A Phase 1 Multicenter Study Evaluating the Safety and Potential Activity Of Two Escalating Doses of *h*Maxi-K Gene Transfer By Direct Injection into the Bladder Wall In Female Participants With Idiopathic (Non-neurogenic) Overactive Bladder Syndrome and Detrusor Overactivity: Double Blind, Imbalanced Placebo Controlled Design Within 2 Sequential Active Treatment Groups

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Sponsor

Ion Channel Innovations, LLC

969 Park Ave., 1G

New York, NY 10028

Tel: 212-639-1561

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Sponsor Representative

Arnold Melman, MD
Directing Member
Ion Channel Innovations

Signature: _____

Date: October 1, 2015

Principal Investigator

I agree to perform this study in accordance with the protocol and Good Clinical Practice (GCP) Guidelines.

Signature: _____

Date: _____

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List of Abbreviations

AE: adverse event	LOCF: last observation carried forward
ALP: alkaline phosphatase	Mg^{++}: magnesium
ALT: alanine aminotransferase (SGPT)	mg: milligram
ANCOVA: analysis of covariance	Min: minute
ANOVA: analysis of variance	mL: milliliter
aPTT: activated partial thromboplastin time	µg: microgram
ARF: acute renal failure	µL: microliter
AST: aspartate aminotransferase (SGOT)	Mm Hg: millimeters of mercury
BMI: body mass index	NIH: National Institutes of Health
BP: blood pressure	Mm or mmol: millimole
BUN: Blood Urea Nitrogen	µmol: micromole
Ca^{++}: Calcium	mEq: milliEquivalents
CBER: Center for Biologics Evaluation and Research	Na^{+}: sodium
CBC: Complete blood count	ng: nanogram
CFR: Code of Federal Regulations	OAB: Overactive Bladder Syndrome
Cl: chloride	OBA: Office of Biotechnical Activities, NIH
cm: centimeter	OHSR: Office of Human Subject Research, NIH
CO_2: Carbon Dioxide	PT: Prothrombin time
CRO: Clinical Research Organization	PTT: Partial thromboplastin time
CPK: creatine phosphokinase	PUO: Partial Urethral Obstruction
E-CRF: electronic case report form	QoL: Quality of Life
CRP: C- reactive protein	QTcF: QTc Fridericia / QTcB: QTc Bazett
CSR: clinical study report	RBC: red blood cell
DBP: diastolic blood pressure	SAE: serious adverse event
DO or DH: Detrusor overactivity	SAP: statistical analysis plan
dL: deciliters	SBP: systolic blood pressure
DLT: Dose limiting toxicity	SD: standard deviation
DRF: discrepancy resolution form	SF-12: Health Status Questionnaire

List of Abbreviations

DSMB: Data Safety Monitoring Board	SGOT: serum glutamic-oxaloacetic transaminase
ECG: electrocardiogram	SGPT: serum glutamic-pyruvic transaminase
FDA: Food and Drug Administration	TEAE: treatment-emergent adverse event
FSH: Follicular stimulating hormone	ULN: upper limit of normal
g: gram	NOBLE: National Overactive BLadder Evaluation
GCP: good clinical practices	UUI: urge urinary incontinence
GeMCRIS: Genetic Modification Clinical Research Information System, NIH	
GGT: gamma glutamyltransferase	
KHQ: King's Health Questionnaire	
K^+: potassium	
LDH: lactate dehydrogenase	
LLN: lower limit of normal	
h: hour	
HR: heart rate	
hpf: high powered field	
ICH: International Conference on Harmonization	
ICIQ-SF: International Consultation on Incontinence Questionnaire- Short Form)	
IEC: independent ethics committee	
IRB: institutional review board	
ITT: intent-to-treat	
kg: kilogram	
L: liter	

Protocol Synopsis

Title	A Phase 1 Multicenter Study Evaluating the Safety and Potential Activity Of Two Escalating Doses of *h*Maxi-K Gene Transfer By Direct Injection into the Bladder Wall In Female Participants With Idiopathic (Non-neurogenic) Overactive Bladder Syndrome and Detrusor Overactivity: Double Blind, Imbalanced Placebo Controlled Design Within 2 Sequential Active Treatment Groups
Study No.	ION 03-OAB
Study Phase	Phase 1
No. of Sites	Up to six clinical sites in USA
Study Objectives	The primary objective of this study is to evaluate occurrence of Adverse Events and their relationship to *h*Maxi-K following multiple intramuscular (IM) injections into the bladder wall. Two dose levels (16000 µg and 24000 µg by IM injection into the bladder wall) in females with moderate OAB/DO of ≥ six months duration are initially planned**. Pending safety and efficacy results for the 2 doses that are planned, the protocol may be amended to investigate additional doses in this study.** The secondary objectives of this study are to evaluate the following safety parameters: • Clinical laboratory tests- changes from baseline • Electrocardiogram change from baseline • Physical Examination- changes from baseline These changes will be evaluated primarily at the individual level, through clinical assessment of outliers supported by descriptive statistics. In addition efficacy parameters will be evaluated (change from baseline compared to placebo), including number of micturitions per day, volume per micturition, incontinence episodes, pad weight, uninhibited contractions during cystoscopy, and general and bladder specific quality of life assessments.
Study Design	This study is a double blind, placebo-controlled, multicenter, sequential active dose, phase 1 study in females with moderate OAB/DO of ≥ six months duration. The proposed study period is approximately 6 months following a single administration of study drug by multiple IM injections into the bladder wall per participant. The protocol sample size of this phase I study is not intended to support the statistical significance of the primary endpoint as outlined in the study objectives. Rather, the sample size takes into account clinical safety considerations. Nine participants per dose level are planned: [6 assigned active treatment and 3 assigned placebo (PBS-20% sucrose only control group)]. Therefore a total of up to 9 participants will be enrolled in each dose group. Each arm of participants will be enrolled sequentially and enrollment into the next higher dose level will be dependent on assessment of safety. The participants chosen will be assigned in a

randomized fashion to either placebo or an active treatment group within each group.

