lactating (breast feeding) or planning a pregnancy during the course of the study, or who are of child bearing potential and not using a highly effective method of birth control (including sexual abstinence). A highly effective method of birth control is defined as one that results in a low failure rate (i.e. less than 1 percent per year) when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine devices (IUDs), sexual abstinence, or a vasectomized partner. Subjects must be willing to continue birth control methods throughout the course of the study.
13. History of cancer within the past 3 years (excluding non-melanoma skin cancer and carcinoma in situ of the cervix).
14. Life expectancy of less than 2 years.
15. Any contraindication or known anaphylactic or severe systemic reaction to either human blood products or materials of animal (bovine, porcine) origin or anesthetic agents.
16. Positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV) assessed at the Screening Visit.
17. Subjects with active tuberculosis (TB) requiring treatment in the past 3 years.
18. Immunocompromised subjects or patients receiving immunosuppressive agents (including patients treated for chronic glomerulonephritis) within 3 months of injection. [Note: inhaled corticosteroids and chronic low-dose corticosteroids [≤ 7.5mg per day] are permitted as are brief pulsed corticosteroids for intermittent symptoms (e.g. asthma).]
19. Subjects with uncontrolled diabetes (defined as metabolically unstable by the PI), or with incapacitating cardiac and/or pulmonary disorders.
20. History of active alcohol and/or drug abuse that in the investigator's assessment would impair the subject's ability to comply with the protocol.
21. Patients with clinically significant hepatic disease (ALT or AST > 3.0 x ULN) at Screening.

22. Patients with bleeding disorders that would, in the opinion of the Investigator, interfere with the performance of study procedures; patients taking coumarins (e.g. Warfarin) or other anticoagulants (e.g. enoxaparin or direct thrombin inhibitors).
23. Any circumstance in which the investigator deems participation in the study is not in the subject's best interest.
24. Use of any investigational product within 3 months of the injection without receiving prior written consent of the Medical Monitor.

Number of Sites: Up to 10 clinical centers will be included in the study.

Study Duration: 18 months NKA injections follow up followed by 24 month long term follow up for a total study duration of 42 months.

Study Enrollment: Up to 30 subjects undergoing NKA injection will be enrolled into the study. Patients who have received a single injection of NKA under previous research protocols may enroll in this clinical trial to receive a single additional injection. Patients who have never received an NKA injection may enroll in this clinical trial for up to a total of two (2) NKA injections, temporally spaced at least 6 months apart. All biopsies are to be taken from a single kidney, and all NKA injections are to be given into the kidney that was biopsied. Patients who complete screening procedures satisfying all I/E criteria will be enrolled into the study immediately prior to the injection. Patients who do not meet all criteria before injection will be considered screen failures. Once a patient has been injected, the patient will have completed treatment and every effort should be made to ensure the patient completes all follow-up visits. Injection dates for the first 3 patients receiving their second NKA injection will be staggered by a minimum of 3 week intervals to allow for assessment of acute adverse events and other safety parameters by the DSMB. Subsequent second injections will continue to be staggered so as to occur no less than 3 weeks apart, but individual DMSB review will not be required. At the completion of the follow-up visits, patients will continue in a long-term follow-up study. Patients will be followed for a total of 36 months following the last NKA injection under this protocol, whether the first or second injection.

Investigational Plan:

Screening: Subjects who satisfy eligibility criteria may be entered into the study. Subjects must have sufficient historical data on renal function to allow for determination of the rate of progression of renal disease prior to injection (Inclusion Criterion 8). Screening procedures will include a full physical examination, electrocardiogram, and laboratory assessments (hematology, serum chemistry, and urinalysis). In addition, an MRI will be performed to assess kidney volume using site standard practices.

Biopsy: Patients not previously enrolled in a Phase 1 trial will require a renal biopsy to obtain the cells for injection. The biopsy specimens obtained from patients previously enrolled in a Phase 1 trial and maintained in a frozen state will be used to generate the second quantum of NKA to be injected under this protocol, if sufficient cells are available after thawing. If the number of cells obtained after thawing the frozen biopsy specimens is insufficient, the patient may need to have an additional biopsy procedure completed for this study.

Injection: Ten to 14 days before the scheduled injection date, subjects will report to the clinic for verification of final eligibility criteria. In addition, a renal scintigraphy study will be performed to obtain a baseline assessment of split kidney function. Subjects who meet appropriate I/E criteria will

be admitted to the hospital/clinical research unit early in the morning on the day of scheduled injection (Day 0). NKA will be injected into the biopsied kidney, using one of two available options: (1) a laparoscopic approach; or (2) a percutaneous approach. The laparoscopic method may utilize robotic assistance to stabilize the kidney while the injection is performed with laparoscopic viewing; while the percutaneous method will employ a standardized technique such as utilized in the ablation of renal masses by radiofrequency or cryogenic methods. Subjects will remain hospitalized for a minimum of 2 nights and up to 4 nights following a laparoscopic injection (or until any procedure- or product-related AE's have resolved or stabilized). Patients may be discharged the same day following a percutaneous injection without complications. An ultrasound study will be performed on Day 1 to verify the lack of subclinical adverse effects for both approaches.

It is anticipated that all patients will be planned to receive 2 injections under this protocol, in order to allow dose-finding and to understand the duration of effect. Under certain circumstances a patient or investigator may decide to postpone or withhold the second dose. In general, if there appears to be any untoward safety risk, in situations including rapid deterioration of renal function, the development of uncontrolled diabetes, or the development of uncontrolled hypertension, or if there is the intercurrent development of a malignancy, the patient should not receive the second dose.

Second injections under this protocol will be staggered so that single injections in different patients occur no less than 3 weeks apart. The DSMB will review the clinical data regarding each of the first 3 second injections under this protocol, and will consult with the Sponsor before the 2nd, 3rd, and 4th second injections are made. Subsequent second injections will continue to be staggered so as to occur no less than 3 weeks apart, but individual DMSB review will not be required.

No staggering will be required for first NKA injections under this protocol.

Post-Injection Follow-up: Subjects will return to the clinic for follow-up safety assessments on Days 7, 14, and 28 post-injection and at 2, 3, and 6 months post-injection. At 6 months post-injection, post-treatment MRI and renal scintigraphy studies will be conducted. After patients complete the 6 month efficacy visit, they will be considered for a second NKA injection. Patients receiving a second dose will follow the same follow up visits that occurred after first injection. Patients 6 month post first injection visit will serve as the patients 14 to 10 day pre- second injection visit. Patients will return for their second injection and return to the clinic for follow up safety assessments on Days 7, 14, and 28 post-injection and at 2, 3, and 6 months post-injection Patients will be followed up to 18 months in the post follow up phase with 6 months after first NKA injection and 12 months after second NKA injection. Refer to the time and events schedule on page 14 for additional post follow up time points.

Long Term Follow-up: Patients will be followed for safety and efficacy for 24 months after the 18 month initial follow up period. Telephone contact will be made 24 and 36 months after the last NKA injection, and visits will be made at 30 and 42 months after the last NKA injection.

NKA Dose: Kidney weight of the target/recipient kidney will be estimated from the results of the MRI taken during Screening. Using the preclinical studies as a guideline, the dose of NKA for this study is 3 x 10^6 cells /g estimated kidney weight (g KW^{est}). Since the concentration of SRC per mL of NKA is 100 x 10^6 cells/mL, the dosing volume would be 3 mL for each 100 g or 6 mL for a 200 g kidney. Based on this dosing paradigm, the following doses of NKA would be administered:

Estimated Kidney Weight (g KW^{est})		Dose Volume (mL)	SRC Delivered (cell number; x 106)
Median Weight (g)	Weight Range (g)		
100	95 – 108	3	300
117	109 – 125	3.5	350
133	126 – 141	4	400
150	142 – 158	4.5	450
167	159 – 175	5	500
183	176 – 191	5.5	550
200	192 – 208	6	600
217	209 - 225	6.5	650
233	226 – 241	7	700
250	242 – 258	7.5	750
	>259	8	800

Safety Monitoring: While unforeseen adverse effects may occur, the greatest recognized risk to subjects enrolled into the study is hemorrhage following the injection procedure. Therefore, precautions have been taken to minimize the risk of excessive bleeding. Patients with abnormal laboratory values predictive of an increased risk of bleeding will not be eligible for the study. Hemoglobin/hematocrit will be monitored a) before, b) 4 hours after, and c) the day after each procedure.

Injection: During the injection procedure, hemoglobin will be monitored on a regular basis and blood pressure will be monitored continuously using standard site practices. Immediately following laparoscopic injection, the subject will remain supine for 8 hours with regular monitoring of blood pressure/pulse and hemoglobin. The subject will remain in the hospital for 2 to 4 nights following laparoscopic injection for observation of adverse events. On Day 1 following injection, an ultrasound study will be performed to verify there are no subclinical adverse effects. If clinically warranted, an ultrasound may also be performed before discharge from the clinic to ensure no adverse events are ongoing. After an injection via the percutaneous route, the patient may be discharged the same day if that is the site's usual practice after similar procedures (i.e. percutaneous ablation), after no less than 2 hours of observation and monitoring. If product- or procedure-related AE's occurred following surgery, the patient should not be released from the hospital until the AE's have either resolved, stabilized, or returned to baseline. After a laparoscopic injection, the patient should be observed in hospital for 2 to 4 nights to assess for AEs. Following discharge, subjects will be monitored at each visit for changes in renal function including the rate of progression of renal insufficiency. Laboratory values predictive of renal function will be closely monitored. Additional imaging studies may be conducted as needed in response to adverse changes in renal function.

Data Safety Monitoring Board: A Data Safety Monitoring Board (DSMB) will be chartered to oversee patient safety, especially as it relates to any unexpected product-related events. The committee will include 3 members with expertise directly related to protocol activities. The members of the DSMB will have no other engagement with RegenMed (Cayman) Ltd. or any of the study

centers, and the DSMB will function independently. In general, the DSMB will advise RegenMed (Cayman) Ltd. on aspects concerning the safety to the patients who have enrolled as research subjects in the clinical trial. The specific activities and responsibilities of the DSMB will be detailed in the DSMB charter. The DSMB's recommendations will be communicated to the study centers, and where required to the IRBs/ECs, and to the regulatory authorities.

Analysis Methods:

Up to 30 subjects will be injected with NKA. As this is a Phase II safety and early efficacy study, formal sample size calculations were not performed. The planned sample size allows for a preliminary characterization of both the safety profile and potential efficacy of the administered dose of NKA in patients with chronic kidney disease.

Subgroup analyses will compare patients receiving a single injection with patients receiving two injections, and patients receiving laparoscopic injections with patients receiving percutaneous injections.

The safety profile will consist primarily of an evaluation of adverse events, including events of special interest, laboratory parameters, including assessments of renal function, imaging results and vital signs.

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Time and Events Schedule: Patients receiving *Second Dose* NKA Only *Laparoscopic* Approach

Event	**Scr**[1]	**Biopsy**[2]				**Pre-Impl.**	**2nd Injection (Hosp.)**			**Follow-up (± 3 day)**			**2nd Injection Follow-up (±7days)**								**Long Term Follow Up**[3] **(±7days)**			
Assessment	≤45d pre-biopsy	3 to 1 day	Biopsy	1 day	4--14 weeks: Renal cell	14 to 10d	D0	Inpt.[4]	Disch.[5]	D7	D14	D28	2mo	3mo	6mo (14 to 10 day)	8mo	9mo	12mo	15mo	18mo[6] EOS	24mo Call	30mo Visit	36mo Call	42mo Visit
ICF[7]	X																							
I/E Criteria[8]	X	X				X																		
Med. Hist.[9]	X	X																						
ECG	X					X						X			X			X		X				
Comp. Phy. Exam[10]	X																							
Interim Phy Exam[10]		X				X						X			X			X		X		X		X
Vital Signs[11]	X	X	X	X		X	X[12]	X	X	X	X	X	X	X	X	X	X	X	X	X				
Ultrasound/CT			X	X		X[13]	X	X	X[14]						X									
MRI	X[15]																			X				
Renal Scint.						X									X			X		X				
Biopsy[16]		X	X[16]																					
Admit to Hosp.			X				X																	
Injection NKA[17]							X																	
Discharge				X					X[5]															
AE's [18]		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ConMeds	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
KDQoL Survey[19]						X						X		X	X		X	X	X	X		X		X

[1] Screening assessments should be conducted within 45 days before the injection; if assessments fall outside of the 45 day window, rescreening should be conducted as discussed in Section 11.1

[2] The biopsy will only be obtained if there are not enough frozen SRC from the previous study. If there is enough for NKA product to be made skip this visit and proceed to Pre-Injection visit (14d to 10d).

[3] Monthly visit dates for follow-up visits should be based on the calendar day (± 7 days).

[4] Inpatient hospital days, Days 1 and possibly Days 2 and 3.

[5] The patient will be discharged as early as Day 2 and as late as Day 4 following laparoscopic injection

[6] Conduct End-of-Study (EOS) assessments at the 18 mo visit post second injection, or when the subject discontinues from the study (See Section 11.4)

[7] The informed consent form (ICF) must be signed prior to conducting any study-specific procedures.

[8] Appropriate selected Inclusion/Exclusion criteria should be reviewed at Screening, and pre-injection.

[9] Medical and surgical history should be obtained at Screening.

[10] The physical examination (PE) is described in Section 12.2.2 .

[11] Vital signs include heart rate, resting BP, and body temperature. (See section 12.2.3)
[12] Vital signs should be measured throughout the procedure.
[13] An ultrasound should be performed 10 to 14 days before injection to verify continued eligibility and on Day 1 following injection to verify lack of adverse events/safety concerns.
[14] If clinically indicated due to, for example, bleeding during the procedure or a drop in hemoglobin, an ultrasound may be performed prior to discharge to assess safety concerns.
[15] To determine kidney size and volume, an MRI study should be performed between screening and the injection.
[16] The patient may be released same day as biopsy per site standard practice, if released same day as biopsy skip day 1 procedures after biopsy.
[17] NKA product should be handled and injected according to procedures described in the Study Reference Manual.
[18] Record NKA-related SAE's, NKA-related and significant CKD-related AE's reported by the patient at the visit, and ongoing CKD-specific medications.
[19] KDQOL Survey will be completed at pre-injection and at day 28 and months 3, 6, 9, 12, 15, 18, 30 and 42 months after second NKA injection.

Time and Events Schedule: Patients receiving *Second Dose* NKA Only *Percutaneous* Approach

Event	Scr[1]	Biopsy[2]			4--14 weeks: Renal cell selection and manufacture of NKA	Pre-Impl.	2nd Injection	Follow-up (± 3 day)				2nd Injection Follow-up (±7days)								Long Term Follow Up[3] (±7days)			
Assessment	≤45d pre-biopsy	3 to 1 day	Biopsy	1 day		14 to 10d	D0[4]	D1	D7	D14	D28	2mo	3mo	6mo (14 to 10 day)	8mo	9mo	12mo	15mo	18mo[5] EOS	24mo Call	30mo Visit	36mo Call	42mo Visit
ICF[7]	X																						
I/E Criteria[8]	X	X				X																	
Med. Hist.[9]	X	X																					
ECG	X					X					X			X			X		X				
Comp. Phy. Exam[10]	X																						
Interim Phy Exam[10]		X				X					X			X			X		X		X		X
Vital Signs[11]	X	X	X	X		X	X[12]		X	X	X	X	X	X	X	X	X	X	X				
Ultrasound/CT			X	X		X[13]	X[14]							X									
MRI	X[15]																		X				
Renal Scint.						X								X			X		X				
Biopsy[16]		X	X[16]																				
Admit to Hosp.			X				X																
Injection NKA[17]							X																
Discharge				X			X[4]																
Telephone Call								X[6]												X		X	
AE's [18]		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ConMeds	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X				
KDQoL Survey[19]						X					X		X	X		X	X	X	X		X		X

[1] Screening assessments should be conducted within 45 days before the injection; if assessments fall outside of the 45 day window, rescreening should be conducted as discussed in Section 11.1

[2] The biopsy will only be obtained if there are not enough frozen SRC from the previous study. If there is enough for NKA product to be made skip this visit and proceed to Pre-injection visit (14d to 10d).

[3] Monthly visit dates for follow-up visits should be based on the calendar day (± 7 days).

[4] The patient may be discharged same day as percutaneous injection.

[5] Conduct End-of-Study (EOS) assessments at the 18 mo visit post second injection, or when the subject discontinues from the study (See Section 11.4)

[6] 24hours post discharge a follow up phone call from the clinic will be conducted to do a wellness check on the subject.

[7] The informed consent form (ICF) must be signed prior to conducting any study-specific procedures.

[8] Appropriate selected Inclusion/Exclusion criteria should be reviewed at Screening, and pre-injection.

[9] Medical and surgical history should be obtained at Screening.

[10] The physical examination (PE) is described in Section 12.2.2 .

[11] Vital signs include heart rate, resting BP, and body temperature. (See section 12.2.3)
[12] Vital signs should be measured throughout the procedure.
[13] An ultrasound should be performed 10 to 14 days before injection to verify continued eligibility and on Day 1 following injection to verify lack of adverse events/safety concerns.
[14] If clinically indicated due to, for example, bleeding during the procedure or a drop in hemoglobin, an ultrasound may be performed prior to discharge to assess safety concerns.
[15] To determine kidney size and volume, an MRI study should be performed between screening and the injection.
[16] The patient may be released same day as biopsy per site standard practice, if released same day as biopsy skip day 1 procedures after biopsy.
[17] NKA product should be handled and injected according to procedures described in the Study Reference Manual.
[18] Record NKA-related SAE's, NKA-related and significant CKD-related AE's reported by the patient at the visit, and ongoing CKD-specific medications.
[19] KDQOL Survey will be completed at pre-injection and at day 28 and months 3, 6, 9, 12, 15, 18, 30 and 42 months after second NKA injection.

Time and Events Schedule: *New Patients* Receiving *Laparoscopic* Approach

Event	**Scr**[1]	**Biopsy**				**Pre-Impl.**	injection **(Hosp.)**			**Follow-up (± 3 day)**			**1st Injection Follow-up (±7days)**		**Pre-2nd Injection**[2]	**2nd Injection (Hosp)**			**2nd Injection Follow-up (± 3 day)**			**Follow-up (±7days)**					**Long Term Follow Up**[3] **(±7days)**			
Assessment	≤45d pre-biopsy	3 to 1 day	Biopsy	1 day	4--14 weeks: Renal cell selection and manufacture of NKA	14 to 10d	D0	Inpt.[4]	Disch.[5]	D7	D14	D28	2mo	3mo	6mo (14 to 10 day)	D0	Inpt. [4]	Disch[5]	D7	D14	D28	8mo	9mo	12mo	15mo	18mo[6] EOS	24mo Call	30mo Visit	36mo Call	42mo Visit
ICF[7]	X																												
I/E Criteria[8]	X	X				X																							
Med. Hist.[9]	X	X																											
ECG	X					X						X			X									X					
Comp. Phy. Exam[10]	X																												
Interim Phy Exam[10]		X				X						X			X									X		X		X
Vital Signs[11]	X	X	X	X		X	X[12]	X	X	X	X	X	X	X	X	X[12]	X	X	X	X	X	X	X	X	X	X				
Ultrasound/CT			X	X		X[13]	X	X	X[14]						X	X	X	X[14]											
MRI	X[15]																									X				
Renal Scint.						X									X									X		X			
Biopsy[16]		X	X[16]																										
Admit to Hosp.			X				X									X													
Inject NKA[17]							X									X													
Discharge				X					X[5]									X											
AE's [18]		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ConMeds	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
KDQoL Survey[19]						X						X		X	X						X		X	X	X	X		X		X

[1] Screening assessments should be conducted within 45 days before the injection; if assessments fall outside of the 45 day window, rescreening should be conducted as discussed in Section 11.1

[2] If at 6 month visit the PI determines a second dose is not going to be completed patient should return to the clinic at month 9 visit and resume TES.

[3] Monthly visit dates for long-term follow-up visits should be based on the calendar day (± 7 days).

[4] Inpatient hospital days, Days 1 and possibly Days 2 and 3.

[5] The patient will be discharged as early as Day 2 and as late as Day 4 following laparoscopic injection

[6] Conduct End-of-Study (EOS) assessments at the 18 mo visit post second injection, or when the subject discontinues from the study (See Section 11.4)

[7] The informed consent form (ICF) must be signed prior to conducting any study-specific procedures.

[8] Appropriate selected Inclusion/Exclusion criteria should be reviewed at Screening, and pre-injection.

[9] Medical and surgical history should be obtained at Screening.

[10] The physical examination (PE) is described in Section 12.2.2 .

[11] Vital signs include heart rate, resting BP, and body temperature. (See section 12.2.3)

[12] Vital signs should be measured throughout the procedure.

[13] An ultrasound should be performed 10 to 14 days before injection to verify continued eligibility and on Day 1 following injection to verify lack of adverse events/safety concerns.
[14] If clinically indicated due to, for example, bleeding during the procedure or a drop in hemoglobin, an ultrasound may be performed prior to discharge to assess safety concerns.
[15] To determine kidney size and volume, an MRI study should be performed between screening and the injection.
[16] The patient may be released same day as biopsy per site standard practice, if released same day as biopsy skip day 1 procedures after biopsy.
[17] NKA product should be handled and injected according to procedures described in the Study Reference Manual.
[18] Record NKA-related SAE's, NKA-related and significant CKD-related AE's reported by the patient at the visit, and ongoing CKD-specific medications.
[19] KDQOL Survey will be completed at pre-injection and at day 28 and months 3, 6, 9, 12, 15, 18, 30 and 42 months after second NKA injection.