Study Population

The study population is women ≥ 18 years old of non-child bearing potential[1] with overactive bladder (OAB) and detrusor overactivity who are otherwise in good health.

The target population is women with idiopathic (non-neurogenic) OAB and detrusor overactivity (DO) who have been unable to tolerate, do not wish to continue, or have had unsuccessful results with, prior therapy for OAB/DO. OAB is characterized by a decreased bladder capacity, frequent voiding, frequent sensations of a strong urge to void, and in some patients, episodes of incontinence. DO is one or more uncontrolled phasic contraction(s) of the detrusor of at least 5 cm/H_20 pressure that are observed during urodynamic testing with or without urinary leakage.

Inclusion criteria will include clinical symptoms of overactive bladder of ≥ 6 months duration including at least one of the following:

a. Frequent micturition (≥ 8/24hrs)
b. Symptoms of urinary urgency (the complaint of sudden compelling desire to pass urine, which is difficult to defer) or nocturia (the complaint of *waking* at night two or more times to void)
c. Urge urinary incontinence (average of 5 per week – Urge urinary incontinence is defined as: the complaint of involuntary leakage accompanied by or immediately preceded by urgency)

Participants must also have a bladder scan at screening demonstrating a residual volume of ≤ 200 ml and detrusor overactivity documented during baseline urodynamic testing of ≥1 uncontrolled contraction(s) of the detrusor of at least 5 cm/ H_20.

[1] All female participants must be of non-childbearing potential (e.g., hysterectomy, tubal ligation or post menopausal defined as last menstrual cycle >12 months prior to study enrollment, or serum FSH >40 mIU/L).

Study Procedures

There will be a total of 9 visits (V) and a 1 and 3 day post dosing telephone contact with the patient to evaluate for any new complaints. Following screening (V1, V1A) and study drug administration at week 0 (V2), eligible participants will be evaluated for safety post dosing with study drug at weeks 1 (V3), 2 (V4), 4 (V5), 8 (V6), 12 (V7), and 24 (V8).

At Screening (V1), Baseline (prior to dosing) and 1 (V3), 2 (V4), 4 (V5), 8 (V6), 12 (V7) and 24 (V8) weeks post dosing participants will have a complete physical exam including urogenital examination. (ECG) will be performed at Screening (V1), at Baseline (V2 prior to dosing and at 2 hours post dosing), at one week post dosing (V3), 4 weeks post dosing (V5), and at the final visit (V8).

Laboratory evaluations including chemistry, hematology, and urinalysis will be performed at Screening (Week -2 (V1), Baseline (prior to dosing; V2) and Weeks 1 (V3), 2(V4), 4 (V5), 12 (V7) and 24 (V8) weeks post dosing. At Baseline (V2) no chemistries required. At V1A and prior to dosing at V2, urinalysis will be done by Dipstick. No chemistry or hematology required at V1A. No chemistry, hematology, urinalysis or urine culture required at V6. Urine cultures will be done at V1 (by catheterization with a urodynamic catheter), at V3 (clean void), at V1A, V2, V5 and V8 prior to cystometry or cystoscopy (by catheterization with the urodynamic catheter) and before discharge by clean void to insure the absence of infection (at V2 it will be the first clean voided urine after study drug dosing).

At Screening (V1) and at 2 (V4), 8 (V6), and 24 (V8) weeks post dosing participants will have a bladder scan to evaluate for residual volume. Participants will be assessed for study drug effects on incontinence at Screening (V1A), and at Week 4 (V5) and Week 24 (V8) by cystometry. Participants will complete a daily diary at home for 7 days prior to the baseline (prior to V2). Prior to V1A they should complete diaries as well which may be less than 7 days, but can be used to evaluate for compliance and inclusion criteria such as incontinence. Diaries will capture number of micturitions, volume of micturitions, number of urge incontinence episodes, and rating of episode, and the number of pads used every 72 hours. The participants will continue to complete this daily diary during the course of the trial and the diary will be evaluated at each follow-up visit. Diaries will be assessed at 1, 2, 4, 8, 12, and 24 weeks after treatment. Micturition diaries will be completed **daily for 7 days prior to treatment and 7 days prior to each visit after treatment. Pads will collected for up to 72 hours prior to V1A, prior to V2 and prior to all visits thereafter.**

The participants' perception of their bladder condition will be rated at Baseline (prior to dosing) and at weeks 1, 2, 4, 8, 12, and 24 post dosing and their opinion of response to treatment assessed after 1, 2, 4, 8, 12, and 24 weeks following the study drug administration. QoL will be assessed with the Kings Health Questionnaire (KHQ) and SF-12 at Baseline (prior to dosing) and thereafter at weeks 4, 8, 12, and 24 weeks post dosing.[1]

All participants will be administered the International Consultation on

	Incontinence Questionnaire – Short Form (ICIQ-SF) at Baseline and at 4, 8, 12, and 24 weeks following administration of study drug. In all participants, **urine and blood specimens** will be collected to assay for the presence of *h*Slo DNA by PCR. If they are positive, for the *h*Slo after 6 months, urine and/or blood specimens will be collected monthly thereafter (post study) until 2 consecutive specimens are negative. On the day of dosing, blood and urine will be collected pre-dosing and 2 hours post-dosing.
Primary Outcome	Safety will be assessed by analysis of adverse experiences, clinical laboratory tests, electrocardiogram, and physical examinations.
Secondary Outcomes	The mean number of micturitions per 24 hours will be evaluated at all visits and changes compared to baseline and placebo. The mean volume per micturition will be evaluated at all visits and changes compared to baseline and placebo. The change in the mean number of urge incontinence episodes per 7 day collection period and the urgency episodes per 24 hours will be evaluated at all visits and compared to baseline and placebo. The change in detrusor overactivity at week 4 and 24 will be evaluated and compared to baseline and placebo. Urgency will be rated with a 4 point validated urgency scale included in the voiding diary. Participants will rate their perceived bladder condition severity using a validated 6-point scale and assess their response to treatment. Improvement will be defined as a decrease of 1 point or more from baseline. Participants' assessment of Quality of Life (QoL) will be evaluated using the Kings Health Questionnaire algorithm and changes compared to baseline and placebo. Changes from baseline at the final visit (24 weeks) in bladder capacity will be evaluated and compared to baseline and placebo.
Study Duration	6 months (24 weeks) per participant with an additional 18 month safety follow-up.
Study Start	November, 2013
Sample Size	Maximum of 18 participants.
Statistical Analysis	Both the safety data and data to assess activity will be analyzed using summary descriptive statistics for the four cohorts and the total study population. The group sample sizes of 6 per arm achieve 80% power to detect a 30% reduction in frequency of voiding to placebo assuming a standard deviation of 2.5 with a significance level of 0.05 using a two-sided, two sample t-test. A comparison to baseline will also be done using a two-sided t- test. Total number of active treatment participants (N=12) will also be compared to total number of placebo participants (N=6).

Table 1. Procedures By Visit

Phase	Screening Phase			Post-Treatment Follow up Visits						
Visit/Period	**Visit 1**	**Visit 1A[n]**	**Visit 2**	**Telephone Follow-up[j]**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**
Day	**-14**	**-14 to -8**	**0 (Baseline)**	**Day 1 & 3**	**8**	**15**	**29**	**57**	**85**	**169 (Final)**
Week	**-2**		**0**	**0**	**1**	**2**	**4**	**8**	**12**	**24**
Visit Window (days)		**+2**	**+2**	**Day 3±1**	**+2**	**+2**	**±2**	**±3**	**±5**	**±5**
Signed Informed Consent	▲									
Evaluation of Inclusion /Exclusion Criteria	▲	▲	▲[f]							
Demographics and Medical / Surgical History	▲									
Physical Examination	▲		▲[f]		▲	▲	▲	▲	▲	▲
ECG	▲		▲[a]		▲		▲			▲
Previous / Concomitant Medication Assessment	▲	▲	▲[f]		▲	▲	▲	▲	▲	▲
Vital Signs [h]	▲		▲[f,l]		▲	▲	▲	▲	▲	▲
Objective OAB /DO Evaluation (Cystometry) [b]		▲[d]					▲			▲
Bladder scan [c]	▲					▲		▲		▲
Dispense Daily Voiding Diary/Urgency questionnaire [i]	▲	▲	▲[f]		▲	▲	▲	▲	▲	
Pad Test [m]		▲	▲		▲	▲	▲	▲	▲	▲
QoL (King's Health Questionnaire) and SF-12			▲[f]				▲	▲	▲	▲
Subjective Evaluation of Disease State [k]			▲[f]		▲	▲	▲	▲	▲	▲
Subjective Evaluation of Response to Treatment [k]					▲	▲	▲	▲	▲	▲
ICIQ-SF			▲[f]				▲	▲	▲	▲
Urinalysis and Urine Cultures [d]	▲	▲	▲[f]		▲	▲	▲		▲	▲
Hematology Laboratory Tests [e]	▲		▲		▲	▲	▲		▲	▲
Chemistry Laboratory Tests [e]	▲				▲	▲	▲		▲	▲
Pharmacokinetic Assessment (urine and blood *hSlo* cDNA)			▲[f,g]		▲	▲	▲	▲	▲	▲[g]
Adverse Event Assessment		▲	▲[f]	▲[j]	▲	▲	▲	▲	▲	▲
Study Drug administered			▲							

a. ECG will be done prior to administration of study drug and at 2 hours post dosing.
b. Cystometry includes: volume at first desire to void, detrusor pressure, abdominal pressure, detrusor pressure at beginning of voiding, detrusor pressure at maximum flow, maximum detrusor pressure, volume at strong urge to void, peak flow rate during voiding, voided volume, volume at DO, post-void residual volume, total bladder volume (voided volume + residual volume), number of detrusor contractions during procedure and duration of DO
c. Inclusion criteria specify residual volume ≤200 ml. Bladder scans at V1 and V8 to be done before catheterization.
d. Urinalysis with microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity at V 1, 3-5 and V7 and V8. At V1A and V2, urinalysis by Dipstick will be done. Urine cultures at V1 (by catheterization with the urodynamic catheter), V3 (clean void); at V1A, V2, V5 and V8 prior to cystometry or cystoscopy (by catheterization with the urodynamic catheter) and before discharge by clean void (at V2 use first voided urine after drug administration). Visit 2 urinalysis by Dipstick will be done prior to dosing and urine culture will be performed both prior to study drug administration and prior to discharge
e. Lab tests to be done at V1, V2 - 5, V7 and V8 include: Hematology- CBC with differential, platelet count, sedimentation rate, PTT, PT (no PT and PTT at V2 and V4), CRP, Antinuclear antibody; Fasting chemistry- BUN, creatinine, Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl^-, albumin, alkaline phosphatase, ALT, AST, GGT, total bilirubin, total protein, CPK, LDH, glucose; Serum Pregnancy Test for beta-HCG required for women of child bearing age who have not had hysterectomy at Screening V1 and on as need basis. In addition, FSH> 40 IU/L if last menstrual cycle not > 12 months prior to study enrollment. HbA1c will be done at screening Visit 1 only. No chemistries will be done at 2 (Week 0). At V4, chemistries will include only BUN, creatinine, electrolytes (Na^+, K^+), CRP, glucose, and ANA. No lab tests will be done at Visit 1A or V6. Lab tests should be taken at the same time of day at all study visits.
f. Test or procedure will be done prior to administration of study drug at Visit 2 . At V2 there is also a urine culture after dosing.
g. At V2, blood and urine pre-dosing and at 2 hour post-dosing. If specimen is still positive at week 24, participant must return monthly until two successive specimens are negative for *hSlo* DNA.
h. Vital signs will include height at V1 only; weight at V1 and V8; oral body temperature at all visits (except V1A). Same arm should be used for all BP measurements and specified.
i. Diaries are to be completed prior to V1A (to test for compliance and inclusion criteria), for 7 days prior to Visit 2 and 7 days prior to each visit, thereafter.
j. Participants will be contacted by telephone on Study Day 1 and 3 (1 day and 3 days ±1**,** following drug administration at Visit 2) for assessment of adverse events.
k. Subjective assessments are based on the following questions in Appendix C: "How bothersome do you consider your bladder problem?" and "Has the treatment been of benefit to you?"
l. BP will be taken every 15 minutes for 2 hour post administration of study drug.
m. Participants will bring in pads/diapers worn for 3 days prior to Visit 1A & 2 (if V1A after screening V1) and 3 days prior to all subsequent visits (Visit 3 to Visit 8); also bring in clean pad/diapers to use as baseline.
n. Visit 1A may occur on same day as V1. In this case all V1A procedures not already to be done at V1 should be completed. Cystoscopy should be performed after all other V1 procedures and post cystoscopy urine culture obtained using clean void. If V1A coincides with V1, then since pad collection and diaries will not be completed prior to V1, these must be checked for compliance at V2.