Time and Events Schedule: *New Patients* Receiving *Percutaneous* Approach

Event	Scr[1]	Biopsy				Pre-Impl.	1st Injection		Follow-up (± 3 day)			1st Injection Follow-up (±7days)		Pre-2nd Injection[2]	2nd Injection		2nd Injection Follow-up (± 3 day)			2nd Injection Follow up(±7days)						Long Term Follow Up[3] (±7days)			
Assessment	≤45d pre-biopsy	3 to 1 day	Biopsy	1 day	4--14 weeks: Renal cell selection and manufacture of NKA	14 to 10d	D0[4]	D1	D7	D14	D28	2mo	3mo	6mo (14 to 10 day)	D0[4]	D1	D7	D14	D28	8mo	9mo	12mo	15mo	18mo[5] EOS	24mo Call	30mo Visit	36mo Call	42mo Visit
ICF[7]	X																											
I/E Criteria[8]	X	X				X																						
Med. Hist.[9]	X	X																										
ECG	X					X					X			X								X		X				
Comp. Phy. Exam[10]	X																											
Interim Phy Exam[10]		X				X					X			X								X		X		X		X
Vital Signs[11]	X	X	X	X		X	X[12]		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X				
Ultrasound/CT			X	X		X[13]	X[14]							X	X[14]													
MRI	X[15]																							X				
Renal Scint.						X								X								X		X				
Biopsy[16]		X	X[16]																									
Admit to Hosp.			X				X								X													
Injection NKA[17]							X								X													
Discharge				X																								
Telephone Call								X[6]								X[6]									X		X	
AE's [18]		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ConMeds	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
KDQoL Survey[19]						X					X		X	X					X		X	X	X	X		X		X

[1] Screening assessments should be conducted within 45 days before the injection; if assessments fall outside of the 45 day window, rescreening should be conducted as discussed in Section 11.1

[2] If at 6 month visit the PI determines a second dose is not going to be completed patient should return to the clinic at month 9 visit and resume TES.

[3] Monthly visit dates for long-term follow-up visits should be based on the calendar day (± 7 days).

[4] The patient may be discharged same day as percutaneous injection.

[5] Conduct End-of-Study (EOS) assessments at the 18 mo visit post second injection, or when the subject discontinues from the study (See Section 11.4)

[6] 24hours post discharge a follow up phone call from the clinic will be conducted to do a wellness check on the subject.

[7] The informed consent form (ICF) must be signed prior to conducting any study-specific procedures.

[8] Appropriate selected Inclusion/Exclusion criteria should be reviewed at Screening, and pre-injection.

[9] Medical and surgical history should be obtained at Screening.

[10] The physical examination (PE) is described in Section 12.2.2 .

[11] Vital signs include heart rate, resting BP, and body temperature. (See section 12.2.3)
[12] Vital signs should be measured throughout the procedure.
[13] An ultrasound should be performed 10 to 14 days before injection to verify continued eligibility and on Day 1 following injection to verify lack of adverse events/safety concerns.
[14] If clinically indicated due to, for example, bleeding during the procedure or a drop in hemoglobin, an ultrasound may be performed prior to discharge to assess safety concerns.
[15] To determine kidney size and volume, an MRI study should be performed between screening and the injection.
[16] The patient may be released same day as biopsy per site standard practice, if released same day as biopsy skip day 1 procedures after biopsy.
[17] NKA product should be handled and injected according to procedures described in the Study Reference Manual.
[18] Record NKA-related SAE's, NKA-related and significant CKD-related AE's reported by the patient at the visit, and ongoing CKD-specific medications.
[19] KDQOL Survey will be completed at pre-injection and at day 28 and months 3, 6, 9, 12, 15, 18, 30 and 42 months after second NKA injection.

4 TIME AND EVENTS SCHEDULE: LABORATORY ASSESSMENTS

Event	Scr[1]	Biopsy[2]				Pre-Impl.	Injection (Hosp.)[3]			Follow-up (± 3 day)			1st Injection Follow-up (± 7 day)		Pre-2nd Injection	2nd Injection (Hosp.)[3]			2nd Injection Follow-up (± 3 day)			Follow-up (± 7 day)					Long Term Follow Up (± 7 day)			
Laboratory Assessment	≤45d pre-biopsy	3 to 1 day	Biopsy	1 day	4 – 14 weeks: Renal cell selection and manufacture of NKA	14 to 10 days	D0	Inpt[4]	Disch[5]	D7	D14	D28	2 mo	3mo	6mo (14 to 10 day)	D0	Inpt[4]	Disch[5]	D7	D14	D28	8mo	9mo	12mo	15mo	18mo EOS[6]	24mo Call	30mo Visit	36mo Call	42mo Visit
Chemistry[7]																														
• Std. panel	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
• Renal	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
• Pregnancy test[8]	X	X				X									X															
• HIV, HBV, HCV	X	X																												
• Lipid Panel	X					X									X															
Hematology[9]	X	X		X		X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X				
• Multiple[10]			X				X									X														
• Coag. Status	X	X				X									X															
Urinalysis																														
• 24 hour	X													X									X	X	X	X				
• Spot urine	X	X				X				X		X	X	X	X				X		X	X	X	X	X	X		X		X
• Test stick				X		X	X[11]								X	X[11]														
Add. Tests:																														
• HbA1c	X																							X		X		X		X
• Beta 2microblobulin[12]	X	X				X						X			X						X			X		X				
• iPTH	X	X				X						X		X	X						X			X		X				
• Research (reserve) Samples[13]	X	X				X						X	X	X	X						X	X	X	X	X	X		X		X

[1] Screening assessments should be conducted within the 45 days prior to the injection; if assessments fall outside of the 45 day window, rescreening should be conducted as discussed in Section 11.1.

[2] Patient may be released same day as biopsy per site standard practice, if released same day as biopsy skip day 1 procedures after biopsy. Note that returning patients may not need a biopsy to be completed and will go from screening to the pre-injection visit.

[3] Patients receiving percutaneous approach will have Day 0 lab assessments and then skip to day 7 assessments.

[4] Inpatient Hospital days, Days 1 and possibly Days 2 and 3 depending on day of discharge.

[5] The patient will be discharged as early as Day 2 and as late as Day 4 following laparoscopic injection, or Day 1 following percutaneous injection. Refer to Section 11.4 for discharge instructions.

[6] Conduct End-of-Study (EOS) assessments at the 18 mo visit, or when the subject discontinues from the study (See Section 6.5). Following the EOS visit, the patient should be rolled into the long-term observational follow-up phase.

[7] The Chemistry panel includes both standard and renal analytes as described in Table 8. Laboratory assessments are described in detail in Section 12.3.

[8] A urine pregnancy test should be performed using a urine dip-strip; if positive, then a confirmatory test should be performed by the central laboratory.

[9] Hemoglobin levels must be verified to be > 9 g/dL within 48 hours of each procedure or per site standard practices for verifying Hb levels prior to an injection or surgical procedure.

[10] On procedure days, HCT and Hb should be measured per site standard practice at the site's local lab so the results are immediately available for clinical care. Samples in lab kits for corresponding visit days should be sent to central laboratory for inclusion in database.

[11] Prior to injection, test stick should be performed to confirm absence of signs of infection.

[12] β 2-microglobulin should be assessed in both serum and urine samples.

[13] Research samples (serum/plasma and urine) will be collected, frozen, and stored for assay of renal specific analytes and/or biomarkers at a future date. Results will not be available for reporting in the CSR for this study.

5 LIST OF ABBREVIATIONS

ACEI	Angiotensin-Converting-Enzyme Inhibitor
ACR	Albumin / Creatinine Ratio
ADL	Activities Of Daily Living
AE	Adverse Event
ALT	Alanine Transaminase
APTT	Activated Partial Thromboplastin Time
ARB	Angiotensin Receptor Blocker
AST	Aspartate Transaminase
ATMP	Advanced Therapy Medicinal Product
AV (fistula)	Arteriovenous
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CKD	Chronic Kidney Disease
ConMed(s)	Concomitant Medication(s)
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CSR	Clinical Study Report
CTA	Clinical Trial Application
CTCAE	Common Terminology Criteria for Adverse Events
CVP	Central Venous Pressure
DMSA	Dimercaptosuccinic Acid
DPBS	Dulbecco's Phosphate Buffered Saline
eGFR	Estimated Glomerular Filtration Rate
ECG	Electrocardiogram
EC	Ethics Committee
EMEA	European Medicines Agency
EOS	End Of Study Visit
ESRD	End Stage Renal Disease
FDA	Food And Drug Administration
FIH	First-In-Human (Clinical Trial)
g	Gram

GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GFR	Glomerular Filtration Rate
HARS	Hand Assisted Retroperitoneoscopic
Hb	Hemoglobin
HbA1c	Glycosylated Hemoglobin
HBV, HBsAg	Hepatitis B Virus, Hepatitis B Virus Surface Antigen
HCV	Hepatitis C Virus
HCT	Hematocrit
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference On Harmonization
I/E	Inclusion / Exclusion
IND	Investigational New Drug
INR	International Normalization Ratio
iPTH	Intact Parathyroid Hormone
i.v.	Intravenous
KDIGO	Kidney Disease; Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
KW_{est}	Kidney Weight, Kidney Weight estimated
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NKA	Neo-Kidney Augment
NOAEL	No-Observed-Adverse-Effect-Level
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
Nx	Nephrectomy, Nephrectomized
PE	Physical Examination
PI	Principal Investigator
PT	Prothrombin Time
PTH	Parathyroid Hormone
QA	Quality Assurance
QP	Qualified Person
RBC	Red Blood Cell
SAE / SAR	Serious Adverse Event / Serious Adverse Reaction
sCr	Serum Creatinine

SMC	Safety Monitoring Committee
SPIO	Superparamagnetic Iron
SRC	Selected Renal Cells
SUSAR	Suspect, Unexpected Serious Adverse Reaction
TB	Tuberculosis
UA	Urinalysis
WBC	White Blood Cell

6 INTRODUCTION AND BACKGROUND

RegenMed (Cayman) Ltd. is a regenerative medicine company whose principals have experience in developing regenerative medicine products (Atala et al., 2006; Basu et al., 2011; Basu and Ludlow, 2012; Genheimer et al., 2012; Jayo et al., 2008a; Jayo et al., 2008b; Jayo et al., 2008c; Kelley et al., 2012; Kelley et al., 2010). RegenMed (Cayman) Ltd. is currently developing a regenerative cell-based product, Neo-Kidney Augment (NKA), designed to augment renal function in patients with chronic kidney disease (CKD). Therapeutic intervention with NKA is intended to delay the need for renal replacement therapy (dialysis or transplant) which at this time, is inevitable in patients with CKD. Therapeutic intervention with NKA entered clinical investigations with two similar Phase 1 studies, one at the Karolinska University Hospital Huddinge in Stockholm, Sweden (6 patients) and one at the University of North Carolina, Chapel Hill, NC (1 patient). To date, 7 patients have been biopsied and injected safely with NKA. This second study is designed to continue those clinical trials and assess the effect of a first and second injection of NKA in type 2 diabetic patients with CKD.

6.1 Chronic Kidney Disease

6.1.1 Background

CKD is characterized by progressive nephropathy that, without therapeutic intervention, will worsen; ultimately the patient may reach end stage renal disease (ESRD). Prevalence data from the U.S. to Europe show that approximately 10% of the general population have stage 1-3 CKD (ERA, 2009; USRDS, 2011). Worldwide, the incidence and prevalence of CKD and ESRD are increasing while therapeutic outcomes remain poor. The greatest cause of ESRD is diabetes mellitus (Postma and de Zeeuw, 2009), and the incidence of CKD continues to increase, primarily due to the increases in the incidence of type 2 diabetes (Postma and de Zeeuw, 2009). CKD is often accompanied by adverse outcomes owing to underlying comorbidities and/or risk factors including hypertension and renovascular disease (Khan et al., 2002). Due to serious comorbidities, patients with CKD are 5-11 times more likely to suffer premature death than survive to progress to ESRD (Collins et al., 2003; Smith et al., 2004). In order to survive, ESRD patients require renal replacement therapy (dialysis or transplantation). Preventing or delaying adverse outcomes of CKD by intervening early-on in the disease is the primary strategy in CKD management. Unfortunately, early therapeutic approaches to prevent disease progression have not been successful.

6.1.2 Stages and Outcomes of CKD

The major causes of CKD are diabetes and hypertension; nearly half of all CKD cases arise from diabetes with or without hypertension (ERA, 2009; Postma and de Zeeuw, 2009). Other causes are glomerulonephritis (the inflammation and damage of the filtration system of the kidneys) caused by post-infectious conditions and autoimmune diseases such as lupus, polycystic kidney disease, long-term use of analgesics, atherosclerosis leading to ischemic nephropathy, ureteral obstruction or stricture leading to renal damage, HIV infection, sickle cell disease, amyloidosis, and chronic kidney infections.

CKD is classified based on cause, GFR category and albuminuria category. KDIGO (Kidney Disease: Improving Global Outcomes) recently published updated clinical practice guidelines with updated staging of CKD based on GFR and albuminuria as shown Table 1(KDIGO, 2013).

Table 1: KDIGO Classification of CKD

A. GFR Categories in CKD		
GFR Category	**Terms**	**GFR (mL/min/1.73m2)**
G1	Normal or high	≥90
G2	Mildly decreased	60-89
G3a	Mildly to moderately decreased	45-59
G3b	Moderately to severely decreased	30-44
G4	Severely decreased	15-29
G5	Kidney failure	<15 (or dialysis)

B. Albuminuria Categories in CKD				
Category	**AER (mg/24 hr)**	**ACR (approximate equivalent)**		**Terms**
		(mg/mmol)	(mg/g)	
A1	<30	<3	<30	Normal to mildly increased
A2	30 – 300	3 – 30	30 – 300	Moderately increased
A3	>300	>30	>300	Severely increased

Abbreviations: CKD (chronic kidney disease), GFR (glomerular filtration rate), AER (albumin excretion rate), ACR (albumin-to-creatinine ratio)

The initial stage of diabetic nephropathy occurs over a period of several years and is marked by microalbuminuria (A2; 30 – 300 mg/24 hours) followed by macroalbuminuria (A3; > 300 mg/24 hours), or proteinuria. As the ability of the kidney to filter blood waste products declines, blood creatinine rises. As kidney damage progresses, rising blood pressures further exacerbates kidney disease. When the kidneys cease to function entirely (ESRD), renal replacement therapy (dialysis or transplant) is necessary.

Mortality in CKD patients increases as GFR declines (Go et al., 2004; Kovesdy et al., 2006). Mortality among CKD patients in 2009 was 56% greater than among non-CKD patients. The unadjusted mortality rate in Medicare CKD patients age 66 and older is approximately 16% (USRDS, 2011). Death commonly results from cardiovascular events and in several studies of patients with advanced CKD was more common than progression to dialysis (Keith et al., 2004; Rahman et al., 2006).

6.1.3 Standard of Care

There is no cure for CKD or therapy which prevents progression of disease. Therefore, treatment of patients with chronic kidney disease is focused on managing comorbid conditions including cardiovascular disease while slowing progression and preparing for kidney failure and kidney replacement.

Co-morbidities: Anemia, mineral and bone disorders, and cardiovascular events are the major comorbidities requiring treatment in CKD patients. Anemia is often treated via recombinant human erythropoietin. In addition, anti-inflammation medications may also be administered to temper inflammatory cytokines. Treatment for mineral and bone disorders focus on maintaining phosphorous and calcium homeostasis (Lee et al., 2007). Initial treatment restricts dietary phosphorus intake or uses specific phosphate binders when phosphate or parathyroid hormone levels begin to rise. For chronic therapy, calcium-based phosphate binders are the most widely prescribed treatments for management of CKD-associated hyperphosphatemia. Additionally,

vitamin D and its related compounds may be administered to raise serum calcium concentration sufficiently to suppress parathyroid hormone secretion. Increased risk of cardiovascular disease can be a complication of CKD or an independent comorbidity, with the former being probable in advanced CKD cases (Gerstein et al., 2001). The use of 1) angiotensin converting enzyme inhibitors (ACEI) and/or angiotensin receptor blockers (ARBs) to reduce proteinuria and control hypertension, 2) insulin titration to achieve appropriate glycated hemoglobin, and 3) statin therapy to counter dyslipidemia, collectively aim to reduce cardiovascular risk and prevent or slow the progression of kidney failure.

Renal replacement therapy: When a patient reaches Stage 5 CKD, renal replacement therapy (i.e. dialysis or kidney transplant) is indicated. The vast majority of Stage 5 individuals receive hemodialysis (Dhingra et al., 2001). Dialysis replaces about 5-15% of kidney function depending on the intensity and frequency of use, and helps restore fluid and electrolyte balance when kidneys fail.

However, the life-expectancy of an ESRD patient initiating hemodialysis is only 4-5 years (USRDS, 2011). Additionally, hemodialysis is associated with multiple and serious complications and quality of life reductions such as arterio-venous graft infections, graft revision, and the need to undergo dialysis up to three times per week.

Kidney transplantation remains the most effective form of therapy at this time; however, there is a chronic shortage of organs. If a patient is able to secure a kidney for transplantation, long-term therapy with immunosuppressive agents is required to prevent rejection. While newer immunosuppressive regimens are more potent leading to a reduction in immune-mediated graft loss, the use of these regimens has resulted in a higher incidence of medication-related problems including increased rates of infection.

Therefore, there is a critical medical need for improved therapies for CKD which could dramatically slow the progression of disease, and significantly delay the need for renal replacement therapy.

6.2Nonclinical Pharmacology Studies

NKA is composed of autologous, homologous selected renal cell population (SRC) formulated in a Biomaterial (gelatin-based hydrogel). SRC are the biologically active component of NKA. The final composition of NKA is:

- SRC: 100 x 10^6 cells per mL of NKA
- Biomaterial: 0.88% solution of gelatin in Dulbecco's Phosphate Buffered Saline (DPBS)

RegenMed (Cayman) Ltd.'s approach to developing NKA is based on extensive scientific evaluation of NKA and its active biological component SRC. SRC are an autologous, homologous renal cell population naturally involved in renal repair and regeneration. In pharmacology, physiology and mechanistic-biology studies, RegenMed (Cayman) Ltd. defined the pharmacological characteristics of SRC and demonstrated their ability to delay the progression of CKD by augmenting renal structure and function. RegenMed (Cayman) Ltd.

subsequently conducted safety pharmacology and GLP toxicology safety studies with NKA in diabetic ZSF1 rats and normal mongrel canines. An overview of the nonclinical studies is presented in Table 2. A summary of these studies is presented below and a detailed description of the studies can be found in the Investigator's Brochure (IB). Of note, injection of NKA in rodent models was accomplished using a syringe affixed to a sharp needle which was used to pierce the capsule and deliver NKA. In canine models, a piercing cannula was used to pierce the capsule and a blunt-ended cannula was used to deposit NKA directly in the kidney.

Table 2: Summary of Preclinical Pharmacology Studies

No	Model	No. Animals	Study Length	Dose per kidney weight[1]	Kidneys Injected (# inj. per kidney)	Total kidney weight Injected[1]	Total Dose[2] Volume	Total SRC	SRC Conc.
				10^6Cell/g	n	g	mL	10^6	10^6/mL
Pharmacology (efficacy, kinetics, migration and persistence) of SRC									
1	*5/6 Nx, Lewis Rat*	*3*	*6 mo*	*5 - 10*	*1 (1)*	*1*	*0.1*	*5-10*	*50-100*
2	*70% Nx, Canine*	*4*	*10 mo*	*6*	*1 (2)*	*57.7*	*5*	*334*	*66.8*
3	*ZSF1*	*7*	*12 mo*	*3*	*2 (2)*	*3.2*	*0.4*	*10*	*25*
Safety Pharmacology (extra-renal effects) of NKA									
4	*5/6 Nx, Lewis Rat*	*77*	*4 d*	*5-15*	*1 (1)*	*1*	*0.1*	*5-15*	*50-150*
5	*Canine*	*1*	*30 min*	*12.5*	*2 (2)*	*120*	*10*	*1500*	*150*
6	*Canine*	*4*	*30 min*	*1.5-9.2*	*2 (1-2)*	*120*	*2.5-10*	*92.7-553.5*	*37-55*
GLP Toxicology of NKA									
7	*ZSF-1 rat*	*5M/5F* *5M/5F* *5M/5F* *5M/5F*	*3 mo 6 mo* *3 mo 6 mo*	*3.13* *3.13* *6.25* *6.25*	*2 (2) 2 (2)* *2 (2) 2 (2)*	*2 2* *2 2*	*0.25* *0.25* *0.25* *0.25*	*6.25* *6.25* *12.5* *12.5*	*25 25* *50 50*
8	*Canine*	*2M/2F* *2M/2F* *2M/2F* *2M/2F*	*1 mo 3 mo* *1 mo 3 mo*	*2.75* *2.75* *11.0* *11.0*	*1 (1)* *1 (1)* *2 (1)* *2 (1)*	*60* *60* *120* *120*	*3* *3* *12* *12*	*330* *330* *1320* *1320*	*110* *110* *110* *110*
9	*Canine*[3] *Dose 1*	*2M/2F*	*n/a*	*5.5*	*2 (2)*	*120*	*6*	*660*	*110*
	Dose 2		*6 mo*	*5.5*	*2 (2)*	*120*	*6*	*660*	*110*

Abbr: Nx, nephrectomy; inj, injection; t, study duration

[1] Estimated kidney weight based on animal model. Actual weights are listed in the study reports where applicable.

[2] Dose refers to SRC or NKA

[3] Two doses of NKA were administered in Study #9; one at 0 months and the second at 3 months

6.2.1 Pharmacodynamics

Proof of principle for SRC as the biologically active component of NKA was established in multiple models of CKD (described in detail in the IB). The 5/6th nephrectomy (Nx) rodent mass reduction model of CKD allowed for an optimized selection of a therapeutically relevant SRC cell population. A 70% Nx canine model of CKD confirmed SRC activity in a large mammal, and the ZSF1 rat served as proof-of-principle for investigating the effects of SRC in the best model for meeting the criteria of human type 2 diabetes (Bilan et al., 2011). SRC injected directly into the kidney cortex in multiple animal models of CKD were capable of affecting a regenerative response in multiple locations of the nephron through direct engraftment or tissue replacement, and through a putative paracrine mechanism involving the effect of secreted factors (Ilagan et al., 2010a; Ilagan et al., 2010b; Kelley et al., 2012; Kelley et al., 2010).