1.1 Introduction

1.2 Overview

Overactive Bladder Syndrome (OAB) resulting in urinary incontinence is a common and significant problem that affects millions of men and women in the United States. Although both age and disease (e.g., benign prostatic hyperplasia, stroke, and diabetes mellitus) contribute to the multifactorial nature of urinary incontinence, bladder overactivity, or involuntary, uncontrolled, spontaneous detrusor contractions, often leading to urge urinary incontinence (UUI) is a particularly prominent manifestation.[2, 3] Incontinence affects all ages, both sexes, and people of every social and economic level. It is estimated that over 17 million men and women in the United States have OAB[4]. It is also estimated that 15 to 30 percent of people over the age of 60 who live at home have incontinence. In addition, at least half of the 1.5 million Americans who reside in nursing homes are incontinent. Nevertheless, while OAB is often perceived as a condition of older people, about half of the people who suffer from this condition are between the ages of 35 and 64 years old.[5] Also, in the past it had been postulated that women were twice as likely as men to have this condition. However, OAB affects men and women at approximately the same rate (prevalence is 16.9% for women and 16.0% for men[6]), although the symptoms of urge incontinence are more prevalent among women (9.3%) than men (2.6%).[4] Men tend to have higher rates of "dry" OAB including symptoms of frequency and urgency. The National Overactive Bladder Evaluation (NOBLE) Program, a survey in over 5000 patients, supports these findings. In this study the overall prevalence of OAB did not differ by sex; however, the severity and nature of symptom expression did differ. Sex-specific anatomic differences may increase the probability that OAB is expressed as urge incontinence among women compared with men. Even so, OAB, with and without incontinence, has a clinically significant impact on quality of life, quality of sleep, and mental health, in both men and women. Despite this effect on quality of life, as many as 40% of the people with OAB do not discuss it with their physician or healthcare professional. Of those that do mention it, only 25% may receive pharmacological therapy.[7]

OAB is characterized by a decreased bladder capacity, frequent voiding, frequent sensations of a strong urge to void, and in some patients, episodes of urge incontinence or UUI which is often used interchangeably with OAB. According to the International Continence Society these symptoms are defined as follows[8:]

- Urgency: the sudden, compelling desire to urinate. This symptom can occur with and without urge incontinence (leakage). Sufferers experience strong, sudden urges to urinate, even if their bladder is not full.
- Frequency: urinating 8 or more times per 24-hour period.
- Urge Incontinence: an involuntary, accidental loss of urine, also called "leakage" or "incontinence."

Standardization of terminology in lower urinary tract function based on the report from the standardization sub-committee of the International Continence Society states that the term "overactive bladder" has replaced the previously used term "unstable bladder". "Detrusor overactivity" is the corresponding urodynamic term, replacing "detrusor instability" and

"detrusor hyperreflexia". Knowledge regarding the epidemiology of the overactive bladder is limited. The myogenic and neurogenic theories of pathophysiology require further evidence.[9]

The parasympathetic nervous system provides the major control of bladder contractility mainly through muscarinic receptors (M_2 and M_3) and is a key target for presently administered pharmacological therapy for patients with OAB/UUI and detrusor overactivity.

The current treatment of choice for OAB/UUI remains the non-selective muscarinic receptor antagonists (e.g. oxybutynin, tolterodine) that are associated with an increased incidence of dry mouth and other side effects. Newer more selective agents also display these adverse events. It is also established that 5-HT_4 receptors at prejunctional parasympathetic terminals can mediate the neurogenic potentiation of cholinergic transmission of human isolated bladder. [10] Mirebegron, a β3-agonist and smooth muscle relaxer, was approved by the FDA in 2012. The reported adverse effects were similar to antimuscarinics, but with a lower incidence of dry mouth compared with tolterodine.[11].

Overall, the mechanistic basis for increased detrusor overactivity is not fully understood, but the general importance of ion channel activity to bladder smooth muscle function is well established [2, 3, 12, 13, 14] The loss of smooth muscle control is likely to be a basic cause of urge incontinence.

pVAX/hSlo (*h*Maxi-K is the name we have established for theGMP product) consists of the gene for the α–pore subunit of the maxi-K channel, *hSlo*, inserted into a plasmid vector. Administration of this gene into the corpora increases expression of the maxi-K channel in the smooth muscle cells and an increased efflux of K^+ across the cell membrane resulting in decreased entry of Ca^{++} ions. A single instillation of 100 µg of *pVAX/hSlo* into the bladder has been shown to restore normal bladder function in rats with documented overactivity in an obstructed female rat model. [15] Decreased bladder spasms were observed in rats transfected with Maxi-K channel DNA and the restoration of smooth muscle demonstrated. As described below, more recent data in male and female rat models of obstruction-induced bladder overactivity also documents the efficacy of *h*Slo gene transfer. Consistent with these observations, Nelson and colleagues[16] have observed robust bladder overactivity in the Maxi-K α-subunit KO mouse. Moreover, this mouse Maxi-K α-subunit KO mouse appears to recapitulate relevant aspects of the corresponding clinical conditions in affected patients. The effect(s) on smooth muscle for OAB/UUI are likely to be very similar to those previously proposed mechanisms that ameliorated erectile dysfunction in the rat model. (See Figure 1 below depicting mechanism of action.)