This intervention strategy elicited a regenerative response that significantly improved survival and stabilized disease progression to renal structure and function, and extended the longevity in both the 5/6th Nx model and the ZSF1 model of CKD. Functional improvements included morphological normalization of multiple nephron structures and functions including, glomerular filtration, tubular protein handling, electrolyte balance and the ability to concentrate urine. Improvement to blood pressure, including reduced levels of circulating renin were also observed in the ZSF1 model. These functional improvements following SRC treatment were accompanied by significant reductions in glomerular sclerosis, tubular degeneration and interstitial inflammation and fibrosis. In each of these models where SRC were evaluated, no toxicologically significant in-life, clinical pathology, or histological changes were observed in the target organ or other tissues. Based on results from multiple preclinical studies conducted in different animal models of CKD, SRC (active component of NKA) are effective in significantly delaying progression of CKD to ESRD when injected in the diseased organ prior to irreversible nephropathy. These results provide a rationale for investigating the effects of this cell-based strategy in patients prior to fulminate, end-stage CKD or ESRD.

6.2.2 Safety Pharmacology

Extra-renal activity: To evaluate extra-renal activity, in particularly the hemodynamic effects of NKA, (SRC formulated in gelatin-based hydrogel) was used in a series of in vivo studies to evaluate the immediate cardiovascular and respiratory effects from procedure under similar conditions reported in the pharmacology studies summarized above. The rodent 5/6th Nx study (Study #4) was designed to test acutely the effects of a lower and higher SRC concentration in varying percentages of gelatin (0.75-1.0%), and the normal canine model was selected to evaluate blood pressure immediately before, during and shortly after NKA injection. No studies on effects on the central nervous system were performed as animals showed normal behavior, and no direct effect on the nervous system is expected from a therapy consisting of injecting intact renal cells into the kidney.

Hemodynamic effects: The rats in the 5/6th Nx study (Study #4) were injected with NKA or controls and the short term effects of the procedure were followed to 4 days post-injection. Among the 77 animals treated in this NKA formulation screening study, 16 animals experienced apnea during or immediately after injection. A total of nine animals died; the causes of death were classified as apnea (n=3), renal hemorrhage (n=2) and deaths associated with CKD (n=4).

Six of the 16 animals that experienced apnea were not pre-treated with atropine; of these animals that experienced apnea two died under the influence of anesthesia prior to the use of atropine, which included two of the three animals that died. Ten of the 16 animals that experienced apnea were treated with atropine and all recovered from the surgical procedure and injection. Importantly, the apnea and bradycardia were not observed following the injection of SRC in the ZSF1 (Study #3), canine pharmacology study (Study #4), or in two (intact) canine pilot studies to assess the short-term effects of volume administration on blood pressure during and following NKA injection (Study #5 and Study #6). This model-specific hemodynamic response can be potentially attributed to 1) altered hemodynamics of the severely mass-reduced rodent remnant kidney (Brenner,

1985), 2) transient changes in kidney interstitial pressure administration triggering a central autonomic response (Montgomery et al., 1950; Swann et al., 1952), and possibly 3) under perfusion of tissue or acute hypoxia from bleeding following injection to the kidney. That pre-treatment with atropine (Field et al., 2010) (Field, 2010), a competitive antagonist of the parasympathetic nervous system, helped mitigate this species-specific volume-induced bradycardia and apnea with the associated death indicates an autonomic response to the injection strategy specific to severely mass-reduced rodent model of CKD.

Dose Volume: Using a range of NKA dose, volume and concentration (Study #5), the normal canine was selected to evaluate blood pressure immediately before, during and after NKA injection. In this study, each pole of each kidney was injected with 2.5 mL of NKA; therefore a total of 10 mL/120 g, or 0.083 mL/g, was injected at a dosage of $12.5x10^6$ cells / gram of kidney mass. Injection was well tolerated, as there were no adverse systemic effects (physical or serological) findings, nor were there any toxicologically significant histomorphological changes indicative of kidney injury or other tissue injury as a result of NKA injection to the kidney. Notably and in contrast to the severe mass reduction rodent models of CKD, there were no hemodynamic changes (i.e. apnea) observed.

6.2.3 Kinetics, Migration and Persistence

Overview: Given the cell-based nature of NKA, RegenMed (Cayman) Ltd. conducted studies to assess migration and persistence within the target organ over selected sampling times (Kelley et al., 2012; Kelley et al., 2010). A brief summary of the studies is presented below; additional information is provided in the references and IB.

No separate study to trace renal cells in tissues outside of the kidney has been performed to date based on: 1) significant renal cell retention following direct targeted injection into the kidney, 2) no negative effects from treatment have been observed with animal survival, and 3) no adverse systemic (physical or serological) findings were observed. These results have been validated by GLP toxicology studies where data from interim QA-validated GLP reports comprehensively showed no gross, serological, macroscopic or microscopic histopathological test article-related adverse findings in the kidney nor in non-kidney tissues, and no traces of extra-renal test product distribution was observed. Our results are in agreement with and supported by recent published reports demonstrating that both intravenous and targeted injection of hundreds of millions of allogeneic stem cells, that were neither native to the recipient or the targeted organ, posed little risk to cardiac insufficiency patients in human clinical trials (Hare et al., 2012; Hare et al., 2009).

Methods: Renal biodistribution was performed via cell engraftment studies in the 5/6th Nx rat model and were determined through cell tracing experiments using male donors in female recipients post treatment through genomic detection of the male SRY gene, and by in situ hybridization detection of the engrafted male chromosome (Y/12) (Kelley et al., 2010).

In the ZSF1 animal model, SRCs were labeled with the Rhodamine-B superparamagnetic iron oxide (SPIO) particle, a contrast agent that is specifically formulated for cell labeling and is readily internalized by non-phagocytic cells. SPIO labeled cells were detected post-injection by MRI and whole organ optical imaging for the SPIO iron-labeling was performed 24 hours following injection. In addition, ZSF1 injected with SRCs labeled with CelSense-19F were also quantified by Nuclear magnetic resonance (NMR) 3h, 24h, and 7 days after injection (Kelley et al., 2012).

Results: Both acute ZSF1 detection and long-term donor cell detection using the 5/6th Nx model of CKD showed significant retention of injected cells. Clinically relevant MRI detection 24 hr following cell injection reveals a region at the anterior pole of the kidney where the cells were injected with the SPIO Biopal ion particle. These data are consistent with whole organ fluorescent imaging highlighting cell detection at and around the site of injection located at the upper cortex of the anterior pole. Sectioning of the whole kidney indicated a bolus of cells migrating and distributing from the cortical injection site, confirming their presence in tubular and peritubular spaces of the cortex and medulla. The robust detection of 19F-labeled cells at 3 and 24 hours following injection confirms their early retention in the kidney and the diminished yet significant detection of 19F 7 days following the transplantation procedure. SRC retention was consistent with the detection of these cells previously reported in the 5/6 Nx model using both SRY gene detection and FISH for Y chromosome (male donor cell detection in female recipients), and cell-membrane PKH26 dye-labeled studies.

Summary:

- Donor SRC frequency estimated at ~1:30,000 at 6 months post-injection
- SRC localized primarily to tubular and peritubular areas
- Cellular proliferation and the 3-6 month half-life of a renal epithelial cell (Nadasdy et al., 1994) in-part accounts for the dilution effect of retention of the SRC label 6 months post-injection

6.3 Toxicology Studies

To assess the safety of NKA, three GLP safety studies were conducted; one in the rat ZSF1 disease model of CKD and the other two in normal canines. Details of the study designs are presented in the IB. A brief description of the results are presented below.

6.3.1 ZSF Single Dose Study

The purpose of this study was to assess the safety of a single administration of NKA in ZSF1 rats; a model of uncontrolled metabolic syndrome including type 2 diabetes, hypertension and severe obesity. Rats were administered either a) high dose NKA, b) low dose NKA, c) PBS (sham-treated), or d) Biomaterial. Each animal received 4 injections of test article, one into each

pole of each kidney. Results were assessed at 3 and 6 months post-treatment. An overview of the results are presented below.

Renal-related findings: No treatment-related kidney findings were noted following evaluation of 8 areas of each kidney (3 stains per area), including evaluation and scoring of 150 glomeruli per kidney. Apart from changes related to injection and/or injection site linear scars, all kidneys were considered normal within the context of the disease model. No test article-related kidney findings were observed at the 3 or 6 month time points. All macroscopic and microscopic kidney changes were considered related to the natural progression of renal disease in the ZSF1 obese rat, or to the injection procedure.

Kidney changes in all groups were more severe in males, and consistent with differences in the disease stage between genders. Overall, there was an apparent trend of lower renal histological severity scores (i.e., lower glomerular injury score, tubule-interstitial injury score, and global nephron score) that was consistently noted in the low-concentration NKA treatment group when compared to the Sham control group 6 months post-procedure.

Based on the absence of differences across study groups, the No-Observed-Adverse-Effect-Level (NOAEL) was the high dose, 6.25×10^6 cells/g KW^{est}.

Non-renal findings: No NKA safety-related findings were observed in non-target tissues. No ureteral or bladder (primary routes of NKA excretion) NKA-related changes were observed. There were no NKA-related effects, and no observable NKA cellular materials, in any of the draining (lymph nodes) or filtering (liver, lung, spleen) tissues examined.

Clinical Pathology: The results of clinical laboratory tests (including hematology, clinical chemistry, and special urinalysis panels) were evaluated for differences between baseline and end of study (3 or 6 months post-treatment), and between treated and control groups. No NKA-related clinically significant laboratory abnormalities were identified. Of note, a less severe decrease in hemoglobin and hematocrit was seen in the both male NKA treatment groups (i.e., high and low) as compared with the male Sham control. Observations in this study support the safety of therapeutic uses of NKA.

Conclusions:

- All animals survived to the end of study (3 or 6 months post-treatment)
- There were no significant safety-related clinical pathology findings attributable to treatment. No test article safety-related findings of toxicological relevance were identified in any of the tissues evaluated.
- No NKA-related clinically significant laboratory abnormalities were identified.

6.3.2 Single Dose Canine Study

The purpose of this study was to assess the safety of single administration of two different doses of NKA compared to PBS (sham treated) and Biomaterial. Test article was delivered into one pole of each kidney approximately 1 month after bilateral renal injection. A total of 32 normal mongrel dogs were entered into the study; 16 assessed at one month and 16 assessed at 3 months. Details of study design are presented in the IB. An overview of the results are presented below.

General results: All 32 animals survived to their designated termination time point (1 and 3-month post-treatment). The animals were in good health throughout the study duration. There were no significant clinical pathology findings over the study duration. None of the animals were azotemic at any point through the study duration and there were no indications of decreased GFR.

Renal-related results: No NKA injection-related macroscopic or microscopic findings were observed at the 1 or 3 month endpoint. No treatment-related kidney findings were noted following enhanced evaluation of 8 areas of each kidney (3 stains per area), including evaluation and scoring of

150 glomeruli per kidney. Apart from changes related to injection site scars (fibrosis/chronic inflammation in the capsule; linear fibrosis/chronic inflammation and inflammatory cells in the cortex/medulla), all kidneys were normal. All macroscopic and microscopic kidney changes were considered background findings or related to the injection procedures.

Non-renal findings: No test article-related findings were identified in other (non-kidney) tissues. All macroscopic and microscopic changes were considered background changes and within normal limits.

Procedure-related findings: The most common abnormalities included swelling at the incision sites (seroma formation) and weight loss at study termination. With regard to incision site swelling, ten of sixteen (10/16) animals had sterile seroma formation post-injection, and nine of sixteen (9/16) post treatment. The animals had varying degrees of swelling at their retroperitoneal incisions, and were treated as deemed necessary by a veterinarian.

Numerous animals had mild inappetence post-operatively, following injection (29 of 32) and treatment (18 of 32) procedures. Numerous (28 of the 32) animals had weight loss over the duration from baseline weight (prior to injection) to termination. Of note, a greater amount of weight loss occurred between baseline (2 weeks prior to the injection) and treatment (day 0; renal injections) than between treatment and termination. The weight loss was also noted across all treatment groups (DPBS, biomaterial, high dose, low dose), was not associated with intermittent inappetence, and was considered to be due to the stressful nature of this study. The animals' feed was increased only if the body condition deteriorated (1/16 animals), and weight gain was observed in those cases.

Conclusions:

- All animals survived to their designated termination time point and were in good general health throughout the study duration based on clinical pathology and veterinary assessment.
- Pathological assessment showed no NKA safety-related (macroscopic or microscopic) findings in either target (kidney) or non-target organs examined.
- NKA-treatment (low and high dose groups) showed no detrimental effects 1 month or 3 months post treatment when compared to biomaterial control and sham (PBS) groups.

- Neither dose of NKA produced macroscopic or microscopic adverse effects. Based on anatomic pathology, the no-observed-adverse-effect-level (NOAEL) is the high concentration of $11.7x10^6$ cells/gram kidney.

6.3.3 Repeat Dose Canine Study

The purpose of this study was to assess the safety of administering two repeat doses of NKA; each dose was delivered into both kidneys at times 0 (baseline) and three months. All animals were subjected to two renal biopsies per kidney (1 cranial and 1 caudal pole) 4 – 6 weeks prior to the initial injection procedure. Control animals were injected with PBS. Animals were monitored for 6 months following the baseline injection.

Study Results: All 8 animals were in good clinical health throughout the study duration and survived to their designated termination time point (6-months). There was mild or insignificant weight loss in 5 of the 8 animals, with only 2 animals losing >3% body weight over the study duration. Greater weight loss occurred between the renal injection and initial treatment than between the initial treatment and termination. No abnormal trends were seen in clinical pathology or urinalysis data. There were no signs of renal insufficiency in clinical pathology (azotemia), and no indications of decreased GFR.

Kidney-Related Findings: No NKA injection safety-related macroscopic or microscopic findings were observed at the 6-month time point. No treatment-related kidney findings were noted following enhanced evaluation of 8 areas of each kidney (3 stains per area), including evaluation and scoring of 150 glomeruli per kidney. All kidneys were essentially normal, apart from changes related to injection site scars (fibrosis/chronic inflammation in the capsule; linear fibrosis/chronic inflammation and inflammatory cells in the cortex/medulla).

Non-Kidney Related Findings: No test article safety-related findings were identified in other (nontarget organ) protocol-required tissues. All macroscopic and microscopic changes were considered background changes and thus, considered within normal limits.

Conclusions:

- All animals survived to their designated termination time point and were in good general health based on clinical pathology and veterinary assessment data.
- Pathological assessment showed no NKA safety-related (macroscopic or microscopic) findings in either the target organ (kidney) or non-target organs examined.
- At the 6-month time point, no detrimental effects of repeat doses of NKA were seen when compared to the sham (PBS- injection) group.

6.3.4 Phase One Clinical Trials – Interim Results

In April 2013, a first-in-human (FIH) clinical trial was initiated at the Karolinska University Hospital Huddinge in Stockholm, Sweden, by Tengion, Inc. The trial was a Phase 1, open-label, safety and injection optimization study of NKA injected into patients with CKD. NKA was manufactured from SRC obtained from a patient's biopsy, formulated with gelatin biomaterial, and injected back into the patient's left kidney. The primary objective of the study was to assess the safety and optimal injection of NKA injected at one site in a recipient kidney as measured by

procedure and/or product related adverse events (AEs) through 12 months post-injection. The secondary objective of the study was to assess changes in renal function over a 12 month period following injection as measured by laboratory assessments. Six patients, recruited from the patient population at Karolinska University Hospital, were enrolled into the study. Each patient's baseline rate of disease progression has been used as the control to monitor for adverse changes in the rate of disease progression following injection. The study was terminated due to a lack of funding on December 10, 2014.

The patients enrolled in the Swedish study were recruited from the clinical practice at the Karolinska Institute and they continue to be closely monitored by the investigators. When RegenMed (Cayman) Ltd. establishes regulatory and ethics committee approval to continue the clinical trial, the patients will be re-consented and further safety and efficacy data compiled, and the opportunity to receive a second injection of NKA offered.

After the Swedish study had been under way, an IND for NKA was opened and a similar study started in the US, with one patient enrolled and treated at the University of North Carolina before the study was terminated due to a lack of funding on December 10, 2014.

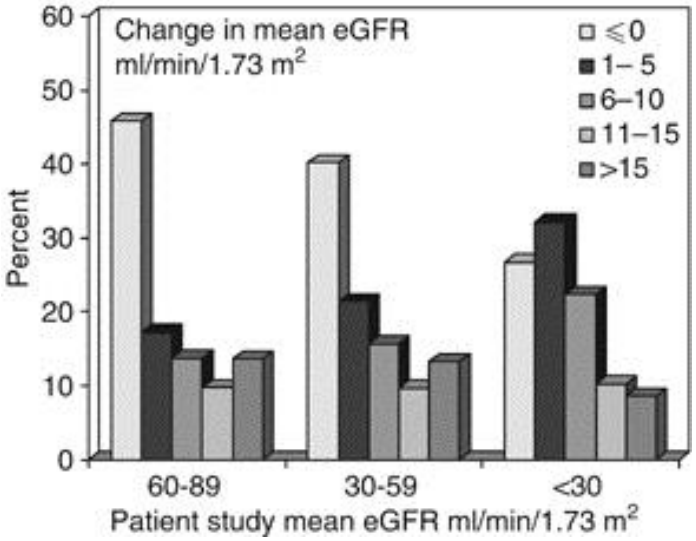
6.4 RegenMed (Cayman) Ltd.Overview of Efficacy

6.4.1 Phase 1 Studies of NKA

Estimated Glomerular Filtration Rate (eGFR)

Elderly male diabetic patients with CKD stage 3b/4 injected with NKA underwent a pre-injection assessment of the progress of their kidney disease (data on file, RegenMed (Cayman) Ltd.). This cohort of patients was consistent with Hemmelgarn Moderate Group decline in eGFR of 5-10ml/min/1.73m3 (Hemmelgarn et al, Kid Intern; 2006).

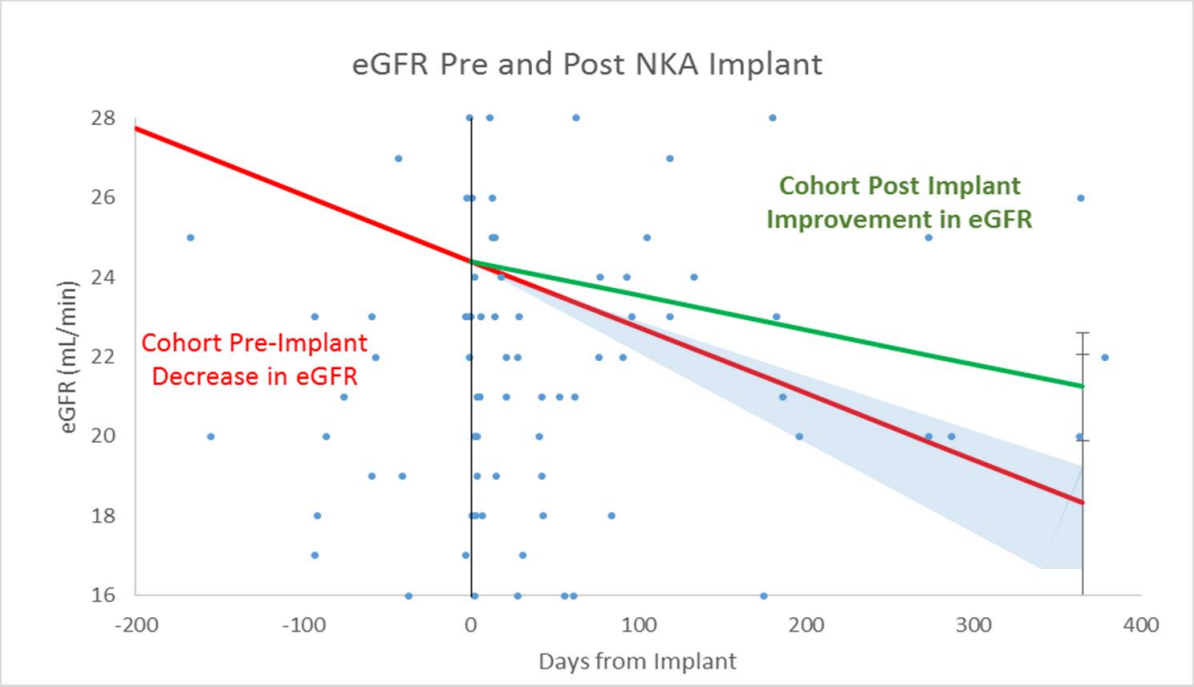
Figure 1: Change in Mean Estimated Glomerular Filtration Rate



This graph from Hemmelgarn 2006 shows the division of community-dwelling elderly patients in 5 groups based on the rate of change in mean eGFR.

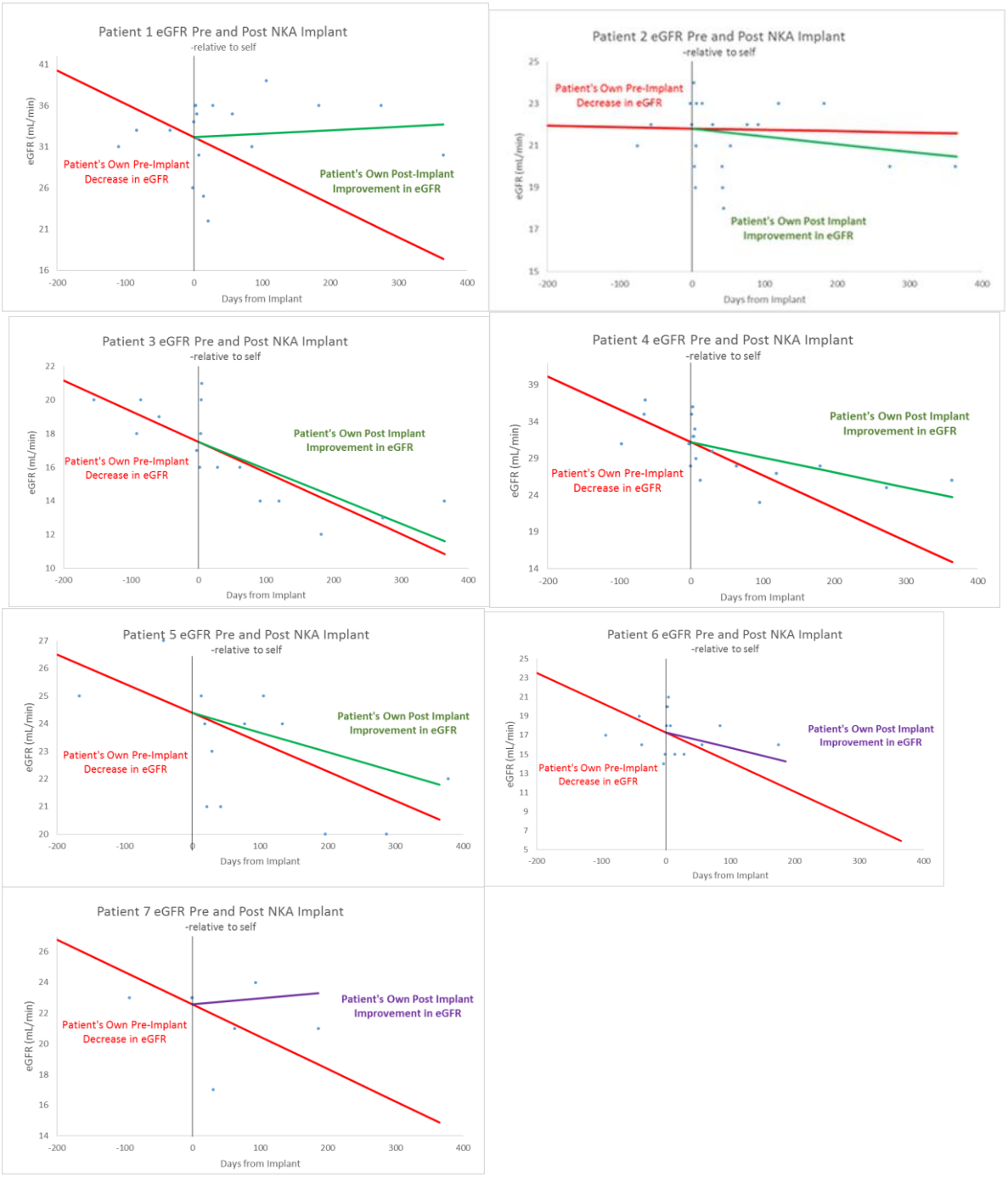
Pre-injection information from this patient cohort indicated that their average decline in eGFR was 6.1 ml/min/year (red-line in graph below). Post NKA administration, eGFR decline for the combined group of 7 patients (6 patients from the Swedish study and 1 patient from the American study) was -3.1 ml/min/year (green line in graph below). The average post-injection rate-of-decline for eGFR in the cohort is shown by the green line and the shaded blue area represents the range of eGFR for the Hemmelgarn group of community-dwelling elderly patients with moderately severe CKD 3b/4 with an annual decline of 5-10 ml/min/year (blue shaded area).

Figure 2: Estimated Glomerular Filtration Rate Pre- and Post-NKA Injected- Entire Cohort



The eGFR changes for individual patients' post-injection of NKA (green) along with the patient's individual pre-injection decline (red-line) demonstrated that 6 of 7 patients had a reduction in the rate of decline in eGFR over the period patients were on study:

Figure 3: Individual Changes in Estimated Glomerular Filtration Rate



The annual rate of eGFR decline pre and post injection for each patient is shown in the table below:

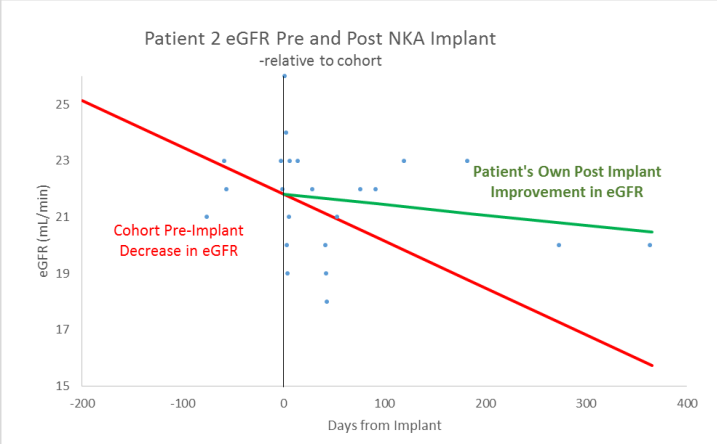
Table 3: Change in Estimated Glomerular Filtration Rate by Patient

	Change in eGFR (mL/min/year)	
Patient #	Pre-NKA	Post-NKA
Patient 1	-14.8	1.5
Patient 2	-0.2	-1.3
Patient 3	-6.7	-5.9
Patient 4	-16.3	-7.5
Patient 5	-3.9	-2.6
Patient 6	-11.4	-5.9
Patient 7	-7.7	1.4

In summary, 6 of the 7 patients had a reduction in the rate (slope) of eGFR decline after NKA injection. In Hemmelgarn's study of community-dwelling elderly patients, patients were divided into 5 groups with different rates of eGFR decline. A moderate group was identified with a decline of 5-10 ml/min/year. The pre-injection rate-of-decline in eGFR for the NKA cohort was 6.1 ml/min/year, consistent with Hemmelgarn's study of community-dwelling elderly patients.

Of interest, patient #2 continued at approximately the same rate of decline as observed during the pre-injection period. Over the short period of eGFR sampling prior to NKA injection in this patient, his eGFR measurements varied over a range of ±3 ml/min/1.73m3. When this patient's eGFR was compared to the overall cohort of 7 patients, changes in his eGFR followed a similar pattern for post-injection as others in the study cohort (see figure). Additionally, this patient's serum creatinine increase was attenuated suggesting a potential stabilization of progression for CKD (see section on sCr below).

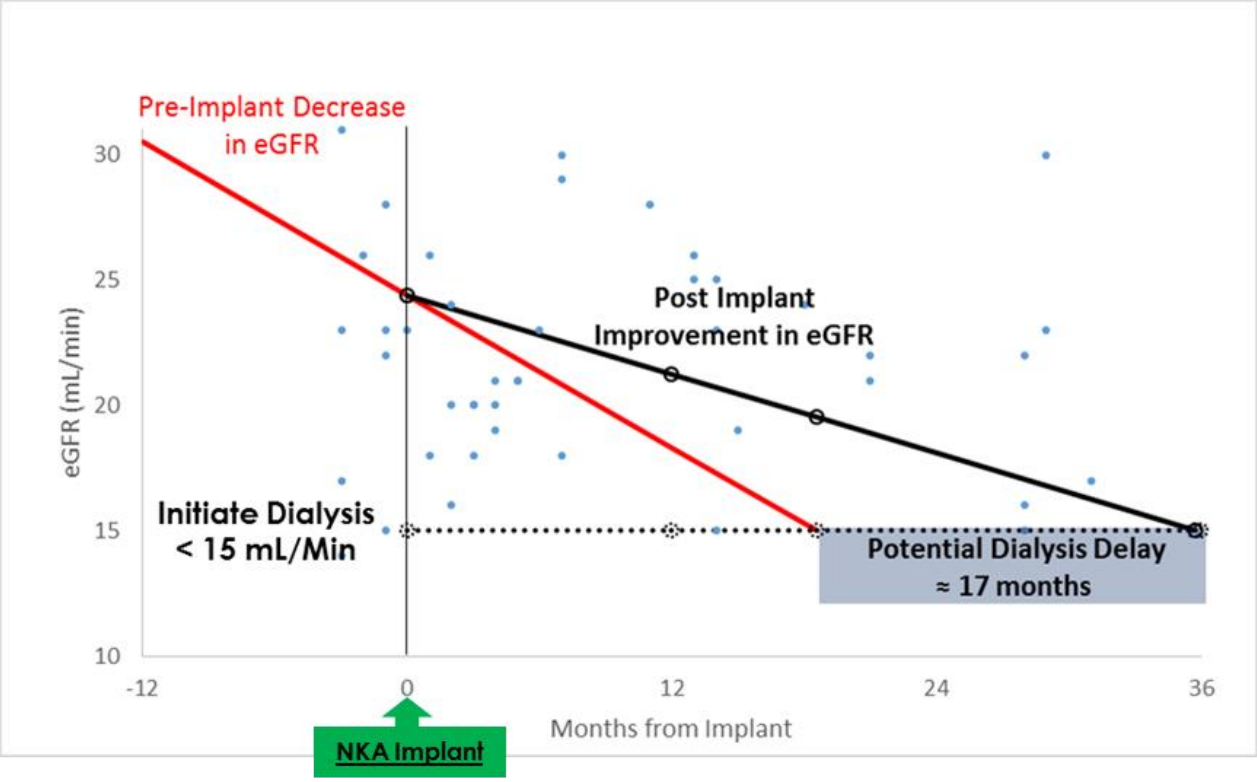
Figure 4: Estimated Glomerular Filtration Rate Pre- and Post-NKA Injected- Patient 2



The Phase I Clinical Trials with NKA were conducted using doses of NKA that were considered likely to be sub-therapeutic, to evaluate the effects of NKA when injected into a single kidney in a small cohort of elderly diabetic pre-dialysis patients with CKD 3b/4. In a diabetic animal model of aggressive chronic kidney disease, the optimal outcomes of delaying death from end-stage kidney disease were obtained when animals were treated in both kidneys. In the interest of safety, in the first clinical studies of NKA, only 1 kidney was injected with NKA, and therefore a

therapeutic signal was not necessarily expected. However, after monitoring the progress of CKD in this cohort of patients for ~ 1 year, the decline in renal function projected for this cohort was modified by a single injection of NKA into a single kidney (left). When the rates of decline of renal function pre- and post-injection are compared, the NKA injected patients have an imputed delay in dialysis of over 1.5 years (see graph below).

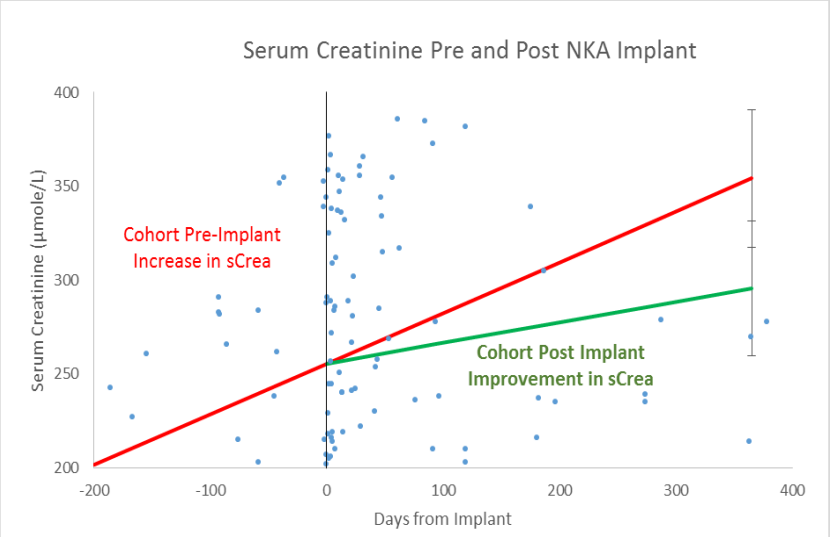
Figure 5: Rate of Decline of Renal Function Pre- and Post-NKA Injection



Serum Creatinine (sCr)

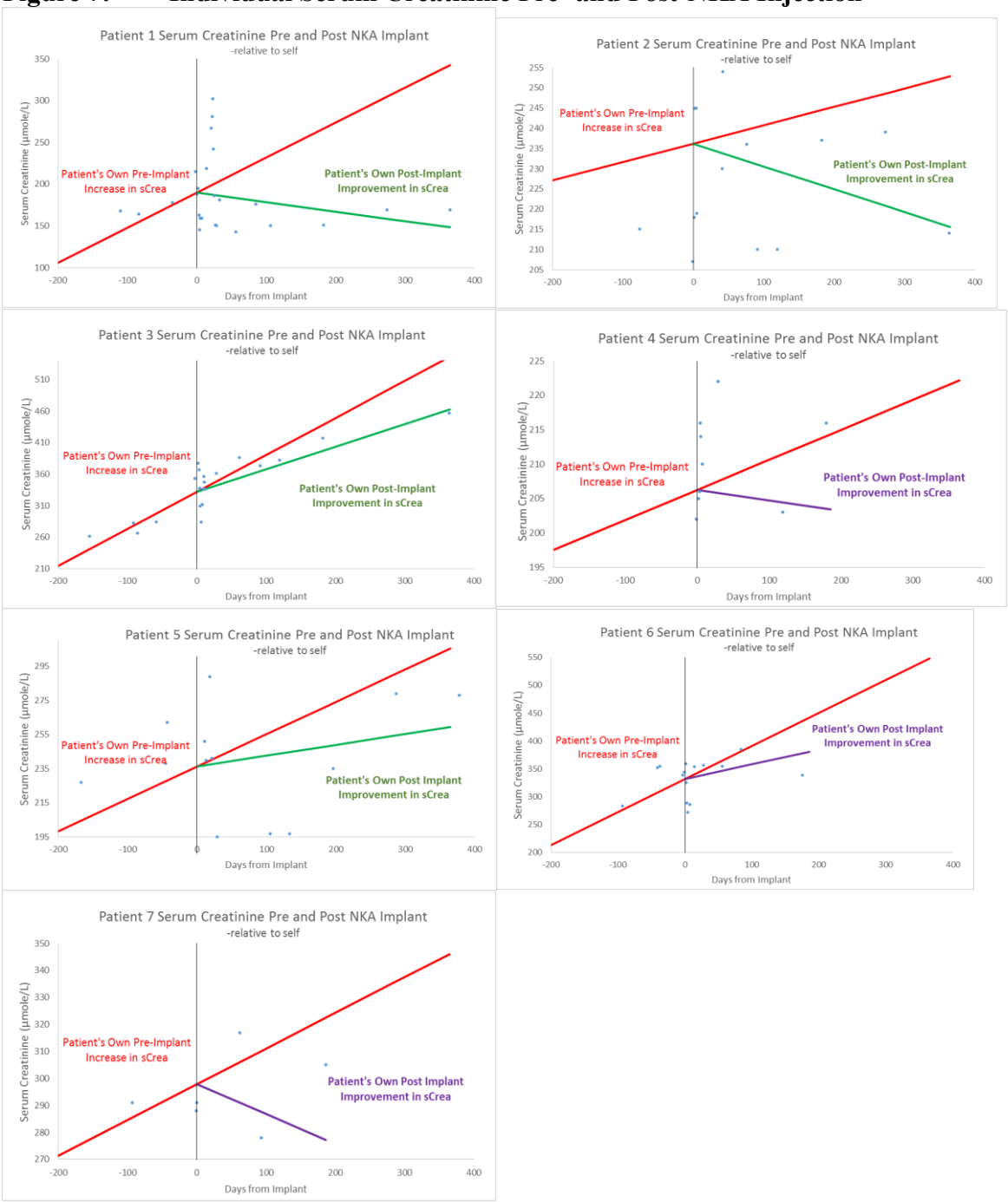
Serum creatinine levels for the cohort of patients pre-injection showed a general increase consistent with what would be expected in diabetic patients with moderate CKD (red-line). The overall pre-injection increase in sCr for the cohort of patients was >100 umole/L/yr. Post-injection the cohort of patients had an increase <50 umole/L/yr (green line). The overall trends in SCr before and after injection are shown below.

Figure 6: Serum Creatinine Pre- and Post-NKA Injection- Entire Cohort



Individual patient serum creatinine changes, post-injection of NKA (green) along with the patient's individual pre-injection decline:

Figure 7: Individual Serum Creatinine Pre- and Post-NKA Injection



The annual rates of increase of serum creatinine before and after NKA injection for each patient are shown in the table below:

Table 4: Change in Serum Creatinine by Patient

	Change in sCr (µmole/L/year)	
PT #	**Pre-NKA**	**Post-NKA**
Patient 1	153	-41
Patient 2	17	-21
Patient 3	214	200
Patient 4	16	-39
Patient 5	69	23
Patient 6	216	95
Patient 7	48	-40

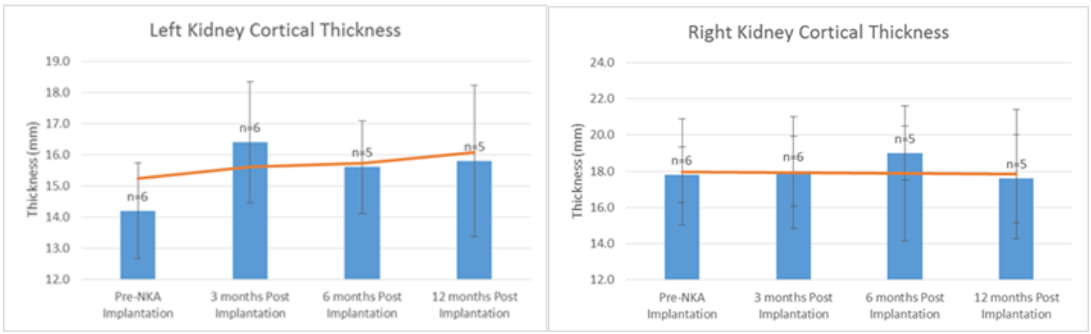
In summary, after NKA injection, all patients had a reduction in their individual rate of increase for sCr compared to the rate of sCr increase that had been observed in the pre-injection period. This change was consistent for each patient and supports the effects observed for eGFR in the after NKA injection period.

Kidney Cortical Thickness

Patients suffering from chronic kidney disease undergo a thinning of the functional portion of the kidney – the cortex. Renal cortical thickness is reduced in CKD as a result of the fibrosis and scarring associated with progression of the disease process.
This region of the kidney contains blood filtering (i.e. glomeruli), blood pressure control (i.e. juxtaglomerular apparatus for production of renin) and most of the metabolically active tubules (i.e. convoluted tubules) that transport electrolytes and other biomolecules scavenged out of the urine by the normal kidney.

An increase in cortical thickness was associated with kidney regeneration in preclinical studies of NKA and was confirmed histologically in all 4 animal species studied (see description of preclinical study results as summarized in Section 6.4). In the clinical studies, cortical thickness was evaluated using imaging technologies – no biopsies were taken to confirm the basis for the increased thickness. Cortical thickness was measured in both the right and left kidney to determine if the injected left kidney exhibited any change in cortical thickness that could be attributed to NKA injection. The right kidney served as a non-injected control.
On average, cortical thickness increased in the left kidney from 14 mm at time of injection to ~16mm after 1 year of injection (Figure 8; Line represents fitted trend). This change in cortical thickness was not sufficient to cause an increase in the total volume of the left kidney (data not presented). No change in cortical thickness was observed in the right kidney cortex (Figure 8).

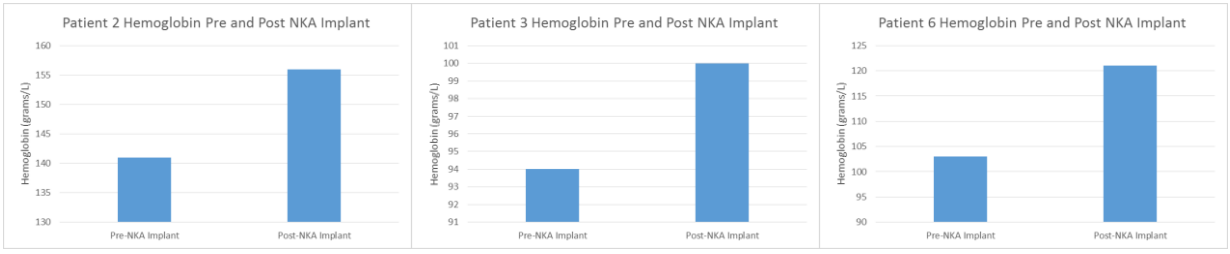
Figure 8: Kidney Cortical Thickness Over Time



Hemoglobin

Chronic Kidney disease can be associated with anemia linked to an alteration in renal erythropoietin production and from various metabolic abnormalities resulting from chronic uremia (Babbit and Lin, Mechanism of Anemia in CKD. J Amer Soc of Nephrology 23: 1631-1634, 2012/ http://jasn.asnjournals.org/content/23/10/1631.full). In the NKA patient trials 3 of the 7 patients showed improved hemoglobin levels after NKA injection and the remaining patients were shown to maintain normal levels during the study (Figure 9).

Figure 9: Hemoglobin Pre- and Post-NKA Injection- Patients 2, 3, and 6



Blood Pressure

Blood pressure was monitored during the course of the clinical study and patients received medication to control their blood pressure. Antihypertensive medication has been reduced in 3 of 6 Swedish patients during the first six months following the injection by NKA (Stenvinkel et al, World Congress of Nephrology, 2015; http://www.abstracts2view.com/wcn/view.php?nu=WCN15L_MON-336).

6.5Study Rationale

When initiating the NKA program, it was hypothesized that autologous injection of renal cells would affect repair/regeneration of renal tissue in diseased organs. Since that time, multiple animal studies conducted using a wide range of doses (3-15 million cells per gram of kidney tissue injected), and extended periods of time post-treatment (up to one year) have demonstrated the ability of NKA to positively affect renal outcomes in different models of renal insufficiency and disease. In GLP safety studies, no unanticipated in-life, hematological, urinological, serological, or histological changes were found in kidney or other tissues.

Thus, the positive results from the pharmacology and toxicology studies presented above support evaluation of NKA as a therapeutic agent designed to slow the rate of disease progression in patients with CKD.

6.5.1 Dose Selection

Nonclinical efficacy and safety data is summarized in Table 5. Based on this data, the NKA dose of $3x10^6$ cells per g KW^{est} will be delivered to patients in this clinical trial. This dose, equivalent to the low dose administered to ZSF1 rats in the GLP safety study, provides a minimum of a two-fold safety margin over doses delivered safely in safety pharmacology and toxicology studies (i.e., approximately two-fold lower than the identified NOAEL, which was the highest dose administered). In addition, this dose demonstrated efficacy in an animal disease model. Therefore, this dose provides a sufficient safety margin while having the potential to provide benefit to patients enrolled in the study.