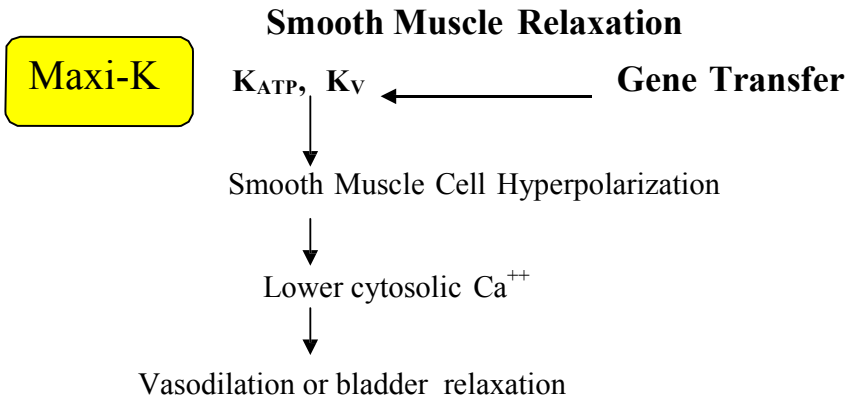


Figure 1. Maxi-K channel effect on smooth muscle

However efficacy over time may vary in different tissues, and moreover, in the bladder, it is conceivable that different cell types (urothelium and perhaps nerves, etc.) may participate in the therapeutic effect.[15] It is proposed that intravesical injections of Maxi-K channel DNA into the bladder of incontinent patients could reduce painful bladder spasm and improve bladder control.

1.2 Preclinical Data on Urinary Incontinence and *pVAX/hSlo*

The pathophysiology of partial urinary outlet obstruction in the rat model recapitulates many relevant aspects of the corresponding lower urinary tract symptoms observed in humans. [17, 18, 19] The noted physiological and pathophysiological similarities make it reasonable to assume that studies on the rat bladder will provide insight into at least some aspects of human bladder physiology and dysfunction.

Because the physiology of the rat bladder parallels many aspects of the human bladder[20, 21] studies examined the potential utility of bladder instilled K channel gene therapy with *h*Slo cDNA (i.e., the maxi-K channel) to ameliorate bladder overactivity in a rat model of partial urinary outlet obstruction. In one study twenty-two female Sprague-Dawley rats were subjected to partial urethral (i.e., outlet, PUO) obstruction, with 17 sham-operated control rats run in parallel. After 6 weeks of obstruction, suprapubic catheters were surgically placed in the dome of the bladder in all rats. Twelve obstructed rats received bladder instillation of 100 µg of *h*Slo/pcDNA in 1 ml PBS-20% sucrose during catheterization and another 10 obstructed rats received 1 ml PBS-20% sucrose (7 rats) or 1 ml PBS-20% sucrose containing pcDNA only (3 rats). Two days after surgery cystometry was performed on all animals to examine the characteristics of the micturition reflex in conscious and unrestrained rats. Obstruction was associated with a three to fourfold increase in bladder weight and alterations in virtually every micturition parameter estimate (see Table 2 below). Obstructed rats injected with PBS-20% sucrose routinely displayed spontaneous bladder contractions between micturitions. In contrast, *h*Slo injection eliminated the obstruction-associated bladder hyperactivity, without detectably affecting any other cystometric parameter. Presumably, expression of *h*Slo in rat bladder functionally antagonizes the increased contractility normally observed in obstructed animals and thereby ameliorates bladder overactivity. These initial observations indicate a potential utility of gene therapy for urinary incontinence. In fact, the dramatic bladder hypertrophy and detrusor overactivity associated with this in vivo rat model recapitulates many relevant aspects of human lower urinary tract symptoms [18, 22, 23, 24] and thus provides an excellent opportunity to further explore the utility of K channel gene therapy for the treatment of this condition and other smooth muscle disorders (i.e., erectile dysfunction, irritable bowel syndrome, and asthma).

Table 2. Summary of treatment effects on mean micturition parameters in 6 week obstructed female rats and sham-operated controls

	WT (mg)	MP	THP	BP	BC	MV	RV	MIP (IP-BP)
Control: unobstructed (n=17)	171 ± 15.0	73.9 ± 4.99	22.3 ± 2.1	12.6 ± 1.09	1.2 ± 0.1	1.13 ± 0.10	0.13 ± 0.04	3.49 ± 0.79
[a]Obstructed: pVAX/hSlo injected (n=12)	*547.6 ± 55.4	*128.9 ± 16.1	*36.3 ± 4.30	*22.1 ± 43.9	*3.44 ± 0.41	*3.22 ± 0.39	*,**0.3 ± 0.10	**5.59 ± 1.05
[b]Obstructed: untreated (n=10)	*473.1 ± 56.6	*132.7 ± 17.9	*39.3 ± 3.6	*18.8 ± 1.9	*2.91 ± 0.62	*2.94 ± 0.65	0.09 ± 0.05	9.37 ± 1.79

[a] 100 µg *pVAX/hSlo* in 200 µl PBS-20% sucrose

[b] 3 of these rats received 1000 µg pcDNA in PBS-20% sucrose. Control: Sham operated, unobstructed age-matched control animals, WT: bladder weight (mg), MP: micturition pressure (cm H_2O), THP: threshold pressure (cm H_2O), BP: basal pressure (cm H_2O), BC: bladder capacity (ml), MV: micturition volume (ml), RV: residual volume (ml), MIP: mean inter-micturition pressure ((cm H_2O; the mean pressure over the entire inter-micturition interval minus the basal pressure on the same animal).