Table 5: Dose Summary: Safety and Efficacy in Animal Studies

Summary of Key Nonclinical Efficacy and Safety Data				
Context	**SRC Dose (10_6 cells per g KW_{est})**	**Dose Volume (mL per g KW_{est})**	**Ratio to Human Dose Regimen [SRC dose (vol)]**	**Study No.** *(ref. Table 2)*
Efficacy (SRC)				
Lowest effective doses tested in 5/6th Nx rats	5-10	0.1	1.67 – 3.33 (3.33)	Study 1
Lowest effective dose tested in ZSF1 rats	3	0.125	1 (4.167)	Study 3
Lowest effective dose tested in dog (70% Nx)	5.2	0.087	1.73 (2.9)	Study 2
Safety (NKA)				
Cardiovascular acute reactions in 5/6th Nx rat	5-15	0.1	1.67 – 5 (3.33)	Study 4
Safe dose /volume load in dog safety pharmacology	12.5	0.08	4.167 (2.67)	Study 5
NOAEL in ZSF1 GLP toxicology study	6.25*	0.125	2.1 (4.167)	Study 7
NOAEL in single dose dog GLP toxicology study	11.0*	0.1	3.67 (3.33)	Study 8
NOAEL in repeat dose dog GLP toxicology study	5.5*	0.05	1.83 (1.67)	Study 9
Reference: Human dose in this study	***3.0***	***0.03***	***NA***	

**Maximum dose administered*

6.5.2 Surgical Techniques

The biopsy will be collected using standard practices at each site. A minimum of 2 tissue cores must be collected in order to provide sufficient material for manufacture of NKA.

For injection, two methods are approved for use in this study. Each investigator team will select the method to be used based on the experience of the personnel at the site.

- Use of laparoscopic surgical techniques allows for direct visualization of the kidney so that any bleeding or other adverse events can be spotted during injection and addressed immediately.
- Use of a percutaneous approach to the kidney has been in use for over a decade, primarily for ablating intrarenal masses. These procedures insert an electrode or cryogenic needle into a defined mass in the kidney, and remain in contact for (typically) 10 to 20 minutes while the lesion is ablated. For injection of NKA, the percutaneous instrumentation is no larger nor more complex, and this approach offers the safety advantages of no surgery (avoiding abdominal puncture wounds and inflation with gas) and minimal immobilization time. Furthermore, the access track can have hemostatic biodegradable material left in place, to further reduce any chance of significant bleeding.

6.5.3 Selection of Kidney for Biopsy/Injection

Laparoscopic surgical techniques can be used to access either the right or left kidney. However, the left kidney is more readily accessible and is most often accessed during nephrectomies, hence the first surgical team had more experience with accessing the left kidney, and the initial patients enrolled underwent left kidney biopsy and left kidney NKA injection. Patients previously enrolled may have a second NKA injection in the left kidney. Newly enrolled patients may have either the left or right kidney biopsied. In any case, the NKA injection should be made into the kidney that was biopsied.

6.6 Benefit: Risk Assessment

6.6.1 Assessment of Potential Benefit

NKA treatment provided a substantial clinical benefit to nephrectomized animals (rats and dogs) in nonclinical studies and in a ZSF1 rat disease model of type II diabetes. The dose selected for this study is equivalent to the lowest effective dose assessed in animal studies (see Table 5). Therefore, the potential exists for patients in this study to realize some clinical benefit from participation; for example, a potential reduction in the rate of progression of CKD.

6.6.2 Assessment of Potential Risks

NKA Product: RegenMed (Cayman) Ltd.'s platform utilizes the injection of the patient's own cells; autologous renal cells obtained from the patient by a kidney biopsy procedure. As a result, the risk of generating an immunological response (e.g., graft rejection) is similar to that seen for autologous stem cell transplants and is extremely unlikely. Furthermore, a dose-limiting toxicity was not identified in animal studies. Specifically, no unanticipated findings were observed in multiple animal studies, species, and disease conditions with administration or re-administration of syngeneic or autologous SRC to diseased or normal kidneys.

Risks associated with escape of NKA to areas outside of the kidney cortex have also been considered; specifically, 1) the sub-capsular space, 2) systemic circulation, and 3) the urinary tract. Leakage of SRC into the sub-capsular space is not expected to pose a risk to subject. The sub-capsular space is commonly used for injecting endocrine tissue, most commonly islet tissue (Medarova et al., 2009). The renal capsule also provides a niche for harboring native stem cells that are capable of migrating into the renal parenchyma (Park et al., 2009).

The possibility that SRC may enter systemic circulation is reduced by the targeted injection process. Potential risk of an injected cell circulating systemically has been evaluated with heterologous allogeneic stem cells (i.e., mesenchymal stem cells) administered intravenously, and has been shown to present no significant risk to patients (Hare et al., 2009). Importantly, direct injection to the target organ, the kidney, reduces the risk of systemically circulating cells and provides a natural route of elimination through the urinary tract.

The Porcine Skin Type B gelatin used in the formulation of NKA meets Pharmaceutical and Edible Gelatin Monograph (European Pharmacopeia 7.0, US Pharmacopeia-National Formulary USP35 NF30) requirements. Gelatin is widely used in pharmaceutical and medical applications, including cellular transplantation for regenerative products, because of its biocompatible nature. The extensive use of Gelatin described in the literature, coupled with the comprehensive evaluation of NKA containing porcine gelatin in GLP toxicology studies support its use in this context and would not be expected to result in an adverse effect in patients. Additional information on the porcine gelatin used in the formulation of NKA can be found in the IB.

NKA Injection: A laparoscopic or percutaneous technique will be used to access the kidney for injection of NKA. Laparoscopic techniques are routinely used to access the kidneys and adverse events resulting from such procedures have been published. Patients will be closely monitored for these events as discussed in Section 8.2.2.

Cain et al. (Cain et al., 1976) reported that renal cell homogenates injected intra-renally into rodent kidneys using a needle produced no significant adverse events. Furthermore, morphological effects of NKA injection of the kidney are consistent with those reported for repeat kidney biopsies taken from canines - a mature connective tissue track and no functional deficits associated with these minimal structural changes (Groman et al., 2004). In isolated cases, increasing intracapsular kidney water volume in canines resulted in elevated intra-kidney pressure and were associated with transient increases in kidney weight and systemic blood pressure (Montgomery et al., 1950; Swann et al., 1952); however, in our pilot canine study where blood pressure was monitored, no changes were observed following volume escalation of up to 6 mL per kidney of NKA.

Therefore, it is anticipated that the most significant risk associated with injection is from the surgical procedure; safety measures will be implemented throughout surgery and post-surgical follow-up to reduce the potential for excessive bleeding and other surgical adverse effects. This version of the protocol introduces an alternative procedure for injection of NKA: a percutaneous approach, rather than the laparoscopic procedure as used to date (see next section). The percutaneous procedure has been used for over a decade in ablation of renal masses, and it provides for a similar injection without requiring abdominal wounds for instrument ports, or abdominal inflation. As the immobilization and recovery periods are generally substantially less, some investigators may find this method could be a safer option for some of their patients. A concise review of this method can be found in Salagierski & Salagierski (2010).

6.6.3 Swedish Clinical Experience

The first-in-human clinical trial with NKA was conducted in Sweden. The patients enrolled in that study (TNG-010) were biopsied and injected in 2013. As of the date of this protocol's preparation (August 2015), all patients are doing well.

Nine SAE's were been reported in the trial in Sweden. A listing of the events is presented in Table 6; all events have resolved. Of note, only two events were considered "possibly related" to NKA by the Investigator. Both were of infectious origins (wound infection and pneumonia) and both resolved without sequelae. As the two events occurred in the first two patients in the same time frame, assessing the events as possibly related to NKA was considered the most conservative approach.

Table 6: Serious Adverse Events Reported in TNG-010

Patient Number	Event Term	Event Intensity	Outcome of Event	Relationship to NKA
001-002	Fatigue	Mild	Recovered/Resolved	Not Related
001-001	Fatigue	Mild	Recovered/Resolved	Not Related
001-001	Postoperative wound infection	Mild	Recovered/Resolved	Possible
001-002	Pneumonia	Moderate	Recovered/Resolved	Possible
001-003	Urinary tract infection	Mild	Recovered/Resolved	Not Related
001-004	Fatigue	Mild	Recovered/Resolved	Not Related
001-005	Volvulus	Moderate	Recovered/Resolved	Not Related
001-003	Fluid retention	Moderate	Recovered/Resolved	Not Related
001-005	Anastomotic hemorrhage	Moderate	Recovered/Resolved	Not Related

7 STUDY OBJECTIVES

7.1 Primary Objectives and Outcome Measures

The **primary objective** of the study is to assess the safety and efficacy of NKA injected in one recipient kidney and determine if two injections of NKA provide stabilization of renal function.

- Primary Safety Outcome Measures: procedure and/or product related adverse events (AE's) through 12 months following the initial NKA injection.
- Primary Efficacy Outcome Measures: serial measurement of serum creatinine and estimation of GFR through 6 months following the second cell injection

The **secondary objective** of the study is to assess the safety and tolerability of NKA administration by assessing renal-specific adverse events over a 12 month period following a patient's first NKA injection.

- Secondary Safety and Tolerability Outcome Measures: renal-specific laboratory assessments through 12 months following the last NKA injection under this protocol, whether first or second.

7.2 Exploratory Objectives

The exploratory objectives of the study are designed to assess the impact of NKA on renal function over a 12 month period following the initial NKA injection.

- Exploratory Outcome Measures: clinical diagnostic and laboratory assessments of renal structure and function (including eGFR, serum creatinine, and proteinuria) to assess changes in the rate of progression of renal disease; and effect of method of injection on these parameters.

Exploratory quality of life outcome measure will be the Kidney Disease Quality of Life survey obtained at baseline and at 1, 3, 6, 7, 9, 12, 15, 18, 30, and 42 months after a patient's first NKA injection.

Additional exploratory endpoints include the following:

1. Changes from baseline in BUN, β2 microglobulin and intact parathyroid hormone (iPTH) at 6 and 12 months post-injection.
2. Changes from baseline in C-reactive protein (CRP) at 6 and 12 months post-injection.
3. Need for initiation of renal replacement therapy.
4. Comparison of pre-injection versus post-injection split functional differences between the left and right kidney based on renal scintigraphy.
5. Comparison of pre-injection and post-injection kidney volume estimates based on comparative MRI assessments.
6. Changes in the patient's perception of quality of life, as measured by serial KDQoL surveys.

8 INVESTIGATIONAL PRODUCT

8.1 Overview

NKA is an injectable product composed of SRC formulated in a Biomaterial (gelatin-based hydrogel). A detailed description of SRC and NKA and the manufacturing process is provided in the IB. A brief description of the process is described below, divided into three steps:

Biopsy: Renal cortical tissue obtained via kidney biopsy is sent to Twin City Bio LLC, Winston Salem, North Carolina, for manufacturing.

Selection of SRC: Renal cells are isolated from the kidney tissue by enzymatic digestion and expanded using standard cell culture techniques. Cell culture medium is designed to expand primary renal cells and does not contain any differentiation factors. Harvested renal cells are subjected to density gradient separation to obtain SRC which are composed a selected population of renal cells, cells well known for their regenerative potential (Humphreys et al., 2008).

Formulation: Gelatin-based hydrogel biomaterial is used to formulate SRC into NKA. Porcine gelatin is dissolved in buffer to form the thermally responsive hydrogel. This hydrogel is fluid at room temperature but gels when cooled to refrigerated temperature (2-8ºC). SRC is formulated

in this gelatin-based hydrogel biomaterial to improve both cellular stability during transport and injection into the kidney cortex.

8.2 Procurement and Manufacture of NKA

NKA will be manufactured in a GMP facility at Twin City Bio LLC in Winston-Salem, NC.

8.2.1 Biopsy: Procurement of Renal Tissue

Those patients enrolling in this clinical trial after participation in the previous single-dose clinical trial will be injected with SRC cells obtained from their initial biopsies. Biopsy samples were obtained for these patients in a Phase 1 trial and maintained in a frozen state. These autologous samples will be used to generate the second dose of NKA that the patients are to receive under this protocol. If there are not enough SRC cells obtained in the remaining sample, patients will be allowed to undergo another biopsy to prepare their second NKA injection dose. Patients who are enrolling in a clinical trial of NKA for the first time will have SRC cells prepared from kidney biopsy tissue obtained according to the usual standard of care procedures.

8.2.2 Product for Injection

Once the biopsy material is received at Twin City Bio LLC, the NKA product will be labeled and strict documentation measures will insure product traceability is maintained. The site will notify Twin City Bio LLC of the date for injection and, following the pre-injection visit, of continuing patient eligibility so that manufacturing can adjust the in-process freezing step to accommodate the date of surgery. If the patient still qualifies, cells will be thawed, NKA manufactured and subsequently shipped. If the patient does not qualify, the PI should notify Twin City Bio LLC staff and renal cells will not be thawed for shipping. In this case, the PI, Twin City Bio LLC, and RegenMed (Cayman) Ltd. staff will discuss available options including, for example, thawing of cells at a later date.

Product will be packaged in sterile packaging in a 10 mL syringe for shipment and shipped to the address provided by the site. It is the responsibility of the site to ensure NKA shipments can be delivered directly to site personnel so that product injection is not delayed. NKA in the syringe must be warmed to 26.5 ± 1.5ºC before injection, in order to liquefy the gelatin-based hydrogel component of NKA. Instructions for warming the product will be provided in the Study Reference Manual. Detailed injection procedures can be found in Section 8.2.2 .

Renal cells/SRC that were frozen but not used to manufacture NKA will be stored at Twin City Bio LLC's GMP facility until the End-of-Study Visit for the patient. At that time, if the cells have not been used and it is determined that they are no longer needed for that patient, then they will be designated for RegenMed (Cayman) Ltd. use and will be used for growth optimization, stability assessments, and other process improvement studies. Some process improvement studies may include assessment of biomarker panels to assist in further characterization of specific cellular phenotypes present in each NKA sample. Renal cells/SRC will be kept for a maximum of 5 years. During the informed consent process, the patient will be alerted to the planned future use of his/her cells. If the patient does not want his/her cells to be used for process improvement research, then during the informed consent process, he/she may request that the cells be destroyed after he/she has completed the study.

8.3NKA Dose

In nonclinical studies, harvested kidneys were weighed directly; dose was calculated based on the average weight of a dog or rat kidney. Using the results from nonclinical studies, each patient will receive a dose of 3.0 x 10^6 SRC/g KW^{est}. For patients in the study, the dose of NKA will be based on kidney volume calculated using MRI. As described in the literature, volume measurements of the kidney in mLs obtained by different methods are approximately 92 – 97% of dry weight measurements in grams obtained by measuring isolated organs trimmed of perirenal fat. Therefore, as a conservative estimation, doses of NKA will be calculated using a conversion of 1 g equals 1 mL. Using this ratio represents the safest approach as it guarantees patients will not receive doses higher than corresponding doses in animal studies. Dosing volumes for a range of kidney weights are shown in Table 7 below. The maximum volume for any patient will be 8.0 mL; that is, if any subject has a left kidney with a calculated weight ≥ 259 g, then that subject will receive 8 mL of NKA.

Table 7: NKA Dosing Paradigm

Estimated Kidney Weight (g KW^{est})		Dose Volume (mL)	SRC Delivered (cell number; x 10_6)
Median Weight (g)	Weight Range (g)		
100	95 – 108	3	300
117	109 – 125	3.5	350
133	126 – 141	4	400
150	142 – 158	4.5	450
167	159 – 175	5	500
183	176 – 191	5.5	550
200	192 – 208	6	600
217	209 - 225	6.5	650
233	226 – 241	7	700
250	242 – 258	7.5	750
	>259	8	800

8.4Accountability

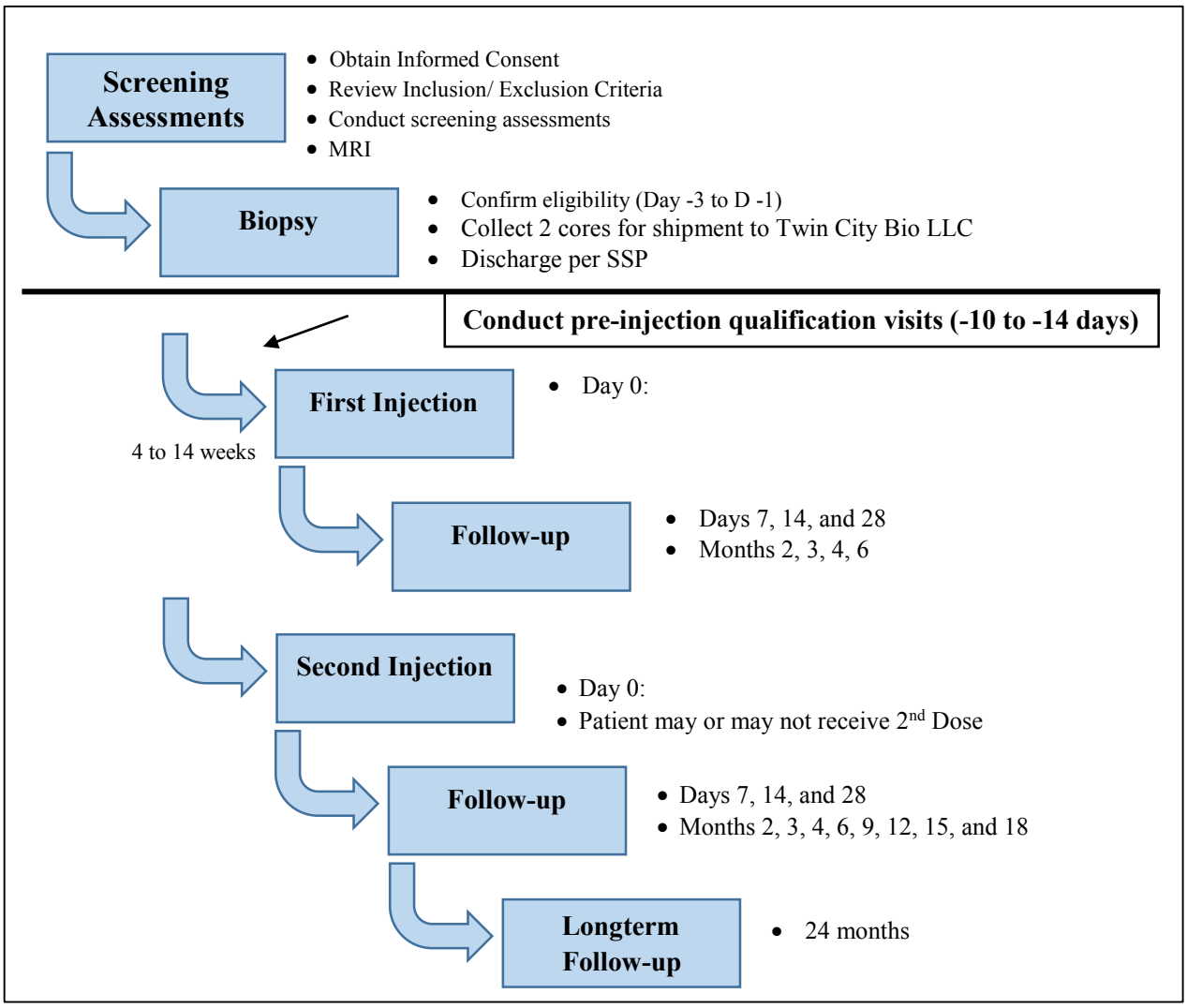
Investigational product will be sent prepackaged in a sterile syringe. The site should verify the subject information matches the subject to be injected. If required by site practices, the site pharmacy may add a site-specific label prior to transport to the operating room. The site should also verify the amount of NKA to be injected based on the kidney weight determined by MRI imaging at screening. During injection, the amount of NKA delivered should be documented in source documents including estimates of the amount of product that leaked out of the kidney following injection, if any.

Following injection, any excess NKA remaining in the syringe will be disposed of in the surgical suite immediately following surgery according to site standard practices.

9 STUDY DESIGN

An overview of the study flow is shown in the diagram below.

Figure 10: Study Design



After patients have signed the ICF, they should be screened for entry into the study. Screening assessments include laboratory assessments, physical examination, and an ECG and MRI study, all of which must be performed before the biopsy is taken. If the patient meets all I/E criteria, a biopsy of the left/right kidney should be taken within 45 days of the first screening assessment. During the biopsy procedure, two tissue cores should be collected and sent to RegenMed (Cayman) Ltd. for manufacture of NKA. If the patient experienced significant AE's/SAE's following biopsy (e.g., excessive bleeding, development of AV fistula) that, in the opinion of the PI would preclude safe injection, then the patient should be discontinued from the study. Biopsy samples that were obtained from previously enrolled patients in a Phase 1 trial and maintained in a frozen state will be used to generate the second dose of NKA that the patients are to receive

under this protocol. If there are not enough cells after thawing then these patients will be allowed to have the biopsy procedure completed for this study.

One week after receipt at Twin City Bio LLC's GMP facility in North Carolina, USA, RegenMed (Cayman) Ltd. will notify the site if the tissue received was of sufficient size and quality for manufacture of NKA. If results are positive, the site should confirm the scheduled date of injection. If the biopsy specimen is not able to be used for manufacture of NKA (for whatever reason), the patient should be discontinued from the study.

Ten to 14 days before the scheduled injection, the patient should report to the clinic for a pre-injection qualification visit including final review of I/E criteria and a renal scintigraphy study. If the patient is still eligible for injection, the site will notify Twin City Bio LLC to manufacture NKA product from frozen renal cells. On the day of injection (Day 0), the patient should be admitted to the hospital and receive an NKA injection into the left (i.e., biopsied) kidney. For injection, the kidney will be accessed either (a) laparoscopic approach, or (b) using a percutaneous approach. The patient will remain in the hospital for two to four nights following the laparoscopic procedure, or 0-1 night following the percutaneous procedure, for observation and safety assessments. The patient should not be discharged until any procedure- and/or product-related AE's which may have occurred have stabilized or resolved.