* Significantly different from sham-op; $p<0.05$.

** Significantly different from control (obstructed but not treated); $p<0.05$, One-Way ANOVA, with Newman Keuls post hoc pairwise comparisons.

Another study examined the ability of *h*Slo gene transfer to alter and/or ameliorate the intermicturition pressure fluctuations observed in an obstructed male rat model. For these studies rats were obstructed for 2 weeks using a perineal approach.[25] Following 2 weeks of obstruction, the rats were catheterized for cystometric investigations as previously described[15, 25] and placed into 1 of 2 treatment groups. Age-Matched Control rats were subjected to a sham obstruction and run in parallel.

The mean values for the micturition parameters in all experimental animals are summarized in Table 3, and the salient features of these findings are graphically depicted in Figures 2 and 3. Importantly, as with the study in the 6-week obstructed female rat, a single intravesical instillation of 100 µg *h*Slo/pVAX was associated with statistically significant changes in several micturition parameters of major physiological relevance.

Table 3. Summary of treatment effects on mean micturition parameters in 2 week obstructed male rats and sham-operated controls.

	Bcap	MV	RV	BP	TP	MP	IMP	SA	Bcom	BW
pVAX (n = 8)[b]	**2.36 ± 0.48**	**1.84 ±0.31**	**0.53 ± 0.21**	**18.65 ± 5.38**	**47.21 ± 8.61[c]**	**91.28 ± 18.52[c]**	**32.49 ± 7.5[c]**	**13.84 ± 2.57[c]**	**0.12 ± 0.04**	**348.3 ± 105.3**
hSlo (n = 16)[b]	**2.48 ± 0.30c**	**2.22 ± 0.26[c]**	**0.27 ±0.12**	**7.66 ± 1.35[d]**	**27.26 ± 3.7[d]**	**54.05 ± 6.28[d]**	**18.13 ± 2.8[d]**	**10.47 ± 1.89[c]**	**0.17 ± 0.03**	**352.3 ± 42.99**
Sham (n = 10)[a]	**1.35 ± 0.14**	**1.32 ± 0.12**	**0.03 ±0.02**	**10.6 ±0.81**	**18.47 ± 0.79**	**46.58 ± 3.34**	**13.96 ± 1.09**	**3.39 ± 0.61**	**0.18 ± 0.018**	**274.4 ± 24.5**

Bcap, bladder capacity (ml); MV, micturition volume (ml); RV, residual volume (ml); BP, basal pressure (cm H_2O); TP, threshold pressure (cm H_2O); MP, micturition pressure (cm H_2O); IMP, mean intermicturition pressure (cm H_2O; the mean pressure over the entire intermicturition interval minus the basal pressure on the same animal); SA, spontaneous activity (cm H_2O); Bcom, bladder compliance (ml/cm H_2O); BW, bladder weight (mg).

[a] 5 of these animals are 2-week sham controls, the other 5 are 1 month older (or 6-week sham controls). However, statistical analysis revealed that there were no significant differences in any of the micturition parameters, and thus, these 2 populations were considered to be homogeneous for the purposes of this analysis.

[b] All treated rats were given 1000 µg pVAX alone or 100 µg *hSlo*/pVAX in 1 ml PBS with 20% sucrose. All data represent the mean ± S.E.M. and were analyzed using a one-way analysis of variance, with a post hoc Tukey's test for all pairwise (multiple) comparisons.

[c] Significant difference from the corresponding sham control value.

[d] Significant difference from the corresponding pVAX value.

As illustrated, 2 weeks of obstruction produces a general increase in bladder pressure. Specifically, BP, TP, MP, IMP and SA are all increased in the 2-week obstructed male rat, relative to the Age-Matched Control Sham-operated rat. The elevated bladder pressures observed in this rat model may well represent the clinical correlates of symptomatic urgency, and furthermore, if left unabated, may well predispose one to urge incontinence. More importantly, a single intravesical injection of 100 µg of *h*Slo/pVAX was associated with a statistically significant decline in the mean BP, TP, MP and IMP observed in pVAX-treated obstructed rats. Of note, the micturition pressure values observed after gene transfer were indistinguishable from those observed in the Sham rats. This is a clear indication of the potentially beneficial effect such therapy might be expected to have on patients experiencing urgency as a result of similar elevations in bladder pressure.

Interestingly, the pathophysiological manifestations observed in the 2-week male rat model of bladder overactivity (as judged by the differences in the measured micturition parameters) are apparently more severe than, and thus, at least in some respects, differ from our previous observations in the 6-week obstructed female rat model. Nonetheless, both models produce a consistent increase in inter-micturition pressures, which are the presumptive clinical correlates of urgency. As such, these data may indicate that *h*Slo gene transfer has therapeutic utility in more than one "type" of urgency and/or urge incontinence.