Post-injection Follow-up: Subjects will return to the clinic for follow-up safety assessments on Days 7, 14, and 28 post-injection and at 3, 6, 9, and 12 months post-injection. At 6 months post-injection, post-treatment MRI and renal scintigraphy studies will be conducted. After patients complete the 6 month efficacy visit, they will be considered for a second NKA injection. Patients receiving a second dose will follow the same follow up visits that occurred after first dose. Patients 6 month post first injection visit will serve as the patients 14 to 10 day pre- second injection visit. Patients will return for their second injection and return to the clinic for follow up safety assessments on Days 7, 14, and 28 post- second injection and at 2, 3, and 6 months post-second injection. Patients will be followed up to 18 months in the post follow up phase with 6 months after first NKA injection and 12 months after second NKA injection. Refer to the time and events schedule on page 14 for additional post follow up time points. Subjects will complete an End-of-Study visit when they discontinue from the study or (for those who complete the study) at 18 months post-second injection.

10 STUDY POPULATION

10.1 Eligibility Criteria

Eligibility criteria should be assessed at Screening. In addition, unless otherwise noted for the specific criterion, eligibility criteria should be also be reviewed before the biopsy and at the Day -10 to -14 pre-injection visit.

Up to 30 subjects will be enrolled in this study, including subjects previously injected in either Phase 1 trial. Note that a subject will be considered enrolled into the study at the time of injection.

10.1.1 Inclusion criteria

Unless otherwise noted, inclusion criteria must be met at Screening, Biopsy, and prior to injection.

1. Male and female subjects, age 30 to 70 years on the date of informed consent.
2. Patients with type 2 diabetes mellitus (T2DM).
3. Patients with a well-established diagnosis of diabetic nephropathy as the underlying cause of their renal disease.
4. At screening, patients not previously injected with NKA with CKD defined as a GFR of 20 – 50 mL/min/1.73m^2 inclusive. Patients previously treated with a single NKA injection with eGFR 15 to 60 mL/min may also enroll in this clinical trial.
5. Microalbuminuria that cannot be explained by an alternative diagnosis. Microalbuminuria is defined as urinary albumin-creatinine ratio (UACR) ≥ 30 mg/g or urine albumin excretion ≥ 30 mg/day on 24 hour urine collection.
6. Prior to biopsy, systolic blood pressure between 105 and 140 mmHg (inclusive) and diastolic blood pressure ≤90 mmHg.
7. Ongoing and stable treatment with ACEI or ARB initiated at least 8 weeks prior to enrollment. Treatment must be stable for the 6 weeks immediately prior to injection. Stable treatment is defined as dose adjustment to no less than ½ of the current dosage and no more than 2X the current dosage over the 6 week period immediately prior to injection; dose interruptions of up to 7 days due to medical necessity are allowed. Patients who are intolerant to ACEI or ARBs may be included as long as they have stable BP within the acceptable limits.
8. Minimum of 2 measurements of eGFR or sCr taken at least 3 months apart (prior to screening) and within the previous 12 months to define the rate of progression of CKD. The patient should have sufficient historical data to provide a reasonable estimate of the rate of progression of CKD as determined following consultation with the Medical Monitor (to insure sufficient data is available). In addition, the rate of progression of CKD must be consistent over time. There is no defined rate of progression that is required to qualify for inclusion.
9. Willing and able to refrain from use of NSAIDs (including aspirin) and clopidogrel, prasugrel, or other platelet inhibitors peri-procedure (i.e., before and after both the biopsy and injection). The wash-out period before and after each procedure should be 7 days. Willing and able to refrain from use of fish oil and dipryridamole for 7 days before and 7 days after each procedure.
10. Willing and able to cooperate with all aspects of the study.

11. Willing and able to give signed informed consent.

10.1.2 Exclusion criteria

Patients may not be enrolled if they meet any of the exclusion criteria listed below. Criteria should be assessed at Screening, before the biopsy, and before injection unless noted otherwise.

1. Type 1 diabetes mellitus (DM).
2. History of a renal transplant.
3. HbA1c > 10% at Screening. Patients with HbA1c > 8% at the time of screening should be offered diabetic teaching and advised to consult their primary physicians for further diabetic management.
4. Hemoglobin levels < 9 g/dL prior to injection. Hemoglobin levels should be measured within 48 hours before the procedure or per site standard practice.
5. Known allergy to kanamycin or structurally similar aminoglycoside antibiotics (as kanamycin is used during manufacture of NKA).
6. Abnormal coagulation status as measured by APTT, INR, and/or platelet count at Screening.
7. Not a good candidate for the injection procedure (based on the assessment of the surgeon who will be performing the injection) including patients who are morbidly obese, have excessive fat surrounding the kidney, have BMI > 45, or who are otherwise at excessive risk for serious complications.
8. Clinically significant infection requiring parenteral antibiotics within 6 weeks of injection.
9. Patients with small kidneys (average size < 9 cm) or only one kidney, as assessed by MRI or renal US at screening or if previously done within 1 year of screening.
10. Patients with a rapid decline in renal function over the last 3 months prior to injection or acute kidney injury.
11. Patients with any of the following conditions prior to injection: renal tumors, polycystic kidney disease, renal cysts or other anatomic abnormalities that would interfere with injection procedure (e.g., cysts in the pathway of the injection for injection), hydronephrosis, skin infection over proposed injection sites, or evidence of a urinary tract infection.
12. Female subjects who are pregnant, lactating (breast feeding) or planning a pregnancy during the course of the study, or who are of child bearing potential and not using a highly effective method of birth control (including sexual abstinence). A highly effective method of birth control is defined as one that results in a low failure rate (i.e. less than 1 percent per year) when used consistently and correctly, such as injections, injectables, combined oral contraceptives, some intrauterine devices (IUDs), sexual abstinence, or a vasectomized partner. Subjects must be willing to continue birth control methods throughout the course of the study.
13. History of cancer within the past 3 years (excluding non-melanoma skin cancer and carcinoma in situ of the cervix).
14. Life expectancy of less than 2 years.
15. Any contraindication or known anaphylactic or severe systemic reaction to either human blood products or materials of animal (bovine, porcine) origin or anesthetic agents.
16. Positive for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV) assessed at the Screening Visit.
17. Subjects with active tuberculosis (TB) requiring treatment in the past 3 years.

18. Immunocompromised subjects or patients receiving immunosuppressive agents (including patients treated for chronic glomerulonephritis) within 3 months of injection. [Note: inhaled corticosteroids and chronic low-dose corticosteroids [≤ 7.5mg per day] are permitted as are brief pulsed corticosteroids for intermittent symptoms (e.g. asthma).]
19. Subjects with uncontrolled diabetes (defined as metabolically unstable by the PI), or with incapacitating cardiac and/or pulmonary disorders.
20. History of active alcohol and/or drug abuse that in the investigator's assessment would impair the subject's ability to comply with the protocol.
21. Patients with clinically significant hepatic disease (ALT or AST > 3.0 x ULN) at Screening.
22. Patients with bleeding disorders that would, in the opinion of the Investigator, interfere with the performance of study procedures; patients taking coumarins (e.g. Warfarin) or other anticoagulants (e.g. enoxaparin or direct thrombin inhibitors).
23. Any circumstance in which the investigator deems participation in the study is not in the subject's best interest.
24. Use of any investigational product within 3 months of the injection without receiving prior written consent of the Medical Monitor.

10.2 Prohibited and Concomitant Medications

Use of investigational drugs is prohibited during the course of the study unless preapproved for use by the Medical Monitor. Investigational drugs are defined as drugs which have not been approved for any use by the FDA.
Patients should avoid taking drugs with nephrotoxic potential during the course of the study, including for example chronic use of ibuprofen and other NSAIDs. Aspirin, up to a dose of 100 mg/day, may be taken for cardiovascular prophylaxis. NSAIDs may be taken PRN but the site should emphasize with patients the need to minimize use of NSAIDs during the course of the study.

Patients who are not intolerant of such agents must be on an ACEI or ARB for 8 weeks prior to enrollment (i.e., at the time of injection) and a stable regimen for 6 weeks prior to injection. Requiring a minimum of 8 weeks treatment prior to enrollment ensures that the patient will be on an ACEI or ARB for a minimum of 12 weeks before administration of the Investigational Product. Stable regimen is defined as a dose no less than ½ of the current dosage and no more than 2X the current dosage over the course of the 6 weeks immediately prior to injection. The ACEI or ARB dose should be the maximum tolerated dose for that patient. In addition, except where medically necessary, no changes should be made to ACEI or ARB dosing regimen from baseline through the 12 month EOS visit. A dose interruption of up to 7 days for medical necessity is allowed. Patients who are intolerant to ACEI or ARB therapy and, therefore, not taking an ACEI or ARB may be enrolled as long as their blood pressure is stable and within the acceptable range.

Medications which specifically interfere with measurements of sCr should be avoided including trimethoprim, dronedarone, and cimetidine. If such medications are required based on medical necessity, then the circumstance should be discussed with the medical monitor and the medication noted on the CRF.

10.3 Subject Withdrawal and Replacement

Subjects may withdraw from the study at any time and for any reason. If the PI determines that continuing in the study is no longer in the best interest of the subject, then the PI should withdraw the subject from the study. If a subject withdraws from the study, the reason for withdrawal should be documented.

If a subject withdraws from the study before having a biopsy, the subject will be considered a screen failure. If a subject withdraws from the study after having the biopsy, but before injection of NKA, the subject will not be a screen failure, but will not be considered enrolled, and may be replaced. If a subject withdraws from the study after injection but before the end of the follow-up period, the subject cannot be replaced. A maximum of 30 subjects, including subjects previously injected in the two previous Phase 1 trials, will be injected with NKA.
Every effort should be made to ensure that patients who have been injected with NKA return for all subsequent follow-up visits and procedures.

11 STUDY VISITS

Subjects will be screened, enrolled, injected and followed according to the Time and Events Schedule and the Time and Events Schedule: Laboratory Assessments.

Before any study specific assessments are performed, the patient must agree to participate in the study by signing the ICF. Informed consent must be obtained following ICH GCP guidelines and 21 CFR Part 50.

11.1 Screening Assessments (Within 45 Days of the Biopsy)

All screening assessments should be scheduled in a timeframe that will allow for scheduling of the biopsy within 45 days of the first screening assessment conducted specifically for this protocol. For example, if a patient signs the consent and then goes to the laboratory for his/her blood draw two days later, then the date of the blood draw would be considered the date of the first screening assessment (not the date the ICF was signed). An MRI study should be conducted as part of the screening assessments. If the patient changes his/her mind after signing the ICF and refuses to have the MRI, then the patient must be discontinued from the study as the MRI will be used to define the dose of NKA.

To qualify for participation in the study, a patient's GFR must be between 20 – 50 mL/min/1.73m^2 inclusive. To define the GFR for entry criteria, the site should use the eGFR assessed during screening procedures and calculated using sCr and the CKD-EPI equation. If a patient does not meet a specific eligibility criterion, but the Investigator believes that the patient would be an excellent candidate for the trial, then that criterion may be reassessed one time. In general, a patient who does not qualify may be rescreened once as long as the Investigator has sufficient clinical justification for rescreening the patient. If the Investigator has any questions concerning appropriateness of rescreening a patient, then he/she should contact the Medical Monitor.

If, for whatever reason, the biopsy cannot be conducted within 45 days of the screening assessments, then the PI and Medical Monitor should discuss and agree upon the need for repeating screening assessments on a case by case basis. In some cases (e.g., if the biopsy will be a few days out of the protocol specified window), the laboratory assessments performed between 1 and 3 days before the biopsy may be used in place of the screening assessments to satisfy final eligibility criteria. In some cases, it may not be appropriate or necessary to repeat the clinical diagnostic procedures including the ECG or MRI studies.

11.2 Biopsy (4 to 14 Weeks before Injection)

The biopsy should be scheduled within 45 days following the first screening assessment. The biopsy should be scheduled so that the injection procedure can be scheduled at 4 weeks or later. Due to shipping and manufacturing schedules, the preferred days for collecting the biopsy are Wednesday or Thursday. Biopsies should not be collected on a Friday.

Patients should report to the site one to three days before the biopsy for pre-biopsy assessments. As much as possible, pre-biopsy laboratory samples may be collected and assessed to verify patient eligibility prior to biopsy.

Following final review of appropriate eligibility criteria, the biopsy should be performed as described below in Section 12.5.1. The biopsy should be sent to RegenMed (Cayman) Ltd. in the shipping container provided by RegenMed (Cayman) Ltd. according to procedures detailed in the Study Reference Manual. After the biopsy, the patient should remain in the hospital overnight for observation. As long as any biopsy-related AEs have resolved, stabilized, or returned to baseline, the patient may be discharged the same day as biopsy per site standard practice.

Once the biopsy is received at RegenMed (Cayman) Ltd., the biopsy will be assessed and processed according to RegenMed (Cayman) Ltd.'s SOPs for product manufacturing. One to two weeks after receiving the biopsy in North Carolina, RegenMed (Cayman) Ltd. will notify the site concerning the adequacy of the sample for manufacture of NKA. Following the notification to proceed, the site can confirm the injection date. If the biopsy cannot be used for manufacture of NKA, the patient should be discontinued from the study.

11.3 Injection (Study Days -14 to 4)

Due to shipping and manufacturing schedules, NKA injection should be scheduled for Wednesday, Thursday, or Friday. If it is necessary to schedule the injection for Tuesday, the site should contact RegenMed (Cayman) Ltd. before scheduling the injection to make sure Tuesday injection can be accommodated. NKA injection should not be scheduled on a Monday.

Patients should report to the clinic ten to 14 days before the scheduled injection (Day -10 to -14) for pre-injection assessments to verify the continued eligibility of the patient. If the patient is still eligible following review of the results of the assessments, the site will conduct the baseline renal scintigraphy study and contact RegenMed (Cayman) Ltd. indicating that NKA product should be produced. If the patient is not eligible for injection, the site should contact RegenMed (Cayman) Ltd. to alert manufacturing staff not to thaw the patient's frozen renal cells and manufacture NKA. The PI should contact RegenMed (Cayman) Ltd. to discuss the reason(s) the patient did not qualify and determine the best course of action. For example, if the patient was excluded for a temporary condition (e.g., active infection) then it may be possible to manufacture NKA at a later date such that the patient could be injected when he/she became eligible. If the patient's results indicate that it is highly unlikely that the patient would become eligible within an acceptable time frame, then the patient should be discontinued from the study. [Note that in the latter case, the patient can be replaced.] If injection is delayed, the renal scintigraphy study, if conducted, does not need to be repeated prior to injection.

For injection, patients should arrive at the clinic the day of the scheduled injection day (Day 0). The patient should be injected according to procedures discussed below (Section 8.2.2). On Day 1 post-injection, an ultrasound should be performed to verify lack of subclinical adverse effects (e.g., swelling, fluid accumulation). If clinically warranted, an additional ultrasound may be performed prior to discharge to verify lack of subclinical events.

Discharge: After an injection via the percutaneous route, the patient may be discharged the same day if that is the site's usual practice after similar procedures (i.e. percutaneous ablation), after no less than 2 hours of observation and monitoring. If product- or procedure-related AE's occurred following surgery, the patient should not be released from the hospital until the AE's have either resolved, stabilized, or returned to baseline. After a laparoscopic injection, the patient should be observed in hospital for 2 to 4 nights to assess for AEs.

11.4 Follow-up (Days 7, 14, and 28, 2month, 3month, 6month ± 3 days first injection and second injection)

The patient should return to the clinic for follow-up visits one week after injection (Day 7) and on Days 14 and 28. The patient should make every attempt to return for the first follow-up visit on the protocol-specified day (i.e., Day 7) although a 3 day window will be allowed in the case of extenuating circumstances. A 3-day window is allowable for visits on Days 14 and 28, and on Months 2, 3, 6, and 12. At Month 6 visit the same procedures as the pre-injection visit should occur to prepare for the second injection of NKA, if the patient and investigator wish to proceed. The month 6 visit should occur -14 to -10 days before second injection and within 3 days of 6 months post first injection. Patients will return to the clinic Days 7, 14, and 28 after second injection and Months 2, 3, 6, 9, and 12 after second injection.

The monthly visit dates should be calculated using the calendar date of injection. For example, if the patient was injected on April 30th, then the protocol-specified date of the 2 month follow-up visit would be June 30th and the 3 month visit date would be July 30th, etc. The visit window for the long-term monthly follow-up visits is ± 7 days.

End-of-Study Visit (EOS): Patients will complete the EOS Visit as noted below:

- If a patient discontinues the study after having the biopsy but before injection, then he/she should complete all EOS assessments except for the imaging studies; the MRI and/or renal scintigraphy should only be performed if the PI feels one and/or the other would provide critical safety information needed for management of the patient. If the patient has an ongoing study procedure-related SAE, then the patient should not be discontinued until the SAE has resolved, stabilized, or returned to baseline. If the SAE continues for 18 months, then the patient should be discontinued from the study at 18 months and followed as discussed in Section 13.3.3.
- If a patient discontinues from the study after injection but before completing all of the protocol-specified follow-up visits, then he/she should have the EOS Visit at the time of discontinuation. For these patients, the PI will determine whether or not it is clinically prudent to perform one or both of the imaging studies (i.e., MRI and renal scintigraphy). If the patient has a procedure- or product-related SAE(s) that is ongoing, the patient should not be discontinued until the SAE(s) has resolved, stabilized, or returned to baseline. If the SAE(s) is still ongoing at the time of the 18 month visit, the subject should be discontinued from the clinical trial and the SAE followed as discussed in Section 13.3.3.
- Patients who complete all visits including all of the follow-up visits will return at 18 months following second injection for the EOS visit. If the patient has any procedure- or product-related SAE's that are ongoing at the 18 month visit (i.e., not resolved, stabilized

or returned to baseline) the subject should be discontinued from the clinical trial and the SAE followed as discussed in Section 13.3.3.

11.5 Long term Follow-up

A limited assessment for long term follow-up will continue for 24 months after the initial 18 month follow up period is completed. Telephone contact will be made with patients at 24 months and 36 months after the injection, and patients will be examined at 30 and 42 months after the injection as long term follow up. At the 24 and 36 month visits, urine and blood specimens will be obtained for standard chemistry and renal panel, spot urine, and HbA1c test, an interim physical examination will be conducted, AEs will be reviewed, and the KDQOL will be completed (see Time and Events Schedule).

11.5.1 Telephone Contact

Patients will be contacted via telephone at 24 and 36 months after the NKA injection in order to inquire about SAEs.

11.5.2 Visits at 30 and 42 Months

Long term follow-up will extend to 30 and 42 months after the NKA injection and will include a physical exam, CBC, blood chemistries, and KDQOL.

12 STUDY PROCEDURES AND ASSESSMENTS

12.1 Demographics, Baseline Values, and Medical History

Demographics and baseline characteristics including medical history will be obtained from each subject. All CKD-related medical history and all other significant medical history should be recorded in the CRF. As a general rule, more recent events are more likely to be considered significant than similar events which occurred in the more distant past. Any items in the history that are still ongoing should be noted. Methods of contraception, if applicable, should be documented in the source documentation.

12.2 Clinical Evaluations

12.2.1 ECG

A 12-lead ECG should be performed at the visits indicated in the Time and Events Schedule. For each assessment, the recording should be obtained after the patient has been seated, both feet on the ground, back supported, and arms comfortably on armrests, with the blood pressure cuff applied but not inflated at the level of the heart for at least 5 minutes. ECG recordings should be assessed by qualified personnel and the results entered into the CRF as explained in the CRF Completion Guidelines found in the Study Reference Manual.

12.2.2 Physical Examination (PE)

The Investigator or designee will perform the PE. The comprehensive examination will include assessment of all pertinent body systems while the interim examination will include specific assessments of those body systems deemed appropriate for that subject by the PI. As a general rule for the interim examination, the PI should review the patient's reported adverse events prior to or in conjunction with the examination and include assessment of related body systems as appropriate. Only clinically significant abnormalities should be recorded in the CRF. Weight should be measured as part of the PE.

12.2.3 Vital Signs

Vital signs will include heart rate, body temperature and blood pressure (BP). Blood pressure should be measured after the subject has rested in a chair with back supported and both feet on the ground for a minimum of 5 minutes. Height should be measured once at screening.

12.2.4 Concomitant Medications

Concomitant medications (ConMeds) should be recorded as follows:

ICF to Biopsy / Biopsy to Injection: Record any *CKD-specific medication, medications that may affect renal hemodynamics, and medications that may affect serum creatinine measurements*. A list of these medications will be provided in the Study Reference Manual. Record *any* ConMed that is *used to treat an AE* that is recorded in the CRF during this time (see Section 13.3.1). Surgical medications used during the injection procedure do not need to be recorded unless their use falls outside of the expected dosages and/or frequencies.

Injection to Month 12 Follow-up: *Any* ConMed taken following injection through the 12 month follow-up visit should be recorded on the CRF. Surgical medications do not need to be recorded unless their use falls outside of the expected dosages and/or frequencies.

Month 12 to Month 18 Follow-up: Following the 12 month visit to the 18 month follow-up visit, record *any* ConMed *given to treat any AE* that is recorded in the CRF as discussed in Section 13.3.1. In addition, record *CKD-specific medications, medications that may affect renal hemodynamics, and medications that may affect serum creatinine measurements* as detailed in the CRF instructions.

Month 18 to Month 42 Long-term Follow-up: Following the 18 month visit to the 42 month follow-up visit, record CKD-specific medications, medications that may affect renal hemodynamics, and medications that may affect serum creatinine measurements that are ongoing at the time of the visit.

12.3 Laboratory Assessments

Laboratory assessments are listed below in Table 8. All samples will be collected as shown in the Time and Events Schedule: Laboratory Assessments will be assayed at a central laboratory designated by RegenMed (Cayman) Ltd. Laboratory results will be graded using the NCI CTCAE grading scale.

Table 8: Laboratory Assessments

Chemistry	**Hematology**
• Standard Panel	Hemoglobin - Hb
Alanine Aminotransferase: ALT	Hematocrit - HCT
Alkaline Phosphatase: ALP	Platelets
Aspartate Aminotransferase: AST	RBC Count
Bilirubin	WBC Count
Creatine Kinase: CK	WBC Differential
Gamma-glutamyl Transferase: GGT	**Coagulation Status**
Lactate Dehydrogenase: LDH	Activated Partial Thromboplastin Time: APTT
• Renal Analytes	INR
Albumin, serum	
Calcium, serum	**Urine Chemistry**
CO2, total	Protein & Albumin
Creatinine, serum	Creatinine
Cystatin-C	Protein & Albumin:Creatinine Ratio (PCR & ACR)
C Reactive Protein: CRP	NeutroPhase 1l Gelatinase-associated Lipocalin (NGAL)
Glucose, serum	**Routine Urinalysis: UA***
Phosphorus, serum	**Additional Selected Analytes**
Potassium, serum	ß2-Microglobulin, serum & urine
Sodium, serum	Hemoglobin (Hb) A1c
BUN	(intact) Parathyroid hormone; PTH
	Virology
Lipid Panel	HIV-1, HBV, HCV
Cholesterol	
LDL	**Research (Reserve) Analytes**
HDL	Serum/plasma and urine sample
LDL:HDL ratio	Example: Fibroblast Growth Factor 23, Pentaxin 3
Triglycerides	**Pregnancy (urine)**
*Routine UA using a urine test strip (dipstick). Microscopic analysis should only be performed if albumin, leukocytes, erythrocytes, or nitrites are positive.	

eGFR: For comparison of all subjects across the study, GFR will be estimated using the CKD-EPI equation. The specific assay for measuring creatinine will be defined by RegenMed (Cayman) Ltd. and the samples will be analyzed by the central laboratory. For comparison to each subject's historical values, it may be necessary to perform a second analysis at the site laboratory used to generate the historical data.

Virology: The biopsy samples collected from the patients will be used for selection of SRC and manufacture of NKA. Therefore, the patient will be tested for viral blood-borne pathogens including HIV, HBV, and HCV.

Urine Chemistry: Over the course of the study, urine will be collected over two different time periods; 24 hour collection and spot urine. Spot urine collections will be used for urine dipstick (test stick) assessments. The times for collection of each type of sample are illustrated in the Time and Events Schedule: Laboratory Assessments. To provide a comprehensive picture of

protein and albumin excretion, both total protein and albumin should be assessed in all samples as appropriate for that type of sample.

Research (Reserve) Analytes: Additional urine and serum/plasma samples will be collected, aliquotted, and stored for analysis of renal specific analytes and/or biomarkers of renal disease at a future time point. Potential analytes include fibroblast growth factor 23 (FGF23) and pentaxin 3 (PTX3). Results from these analyses will not be available, nor will they be included in the Clinical Study Report (CSR) for this study.

Pregnancy: A urine pregnancy test will be performed at the site using a test-strip. If the test is positive, then a confirmatory test will be performed at the clinical laboratory. If site practices do not accept the results of a test-strip, then a urine sample should be sent to the central laboratory for analysis.

12.4 Renal Imaging

12.4.1 Ultrasound

Ultrasound will be performed according to standard site procedures and will be used to assess safety during injection, prior to and following injection. An ultrasound may be conducted at other times if required for safety assessment or guidance of instrumentation during procedures. Findings from the ultrasound (e.g., resistance index, length, etc.) will be recorded on the CRF.

12.4.2 Magnetic Resonance Imaging

MR imaging will be performed according to site standard practices. During the site initiation visit, the MRI process will be defined for each site as dependent upon the MRI equipment in use. Generally, a 1.5-T unit should be used. Images will be used to determine kidney volume (for dosing calculations) and may be used to measure renal cortical thickness. MRI will be performed using standard sequences without injection of contrast agents. Volume measurements may be calculated, for example, using a fast 3D gradient-echo sequence, VIBE, with an acquisition time of 22 sec. and spatial resolution of 2x1.4x1.2 mm. The imaging parameters may be adjusted between patients; but the same parameters must be used for before and after images on any one patient. The specific parameters used will be recorded in the source documents and appropriate fields completed in the CRF.

12.4.3 Renal Scintigraphy

Renal scintigraphy has been used for a long time to measure relative kidney function. Historically, the method was performed with different radiopharmaceuticals such as dimercaptosuccinic acid labeled with Technetium-99m (99mTc-DMSA), diethylenetriamine pentaacetic acid (99mTc-DTPA), mercaptoacetyltriglycine (99mTc-MAG3), ethylenedicysteine (99mTc-EC) and orthoiodohippurate labeled with 131-J (131J-OIH). Among these, 99mTc-DMSA, a static renal agent, is considered as the most reliable method to measure relative renal function and is the preferred agent for this study.

Renal scintigraphy using 99mTc-DMSA is being advocated as the preferred method for assessment of renal function following several types of kidney disease. Its uptake correlates with effective renal plasma flow, glomerular filtration rate and creatinine clearance. Its quantitative measurement is therefore a good index for relative renal function. Previous studies have shown

that 99mTc-DMSA uptake differentiates normal from diseased kidney. If the site routinely uses a different agent, then the method should be reviewed at the site initiation visit.

The site should use their standard site procedure. Outline of an example procedure is below:

- Patient should receive an intravenous injection of 50MBq 99mTc-DMSA with imaging performed 3 hours after injection.
- Patient will be placed in supine position and an acquisition of posterior view with preset time of 15 minutes, 256x256 matrix will be performed with ultra- high resolution collimator.
- Differential renal function will be calculated using region-of-interest drawings.

Note: If site standard practice is to use a labeled agent other than 99mTc-DMSA, then the site must discuss the procedure with RegenMed (Cayman) Ltd. staff prior to site initiation. If the procedure is considered sufficiently equivalent to the procedure listed in the protocol, then the site will be allowed to use their standard procedure. In this case, the procedure will be signed by the RegenMed (Cayman) Ltd. Project Leader and a copy kept in the site's regulatory binder.

12.5 Surgical Procedures

12.5.1 Biopsy

The biopsy should be collected from the left/right kidney under sterile conditions using an ultrasound or CT guided method as dictated by site standard practices. The only difference from the standard procedure may be collection of 2 tissue cores and use of a 16 gauge needle. Two biopsy renal tissue cores are needed to insure sufficient cortical tissue is collected for selection of SRC and manufacture of NKA. Likewise, a 16 gauge biopsy needle should be used to insure sufficient cortical material is collected for manufacture of NKA. If site standard practices dictate use of a 15 gauge biopsy needle, then a 15 gauge needle may be used following consultation with the Medical Monitor. In any case, it is imperative that as much cortical tissue is collected as possible. If available at the site, bedside examination of the biopsy cores may be performed to ensure sufficient cortical material is obtained.

It is important to remember that the biopsy tissue will be used to manufacture NKA, an injectable product. Therefore, the site should ensure that individuals collecting the biopsy are aware that the tissue cores must be harvested using sterile conditions so that the risk of contamination during cell expansion and selection is minimized. Product with microbial bioburden cannot be released for injection, so contamination of the tissue cores during collection could significantly jeopardize RegenMed (Cayman) Ltd.'s ability to manufacture an NKA product for that patient.

Guidance on wound care and pain management following the injection procedure will be provided in the Study Reference Manual. Importantly, pain medication administered to the patient post-biopsy should selected carefully, avoiding as possible medications with nephrotoxic potential as discussed in Section 10.2. Specialized patient care surrounding the injection will focus on minimizing potential bleeding events. The subject will remain supine for 4 hours with monitoring of hemoglobin, blood pressure, gross hematuria, abdominal/flank pain, and flank

ecchymosis. In addition, the patient may be discharged the day of biopsy per site standard practice.

If a subject experiences significant adverse events following the biopsy that, in the opinion of the PI would put the subject at increased risk for significant adverse effects following biopsy, then he/she will not be injected with NKA, but will be followed until resolution of the event(s) and then discontinued from the study.

12.5.2 Injection

NKA in a sterile syringe will be sent to the site from RegenMed (Cayman) Ltd. Once received at the site, NKA should be stored in the Shipping Container until it is transported to the surgical suite. NKA must be equilibrated to 26.5±1.5 ºC for a minimum of 30 minutes but not more than 120 minutes immediately prior to injection into the patient. Instructions for equilibration will be provided by RegenMed (Cayman) Ltd. in the Study Reference Manual.

Patients will be injected with NKA using either: (1) a percutaneous minimally-invasive image-guided direct-needle approach, or (2) a laparoscopic technique similar to that used to deliver NKA in the canine studies and already utilized 6 or more times in the Swedish study in humans. The objective in either case is to approach at an angle allowing deposition of NKA in the renal cortex, distributed as widely as feasible. This could require imaging the kidney in a longitudinal or transverse approach, depending upon individual patient characteristics. Ideally the injection will involve multiple deposits as the injection needle/cannula is gradually withdrawn. The volume to be injected is determined by the weight of the kidney as estimated from the MRI, up to a maximum of 8 mL. For an 8 mL injection, it is anticipated that one to two mL will be deposited in 8 to 4 increments, respectively. Up to two entry points may be used to deposit the full volume of NKA into the kidney (two entry points per kidney were routinely used in all of the animal studies). NKA must be administered slowly at a rate no faster than 2 mL/min, ensuring viability of the NKA. If possible, the injection procedure will be viewed by the sponsor and actual injection of the kidney will be recorded on video for use as an educational/training tool.

Prior to surgery, the site must document the specific procedural approach that will be used to access the kidney.

- **Percutaneous procedure:**

Prior to the procedure, the operating physician will evaluate the patient, including:

a. Physical evaluation, to determine the feasibility of the procedure in general;
b. Evaluation of bleeding parameters, including coagulation panel, INR, platelets, hemoglobin, hematocrit, and other pertinent laboratory studies as indicates;
c. Review of available imaging studies, including ultrasound, MRI, and/or CT, to determine route of access, depth of kidney, and appearance of cortical-medullary junction. Mapping of potential sites of NKA cell deposition will be performed.
d. Determination of ASA class from airway assessment, medical history, allergies, and medications.
e. Interview of patient and family/supporters to discuss the procedure, its risks and possible complications, sedation, answer questions, and obtain documented informed consent.

Procedural technique: Specifically, a co-axial technique will be utilized (details below).

Image guidance: The operator will determine which kidney was biopsied, and will plan his approach to that kidney via longitudinal, transverse, or both reference planes. Imaging options during the procedure include ultrasound alone or ultrasound with complementary CT; the operator will verify and document the availability of adequate functionality, including color Doppler, measuring ability, probe frequency, and overall design.

Prior to the procedure, abnormal coagulation values will be corrected. Prophylactic antibiotics will be given intravenously according to the usual practices at the site. An initial CT scan may be ordered if necessary, for evaluation of adjacent viscera, renal location, presence of renal cysts, and for determination of the cortical-medullary junction in conjunction with ultrasound.

During the procedure, moderate conscious sedation will be employed, and patient monitoring will be continuous.

NKA is targeted for injection into the kidney cortex via a needle (cannula) compatible with cells. The intent is to introduce NKA via penetration of the kidney capsule and deposit into multiple sites of the kidney cortex. Initially, the kidney capsule will be pierced using a 15-20 gauge access trocar/cannula inserted approximately 1 cm into the kidney cortex (but not advanced further into the kidney). NKA is contained in a syringe that will be attached to a blunt tipped inner needle or flexible cannula (18-26 gauge, as suitable for the access cannula). In the Phase 1 clinical study, NKA was delivered via an 18G needle. The proposed Phase II study will utilize an 18 gauge or smaller needle (18-26 gauge) for NKA injection. The needle will be threaded inside the access cannula and advanced into the kidney, from which the NKA will be administered. Injection of the NKA will be at a rate of 1-2 ml/min. After each 1-2 minute injection, the inner needle will be retracted along the needle course within the cortex to the second site of injection; and so forth until the needle tip is at the end of the access cannula or the entire cell volume has been injected. This procedure can be used for both laparoscopic (used previously in Phase I study) and percutaneous injection of NKA. For percutaneous injection of NKA, the placement of the access cannula/trocar and needle will be performed using direct, real-time image guidance. Injection of the NKA will be monitored with ultrasound image guidance to visualize the microbubble footprint of cell deposits.

NKA injection will cease if there is imaging evidence of cell extravasation into central or peripheral renal blood vessels, the medullary portion of the kidney, or through the renal cortex and into the retroperitoneal soft tissues, or evidence of active bleeding. At this point, the needles are withdrawn. Additional renal injection sites may be chosen if NKA remains to be injected, along the same needle track, or at a new site in a different location in the kidney.

Following completion of NKA injection, the inner needle will be withdrawn and the outer cannula will remain in place for track embolization. During removal of the outer cannula (trocar), the site of the renal cortex puncture and needle track through the retroperitoneum will be embolized with absorbable gelatin particle/pledgets (e.g. Gelfoam [Pfizer]) or fibrin sealant (e.g. Tisseel [Baxter]) or other suitable agent to prevent renal bleeding.

At completion of the procedure, non-contrast CT scan or ultrasound with color Doppler evaluation will be performed to assess for puncture site cell injection and any hematoma or bleeding.

For 2 to 3 hours post-procedure there will be observation in a recovery-room environment with nursing assessment and vital-sign monitoring. The patient may be discharged after that, if all indices are normal.

A follow-up phone call will be conducted at 24 hours post-discharge, and follow-up in clinic will continue per protocol.

Minimally-Invasive Laparoscopic Procedure:

Using the second method, the kidney will be accessed while the patient is under full anesthesia, using a robotic- or hand-assisted laparoscopic approach. (The site may choose to use HARS as described in Wadstrom et al., 2011a and 2011b, or else a standard robotically-assisted method). Using a robotic- or hand-assisted approach allows the surgeon to place the kidney in an optimal position for the injection. It also allows the surgeon to visualize and stop bleeding if this should occur. Blood pressure will be monitored continuously during surgery using standard site surgical practices.

Once the kidney is accessed, NKA will be injected at an angle that allows for deposition of NKA into the kidney cortex. The cannula should be inserted to a depth that allows for multiple depositions of NKA as the cannula is retracted from the kidney. Entry points should be off the mid line of the kidney and angled to maximize deposition of NKA into kidney cortex.

Immediately after injection, the patient will recover in a post-operative surgical recovery unit under supervision. The patient will not be taken to his/her room until all vital signs are stable. Overall, the patient will be kept in the bed for at least 8 hours with monitoring of blood pressure and pulse. The patient should be closely monitored for 24 hours by a skilled team of caregivers used to dealing with post-operative problems. If pain, fever, dramatic decreases in blood pressure/hemoglobin, or any other clinical sign/symptom indicates potential adverse reactions/events, then further physical examinations (potentially including x-rays or ultrasound investigations) must be carried out liberally and swiftly for diagnostic purposes.

In addition to standard safety measures, hemoglobin will be monitored before, 4 hours after, and then daily while hospitalized following injection. Patients will remain in the hospital for 2 to 4 nights following surgery. Patients will not be released from the hospital until procedure- and/or product-related AE's have resolved, stabilized, or returned to baseline.

Post-procedure Evaluation of the injection:

With either procedure, if NKA leaks from the kidney during deposition, then the amount leaked should be estimated and recorded in the CRF. To prevent further leakage, the rate of injection may be slowed.

- As part of this protocol, injection parameters including (but not limited to) rate of injection and angle of injection may be adjusted for optimization.

13 SAFETY ASSESSMENTS AND MANAGEMENT

13.1 Event Definitions

13.1.1 Adverse Events

An Adverse Event (AE) is any untoward medical occurrence associated with the use of a drug or with study participation, regardless of the relationship of the occurrence to study drug or protocol. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the drug, whether or not considered related to the drug. An AE can arise from any use of the drug, and from any route of administration, formulation or dose, including an overdose.

Disease or other specific clinical signs and symptoms which were ongoing prior to study entry (i.e., a "pre-existing" conditions) should be recorded on the Medical History CRF and are not considered AE's unless the condition unexpectedly worsens during the study. Specifically, CKD signs and symptoms which were present at study entry and had been worsening over time are not considered AE's unless there is an unexpected worsening of the events. This would include, for example, an unexpected increase in the rate of disease progression.

Treatment emergent abnormal clinical laboratory findings or other abnormal assessments that are judged by the PI as clinically significant, involve therapeutic medical intervention, or lead to study discontinuation will be recorded as AE's or SAE's if they meet the definition of an AE or SAE presented in this section.

To determine the presence of adverse events, patients should be questioned about the event in a general way, without asking about the occurrence of any specific symptom. The investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases the diagnosis should be documented as the AE and not the individual signs and/or symptoms.

Following questioning and evaluation, all AE's, whether believed by the investigator to be related or unrelated to study procedures and/or NKA, must be documented in the patient's medical records in accordance with the investigator's normal clinical practice and on the AE CRF. Each AE is to be evaluated for nature, duration, severity, seriousness, and causal relationship to NKA administration.

All events will be managed and reported in compliance with all applicable regulations, and included in the final study report.

13.1.2 Serious Adverse Reaction/Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening adverse event,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

- A congenital anomaly/birth defect
- An important medical event

Life-threatening event: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. **Disability** is defined as a substantial disruption in a person's ability to conduct normal life functions. **Hospitalization** for elective treatment of a preexisting condition that did not worsen during the clinical investigation is not considered an AE/SAE. Admittance to an emergency room for observation without being admitted to the hospital may be an AE but is not arbitrarily classified as an SAE. Hospitalization or nursing home admission for the purpose of caregiver respite is not considered an AE/SAE. Complications that occur during hospitalization are AE's, and if a complication prolongs hospitalization, the event is considered serious.
Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

13.2 Assessment and Grading of AE's/SAE's

13.2.1 Relationship Assessment

For each reported AE, the Investigator must make an assessment of the relationship of the event to study procedures and/or NKA using the following criteria:

- **Unrelated:** applicable to an AE that occurs when the subject was not exposed to study treatment or another cause is obvious.
- **Unlikely to be related:** applicable to an AE that meets the following criteria:
  - Does not follow a reasonable temporal sequence from injection of NKA
  - May readily have been produced by the patient's clinical state, environmental, or toxic factors, or other therapy administered to the patient
  - Does not follow a known pattern of response to injection (based on animal data)
- **Possibly related:** applicable to AE's where connection with NKA injection appears unlikely but cannot be ruled out. Applicable to AE's where:
  - It follows a reasonable temporal sequence following injection of NKA
  - It follows a known pattern of response to injection of NKA (based on animal studies)
- **Probably related**: applicable to AE's that are considered, with a high degree of certainty, to be related to NKA. Applicable to AE's where
  - It follows a reasonable temporal sequence from injection of NKA
  - It cannot be reasonably explained by the know characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy
  - It follows a known patter of response to NKA injection (based on animal data).

13.2.2 Severity of Adverse Events

All AE's including SAE's will be assessed for severity, using the following grading scale:

Severity of symptoms and abnormal findings will be assessed in this study using the Common Terminology Criteria for Adverse Events (NCI CTCAE, version 4.03).

In the event that an AE is not covered by the NCI-CTCAE grading system, the following definitions will be used:
 Grade 1 - Mild: awareness of sign, symptom, or event, but easily tolerated; does not interfere with usual daily activities or tasks
 Grade 2 - Moderate: discomfort enough to cause interference usual activity and may warrant therapeutic intervention
 Grade 3 - Severe: incapacitating with inability to perform usual activities and daily tasks; or significantly affects clinical status, ; requires therapeutic intervention
 Grade 4 - Life-threatening: immediate risk of death
 Grade 5 - Death

13.3 Recording, Follow-up and Reporting of Adverse Events

13.3.1 Recording of Adverse Events

Subjects will be required to report any AE that occurs after informed consent is signed. All AEs that occurred from the time of informed consent until the 18-month follow-up visit will be recorded with the following precisions.

ICF to Biopsy: Any AE occurring after the patient has signed the ICF but before biopsy should only be recorded on the AE page of the CRF if it is *directly related to study procedures* (e.g., nausea resulting from an agent used for an imaging study). *Any CKD specific event* occurring during this time should be recorded (or updated) on the Medical History page.

Biopsy to Injection: *Any* AE occurring during or after the biopsy and before injection should be recorded on the AE page *only if the event was considered possibly or probably related to the biopsy or other protocol-specified procedure*. CKD specific events should be recorded (or updated) on the Medical History page unless, in the opinion of the Investigator, the worsening of the CKD event could be related to the biopsy or other study procedure.

Injection to Month 12 Follow-up: *Any* AE occurring during or after injection until the 12 month follow-up visit should be recorded on the CRF.

Month 12 to Month 18 Follow-up: Following the 12 month visit to the 18 month follow-up visit, *all AE's assessed as product- or procedure-related* should be recorded in the AE CRF. In addition, a*ny AE's with a severity assessment of "severe" or "life-threatening"* should be recorded in the CRF, regardless of relationship. *Any CKD-related event that meets the definition of an AE* (e.g., clinically significant increase in sCr) should be recorded in the AE CRF. All other AE's do not need to be recorded in the CRF; however, as the PI is responsible for patient safety, the site should record any event that the PI determines should be recorded in the AE CRF.

13.3.2 Recording of Serious Adverse Events

All SAE's should be recorded in the AE CRF from first study procedure through the 6 month follow-up visit. In addition, any SAE's that occur during the long-term observational phase AND are considered related to investigational product, should be recorded in the CRF. All SAE's should be reported according to the procedures discussed below (Section 13.3.4).

13.3.3 Follow-Up of AE's/SAE's

All AE's that are assessed as related to Investigational Product (i.e., NKA) or study procedures (e.g. biopsy, laparoscopic surgery, or surgical or percutaneous injection) should be followed by the Investigator until the event resolves, stabilizes, or returns to baseline. The investigator should continue to report any significant follow-up information to the Sponsor or Sponsor's designee up to the point the event has been resolved.