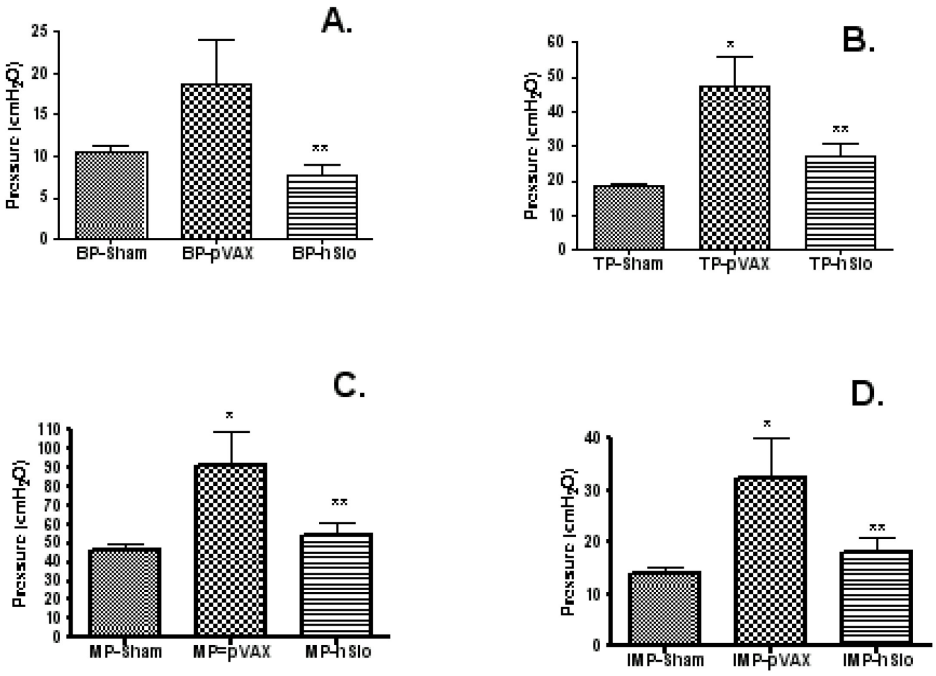
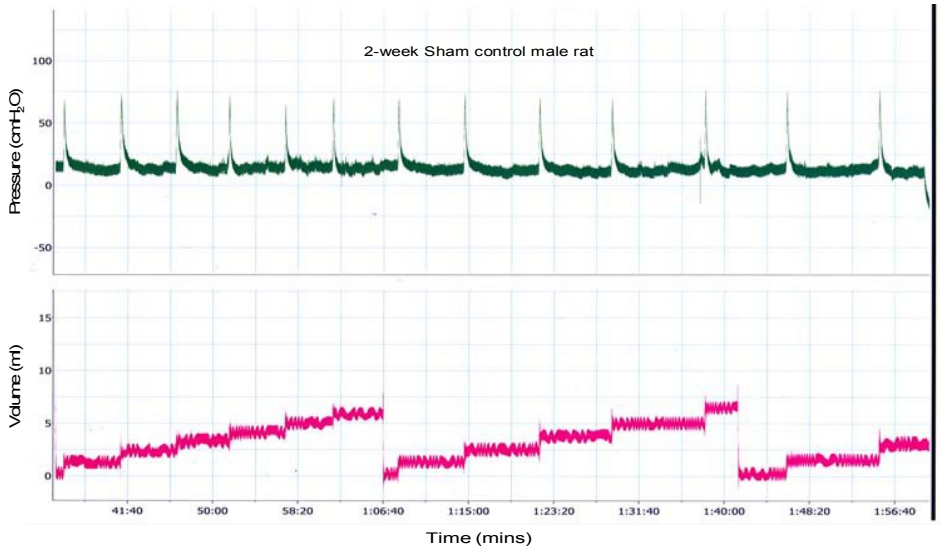


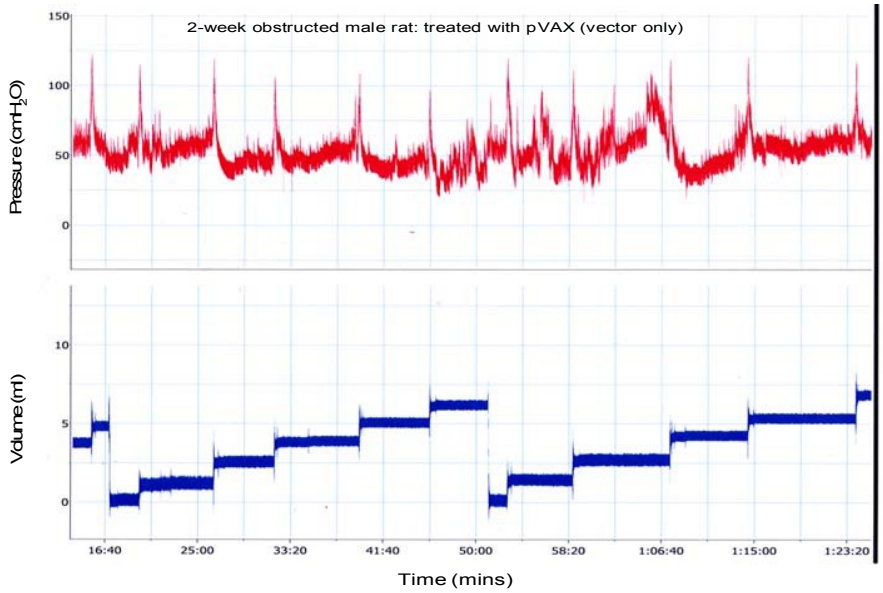
Figure 2. Graphical depiction of the impact of 2 weeks of obstruction on the relevant micturition parameters in the two treatment groups, relative to the Sham-operated, Age-Matched Control rats. Panels A-D represent the data summarized in Table 3.

Below, Figure 3 shows representative examples of cystometric recordings from distinct rats in each treatment group (Graphs A-C).

Graph A



Graph B



Graph C

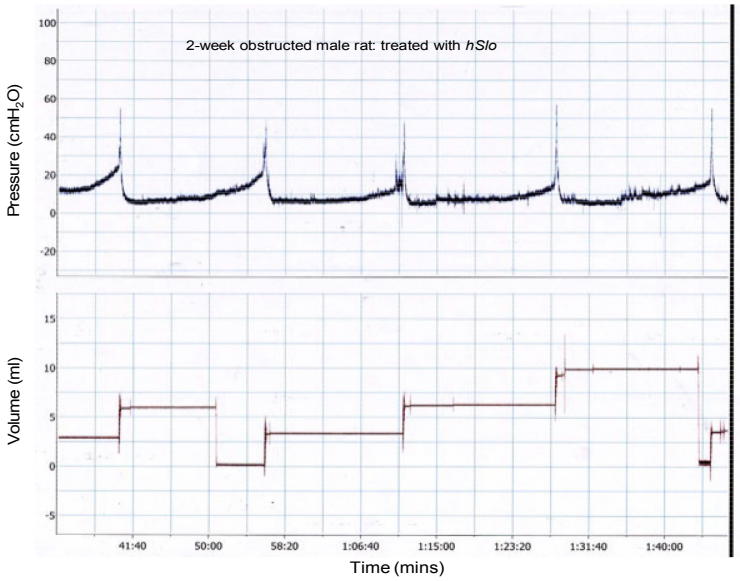


Figure 3. Graphs A-C are representative examples of approximately 1 hour of cystometric recordings from distinct rats in each treatment group.