SAE's should be followed until the event resolves, stabilizes, or returns to baseline. If the patient insists on discontinuing from the study prior to resolution/stabilization/return to baseline of the SAE, then the patient will continue to be followed as part of the SAE reporting system. That is, the Investigator will continue to follow the patient on a schedule dictated by the clinical situation and submit appropriate follow-up SAE reports as discussed in Section 13.3.4. If the patient refuses to continue to follow-up with the Investigator, then the Investigator may follow the patient through consultation with the patient's primary physician if allowed by the site-approved ICF and applicable regulations.

SAE's that are identified on the last scheduled visit must be recorded in the AE CRF and reported to the Sponsor or Sponsor's designee as outlined in Section 13.3.4. Subjects with unresolved, previously reported serious adverse events, or new serious adverse events identified on the last scheduled visit, should be followed by the investigator until the events are resolved, stabilized, or returned to baseline.

13.3.4 Reporting of Serious Adverse Events

8.3.4.1 Reporting of SAEs to the Sponsor

It is the responsibility of the PI to insure all SAEs and follow-up SAEs, occurring from the signature of the ICF until the 18-month follow-up visit, are reported to the Sponsor or Sponsor's designee within 24 hours of discovery or notification of the event or the follow-up information. For each SAE and follow-up to an SAE the site should ensure that critical AE CRFs are completed as of the onset date for the SAE (e.g. demographics, concomitant medications, and medical history) as described in the Study Reference Manual.
At any time after completion of the AE reporting period, if the Investigator becomes aware of an SAE that is suspected by the Investigator to be related to the investigational product, the SAE must be reported to the Sponsor or Sponsor's designee within the same timelines.

SAEs shall be sent to CTI Safety preferably by Facsimile transmission, via secure e-fax specific to this study, with the SAE Report Form. In circumstances where facsimile equipment is not available, notification by email to CTISafety@CTIFacts.com is acceptable.

SAEs shall be sent by eFAX with the SAE report form to: CTI Safety

Drug Safety Country-Specific Contact Numbers

Country	E-Fax	SAE Hotline
United States	**1-800-541-1501**	**877-755-0742 / 513-212-6804**
Sweden	**46 850 127 343**	
CTI GSP EMAIL	CTISafety@CTIfacts.com	

In circumstances where it is not possible to send the notification by the above-mentioned method, notification by telephone is acceptable, with a copy of the SAE Report Form sent by overnight mail. Initial notification via the telephone does not replace the need for the investigator to complete, sign and send the SAE form within the time frames outlined above.

8.3.4.2 Reporting of SAEs to Competent Authorities

SAEs that are considered as possibly or probably related to the investigational product, and as unexpected, will be reported to the Competent Authorities by the Sponsor or Sponsor's designee as required by applicable local regulations; therefore it is critical that site personnel record SAEs in the eCRF in a timely manner. Per regulation, any unexpected fatal or life-threatening AEs assessed as possibly or probably related to NKA will be reported to the Competent Authorities by the Sponsor or Sponsor's designee within 7 calendar days. Additional information will be reported to the Competent Authorities within an additional 8 calendar days. The Sponsor or Sponsor's designee is required to submit any other unexpected SAEs assessed as possibly or probably related to NKA to the Competent Authorities within 15 calendar days of notification.

13.4 Events of Special Interest

The following events are considered "events of special interest" as they are events that have been reported in the past following one or more of the procedures performed during this study. Patients should be carefully monitored for appearance of these events at any time during the study.

13.4.1 Procedure-related Events

Post-procedure Pain: If the subject experiences pain following the biopsy or injection, simple analgesia with paracetamol or paracetamol-codeine combinations usually suffices to control the pain. More severe pain in the loin or abdomen requires ultrasonography to exclude significant perirenal hemorrhage. Patients will be followed with regular hemoglobin and blood pressure monitoring. If severe pain occurs, opiates may be necessary. If analgesic doses higher than the maximum authorized doses are required to alleviate pain, then the Investigator must perform additional clinical evaluations to ascertain the probable cause(s) of excessive pain.

Hemorrhage: A degree of perirenal bleeding accompanies almost every renal biopsy. According to the literature (Whittier and Korbet, 2004b), mean hemoglobin decreases approximately 1.0 g/L to about 10 g/L after injection biopsy. Macroscopic hematuria and painful hematomas are seen in about 3 % of patients after renal biopsy. Therefore, following the biopsy and injection, patients will be followed with regular hemoglobin and blood pressure monitoring. Patients will be confined to bed and monitored for maintenance of normal coagulation indices. If bleeding is brisk and the patient is hypotensive despite bed rest, blood transfusion will be considered. If the bleeding is still not controlled, surgery may be considered. In rare cases, renal angiography should be performed to identify the source of bleeding and coil embolization can be performed in such cases during the same procedure.

Arteriovenous (AV) fistula: According to the literature (Abutaleb and Obaideen, 2007; Arora et al., 2012; Ham et al., 2012; Huang et al., 2011; Whittier and Korbet, 2004a; Whittier and Korbet, 2004b), AV fistulas can be found in about 18% of patients that have undergone renal biopsy. However, as more than 95% of these resolve within 2 years, they should not be routinely pursued. If patients have recurrent hematuria after a renal biopsy or injection, AV fistulas could be considered. In these cases embolization may be indicated. If an AV fistula is detected by ultrasound at the pre-injection qualification visit, then the PI should consider discontinuing the patient prior to injection.

Other complications: In very rare cases other organs, such as liver, gallbladder and lungs may have been penetrated during the procedure. In these cases appropriate treatment and follow-up will be discussed with surgeons.

Death: Deaths resulting from renal biopsies occur in <0.01% of patients (Hergesell et al., 1998; Lin et al., 2006; Walker, 2009) but could be due to uncontrolled bleeding especially in patients with severe renal impairment. Strict inclusion/exclusion criteria will be followed to ensure that patients who may be predisposed to uncontrolled bleeding would not be enrolled in the trial.

13.4.2 Product-related Events

No product-related events are expected to occur. This is based on the autologous nature of NKA (i.e., NKA is manufactured from renal cells isolated from the same kidney to which they are to be returned), the lack of product-related events observed in the animal studies, and the lack of specific NKA related events seen during and following injection of the first 5 patients in Sweden (See Section 6.5.1). However, patients will be extensively monitored throughout the course of the study for any unexpected events. If an event occurs which is assessed as related to the investigational product, NKA, then the event will be immediately reviewed by the Medical Monitor and PI.

13.5 Data Safety Monitoring Board

Given the uniqueness of this product, an external data safety monitoring board (DSMB) will be convened to oversee patient safety, especially as it relates to any unexpected product-related events. The committee will be composed of a minimum of 3 members whose expertise is such that the Board has adequate familiarity with reconstructive urological surgery, renal medicine, and monitoring emerging safety issues in clinical studies. The DSMB will be an independent committee that is not otherwise engaged in the conduct of the clinical study and who do not have conflicts of interest.

The DSMB will be governed by the DSMB charter. The specific mandate of the DSMB will be to review any AE's/SAE's assessed by the PI as related to the investigational product, NKA. Thus, any events assessed as related to NKA will be forwarded to the DSMB by the study site or CRO. If the event is rated mild or moderate, the DSMB will work with the PI and Medical Monitor to determine the best course of action. The "best course of action" may range from no changes to adaptations of procedures to prevent future events, etc. If the event is severe or life-threatening, then the DSMB may consider halting future injections until a best course of action is devised. If the event is an SAE that is considered severe or life-threatening, then no future patients may be injected until approved by the DSMB. In addition to the above, the DSMB will

be charged with reviewing the safety data from the first two patients injected and providing consultation on enrollment of subsequent patients.

The DSMB will be expected to advise RegenMed (Cayman) Ltd. regarding the study design and study conduct, and may recommend early study termination for safety reasons. The DSMB will be expected to request additional information and analyses as they require. The DSMB's recommendations will be communicated to the study centers, and where required to the IRBs/ECs and the Competent Authorities. If the DSMB recommends stopping the study, the Competent Authorities and the IRBs/ECs will be notified.

The surgical procedures used in this study, renal biopsy, percutaneous needle insertion in the kidney, and laparoscopic or retroperitoneoscopic surgery, are established procedures with known adverse event profiles. However, if unexpected procedure-related events occur, the DSMB will review the events on a case-by-case basis.

13.6 Stopping Criteria

Approximately two to three weeks following each of the first two injections, the PI, Medical Monitor, DSMB and RegenMed (Cayman) Ltd. staff will review the case to assess both positive and negative results of the injection. If no clinically significant unexpected events are identified, the next patient may be injected such that the timing between the first 3 injections will be staggered by 3 weeks. If any of the following events occur, no additional patients can be injected until review by the DSMB is completed:

1. An SAE that is rated as severe or life-threatening and considered related to NKA or study procedures by the PI
2. Death of an enrolled patient
3. Similar SAE's in more than one patient that are considered related to NKA by the investigator
4. Inability to deliver a minimum of 50% of the dose of NKA in more than one patientdue to surgical or other issues
5. DSMB recommendation to temporarily stop enrollment to allow for more precise review of case(s)

14 STATISTICAL CONSIDERATIONS

14.1 Samples Size

Up to 30 subjects will receive up to two doses with NKA. As this is an early Phase II safety and efficacy study, formal sample size calculations were not performed. The planned sample size allows for a preliminary characterization of the safety profile of the NKA in patients with chronic kidney disease.

14.2 Analysis Objectives

The **primary objective** of the study is to assess the safety and efficacy of NKA injected in one recipient kidney and determine if two injections of NKA provide stabilization of renal function.

- Primary Safety Outcome Measures: procedure and/or product related adverse events (AE's) through 12 months following the initial NKA injection.
- Primary Efficacy Outcome Measures: serial measurement of serum creatinine and estimation of GFR through 6 months following the second cell injection

The **secondary objective** of the study is to assess the safety and tolerability of NKA administration by assessing renal-specific adverse events over a 12 month period following a patient's first NKA injection.

- Secondary Safety and Tolerability Outcome Measures: renal-specific laboratory assessments through 12 months following the last NKA injection under this protocol, whether first or second.

The **exploratory objectives** of the study are designed to assess the impact of NKA on renal function over a 12 month period following the initial NKA injection.

- Exploratory Outcome Measures: clinical diagnostic and laboratory assessments of renal structure and function (including eGFR, serum creatinine, and proteinuria) to assess changes in the rate of progression of renal disease; and effect of method of injection on these parameters.

Exploratory quality of life outcome measure will be the Kidney Disease Quality of Life survey obtained at baseline and at 1, 3, 6, 7, 9, 12, 15, 18, 30, and 42 months after a patient's first NKA injection.

Additional exploratory endpoints include the following:

- Changes from baseline in BUN, β2 microglobulin and intact parathyroid hormone (iPTH) at 6 and 12 months post-injection.
- Changes from baseline in C-reactive protein (CRP) at 6 and 12 months post-injection.
- Need for initiation of renal replacement therapy.
- Comparison of pre-injection versus post-injection split functional differences between the left and right kidney based on renal scintigraphy.
- Comparison of pre-injection and post-injection kidney volume estimates based on comparative MRI assessments.
- Changes in the patient's perception of quality of life, as measured by serial KDQoL surveys.

14.3 Endpoints

The primary endpoint of the study is to assess the safety and efficacy of NKA injected in one recipient kidney and determine if two injections of NKA provide stabilization of renal function.

- Primary Safety Outcome Measures: procedure and/or product related adverse events (AE's) through 12 months following the initial NKA injection.
- Primary Efficacy Outcome Measures: serial measurement of serum creatinine and estimation of GFR through 6 months following the second cell injection

Secondary Endpoint

The secondary endpoint of the study is to assess the safety and tolerability of NKA administration by assessing renal-specific adverse events over a 12 month period following a patient's first NKA injection.

- Secondary Safety and Tolerability Outcome Measures: renal-specific laboratory assessments through 12 months following the last NKA injection under this protocol, whether first or second.

Exploratory Endpoints

The exploratory endpoint of the study are designed to assess the impact of NKA on renal function over a 12 month period following the initial NKA injection.

- Exploratory Outcome Measures: clinical diagnostic and laboratory assessments of renal structure and function (including eGFR, serum creatinine, and proteinuria) to assess changes in the rate of progression of renal disease; and effect of method of injection on these parameters.

Exploratory quality of life outcome measure will be the Kidney Disease Quality of Life survey obtained at baseline and at 1, 3, 6, 7, 9, 12, 15, 18, 30, and 42 months after a patient's first NKA injection.

- Changes from baseline in BUN, β2 microglobulin and intact parathyroid hormone (iPTH) at 6 and 12 months post-injection.
- Changes from baseline in C-reactive protein (CRP) at 6 and 12 months post-injection.
- Need for initiation of renal replacement therapy.
- Comparison of pre-injection versus post-injection split functional differences between the left and right kidney based on renal scintigraphy.
- Comparison of pre-injection and post-injection kidney volume estimates based on comparative MRI assessments.
- Subgroup analyses will compare patients receiving a single injection with patients receiving two injections, and patients receiving laparoscopic injections with patients receiving percutaneous injections.

14.4 Analysis Conventions

14.4.1 Analysis Sets

Full Analysis Set: The Full Analysis Set (FAS) will consist of all subjects who are enrolled in the study.
Injection Analysis Set: The Injection Analysis Set will include all patients who undergo a successful NKA injection.

14.4.2 Handling Missing Data

Values for missing data will not be imputed.

14.5 Demographic and Baseline Characteristics

Demographic data and baseline characteristics will be summarized using a typical 8-number summary (sample size, mean, standard deviation, median, Q1, Q3, minimum, and maximum) for continuous variables and frequency and proportion for categorical variables. These summaries will be produced for both the FAS and the Injection analysis set. Generating inferential statistics is not planned. Demographic and baseline characteristics information will be provided in a data listing.

14.6 Efficacy Analysis

Not applicable.

14.7 Safety Analysis

14.7.1 Biopsy, NKA Injection, Patient Disposition

Biopsy and NKA injection data will be provided in a data listing. Patient disposition (e.g., Screen Failure, Enrolled/Underwent Injection, Withdrawn Pre-injection, Successful injection, Discontinued Early Post-injection, Completed Study) will be summarized by frequency and proportion and will be provided in a data listing.

14.7.2 Adverse Events

Clinical and laboratory AE's will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System Organ Class (SOC), High-Level Group Term (HLGT), High-Level Term (HLT), Preferred Term (PT), and Lower Level Term (LLT) will be attached to the clinical database. Adverse events will be graded using the NCI CTCAE scale.

A treatment-emergent AE will be defined as any adverse event that begins on or after the date of enrollment up to 18 months post-injection.

Summaries (frequency and proportion) of treatment-emergent AE's will be presented by SOC and PT.

Additional summaries will include, but are not limited to, treatment-emergent AE's by severity grade, Investigator's assessment of relationship to procedure and/or product, serious AE's (SAE's), related SAE's, and deaths. AE summaries will be provided for both the FAS and the Injection analysis set. There are no plans to generate inferential statistics. AE data will be provided in a data listing.

14.7.3 Laboratory Evaluations

Baseline values will be collected immediately prior to injection. Observed and change from baseline laboratory data will be summarized using a typical 8-number summary (sample size, mean, standard deviation, median, Q1, Q3, minimum, and maximum) for continuous variables and frequency and proportion for categorical variables. These summaries will be produced for both the FAS and the Injection analysis set. Generating inferential statistics is not planned.

Graded laboratory abnormalities will be defined using the NCI CTCAE grading scheme as defined in Section 12.3. Incidence of treatment-emergent laboratory abnormalities, defined as values that increase at least 1 toxicity grade from baseline at any time post baseline up to six months post-injection will be summarized. If baseline data are missing, then the latest value collected between biopsy and injection will be used as the baseline value. If baseline and pre-injection data are missing, then any graded abnormality, i.e., at least a Grade 1, will be considered treatment emergent.

Laboratory data summaries will be provided for both the FAS and the Injection analysis set. There are no plans to generate inferential statistics, except for variables concerning the rate of renal insufficiency progression. A paired t-test may be performed to assess the change in the rate of progression of renal insufficiency. Laboratory data will be provided in a data listing.

14.7.4 Other Safety Evaluations

Data from ECG, physical exam, medical history, concomitant medication, vital signs, ultrasound, renal scintigraphy, and MRI assessments will be provided in a data listing. Descriptive statistics for these evaluations will be generated as warranted.

14.8 Exploratory Analysis

As this study is a Phase II evaluation, hypothesis generating exploratory analyses may be conducted to help design future clinical studies. Subgroup analyses will compare patients receiving a single injection with patients receiving two injections, and patients receiving laparoscopic injections with patients receiving percutaneous injections.

15 STUDY ADMINISTRATION

15.1 Ethical and Regulatory Standards

This study will be conducted in accordance with ICH E6 GCP guidelines, ethical principles outlined in the Declaration of Helsinki, and all appropriate US regulations including 21 CFR Parts 11, 50, 54, and 56, and all appropriate EU regulations.

15.1.1 Institutional Review Board and Ethics Committee

It is the Investigator's responsibility in the US and the sponsor's (or sponsor's designee's) responsibility in the EU to ensure that this protocol is reviewed and approved by an appropriate IRB which conforms to US regulations found in 21 CFR Part 56, as well as EU and national regulations. The PI in the US and sponsor/sponsor's designee in the EU must also submit the ICF, any other written documentation provided to the subject and all advertisements that may be used for study-specific activities to the IRB/EC for approval. If it is necessary to amend the protocol during the study, then it is the responsibility of the PI in the US and sponsor/sponsor's designee in the EU to ensure that IRB/EC approval is obtained before implementation of the amended procedures. It is also the responsibility of the PI in the US and sponsor/sponsor's designee in the EU to provide the IRB/EC with any SAE or safety reports.

15.1.2 Subject Informed Consent

Prior to conducting any study-specific procedures, the site must obtain the informed consent of the patient, as indicated by his/her signature on the ICF. The Investigator is responsible for informing the patient about the study and obtaining informed consent from the patient as indicated by his signature on the ICF to verify the task. The ICF must contain all elements required by ICH guidelines and 21 CFR Part 50 in the US and EU and national regulations be approved for use in the study by the appropriate IRB/EC.

15.2 Data Collection and Review

15.2.1 Data Collection

All observations relating to the study must be recorded by study site personnel in source documents. In addition, specific Case Report Forms (CRFs) will be provided for this study. A CRF must be completed for every patient who signs the ICF and has at least one protocol-specified, study-specific assessment conducted. CRF completion instructions will be provided to the site in the Study Reference Manual. Every attempt should be made to enter patient visit data into the CRF within 3 days of the patient's visit. After the patient has completed the study, the PI must review and sign the CRF indicating that he has reviewed the completed CRF and pertinent clinical data for that patient and that, to the best of his knowledge, all data recorded in the CRF accurately reflects the patient's clinical performance in the study.

15.2.2 Study Monitoring

A study monitor appointed by the Sponsor will periodically contact the site and conduct on-site monitoring visits as described in the Study Monitoring Plan. The study monitor will insure that the investigation is conducted according to the protocol and regulatory requirements including ICH GCP guidelines.

In addition, a DSMB will be convened to oversee patient safety. The composition, role, and responsibilities of the DSMB will be as discussed in Section 13.5 and detailed in the DSMB charter.

15.2.3 Quality Assurance

At its discretion, RegenMed (Cayman) Ltd. or its designee may conduct a quality assurance audit of this study. If such an audit occurs, the Investigator will give the auditor direct access to all relevant documents, and will allocate his time and the time of his staff to the auditor as required. In addition, regulatory agencies may conduct an inspection of this study. If such an inspection

occurs, the Investigator will allow the inspector direct access to all source documents, CRFs, and other study documentation for source data check and/or on-site audit inspection.

During any audit, the confidentiality of the data and the protection of patient confidential information will be respected and maintained.

15.3 Retention and Disclosure of Information

15.3.1 Records Retention

Results from this study will be included in a US IND submission, therefore US FDA regulations for record retention will apply. These regulations require that records and documents pertaining to the conduct of this study, including CRFs, consent forms, and laboratory test results must be kept on file by the PI for a minimum of two years after notification by RegenMed (Cayman) Ltd. that a marketing application has been approved. If no application is filed or approved, these records must be kept for two years after the investigation has been discontinued and the U.S. FDA and applicable foreign authorities have been notified.

EU regulations for record retention, in particular Directive 2005/28/EC, also apply and will be followed.

15.3.2 Disclosure of Information

Disclosure of Data: The Investigator agrees by his/her participation that the results of this study may be used for submission to national and/or international registration and supervising authorities. If required, these authorities will be provided with the names of Investigator, his address, qualifications and extent of involvement. It is understood that the Investigator is required to provide RegenMed (Cayman) Ltd. with all study data, complete reports and access to all study records.

Data generated by this study must be available for inspection by regulatory authorities, by RegenMed (Cayman) Ltd., and the US IRBs as appropriate. At a patient's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Patient medical information obtained during the course of this study is confidential and disclosure to third parties other than those noted above is prohibited.

Confidentiality: All information and materials disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the study, including but not limited to the Investigational Product, Shipping Materials, protocol, CRFs, IB and the results obtained during the course of the study are confidential. The Investigator and any person under his/her authority agree to undertake to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor.

The Sub-investigators shall be bound by the same obligation as the Investigator. The Investigator shall inform the Sub-investigators of the confidential nature of the clinical trial. The Investigator and the Sub-investigators shall use the information solely for the purposes of the clinical trial, to the exclusion of any use for their own or for a third party's account.

Publication Policy: Following completion of the study, the Investigators and Sponsor are expected to publish the results of this research in a scientific journal.

Property Rights: All information, documents and Investigational Product provided by the Sponsor or its designee are and remain the sole property of the Sponsor.

The Investigator shall not mention any information or the Investigational Product in any application for a patent or for any other intellectual property rights.

All the results, data, and documents, which arise directly or indirectly form the study in any form, shall be the immediate and exclusive property of the Sponsor.

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