A third study evaluated the effects of *hSlo* gene transfer following 2 weeks of partial urethral outlet obstruction in *female* rats. In order to create a partial urethral outlet obstruction (PUO), a ligature was placed on the urethra of female Sprague-Dawley rats weighing 200-250g (Christ et al., 2001) as described above. Two weeks after placement of the ligature, the rats were subjected to surgery for placement of a suprapubic catheter. Two days later, bladder function studies (i.e., cystometry) were performed on conscious, unrestrained rats in metabolic cages. As illustrated in Table 4 and Figure 4 following the 2 weeks of partial urethral outlet obstruction, female rats exhibit significant changes in bladder function, as evidenced by the more than 2-fold increase in bladder capacity and the appearance of significant spontaneous bladder contractions. The increased spontaneous bladder contractions were observed as pressure fluctuations between micturitions (see Figure 4), and can be quantified as shown in Table 4 by the corresponding increases observed in the SA and IMP values. A single intraluminal bladder injection of 300 µg and 1000 µg of pVAX/*hSlo* (in 1 ml PBS-20% sucrose) resulted in a nearly complete ablation of detrusor overactivity. This effect is reflected by the significant decrease in IMP and SA in the *hSlo*-treated, obstructed rats when compared with the rats treated with pVAX vector only (see Table 4). Although, a true DO effect relationship for *hSlo* gene transfer was not shown in this model, this study did demonstrate that over a 1-log unit variation in DO (from 100 to 1000 µg), there is a statistically significant, and moreover, physiologically relevant, diminution in DO, in the absence of any detectable effect on the ability of the bladder to empty. That is, in this animal model, pVAX/*hSlo* is able to ameliorate the pathophysiological effects of outflow obstruction-related DO, without having any detrimental effect on bladder function. Similar effects were observed after instillation of 100 µg *pVAX/hSlo* in the 6-week obstructed female Sprague Dawley rats, which are shown below.

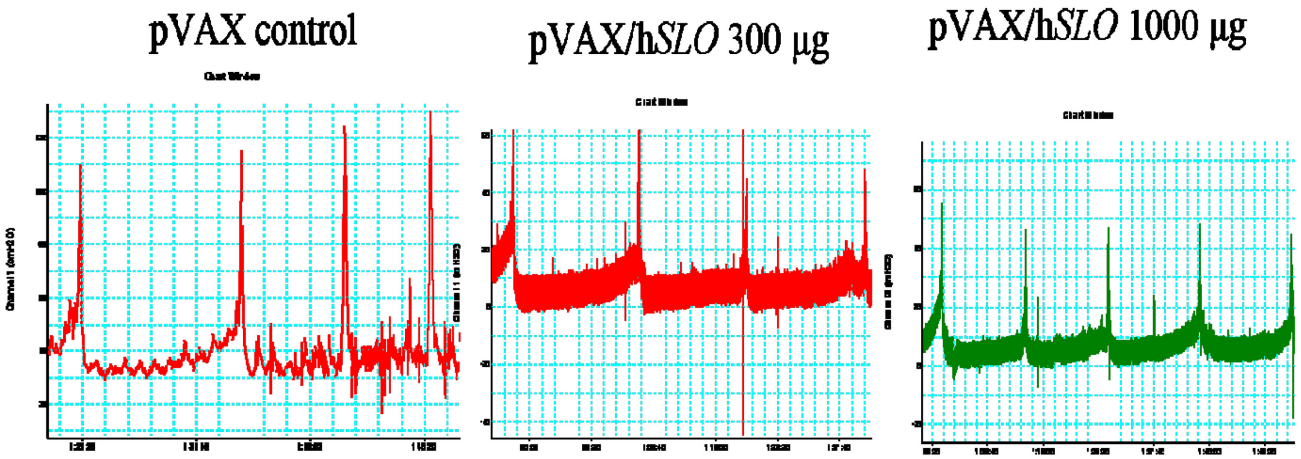


Figure 4. Representative tracing of a cystometric recording in a rat given vector alone (pVAX), and 300 and 1000 µg of pVAX/hSLO. Note the regular, periodic emptying and the virtual absence of intermicturition pressure fluctuations in the treated animals.

Table 4. Summary of treatment effects on mean micturition parameters in 2 week obstructed female rats

	MP	TP	BP	BC	MV	RV	MIP (IP-BP)	MF	SA	BCO
Control: pVAX (n=10)	68.1±8.1	34.2±4.9	9.1±1.9	2.3±0.3	2.2±0.3	1.1±0.0	24.0±4.6	4.6±0.5	14.9±3.4	0.1±0.02
[a]Obstructed: 10 µg pVAX/hSlo injected (n=7)	65.3 ± 10.5	30.3 ± 3.6	7.2 ± 1.0	2.5 ± 0.3	2.4 ± 0.3	0.2 ± 0.1	20.0 ± 3.5	4.4 ± 0.5	12.8 ± 3.0	0.1 ± 0.02
[b]Obstructed: 30 µg pVAX/hSlo injected (n=9)	81.1 ± 7.3	36.6 ± 4.4	11.8 ± 2.6	3.2 ± 1.0	2.7 ± 0.4	0.4 ± 0.2	27.1 ± 3.5	4.3 ± 0.4	15.3 ± 1.5	0.1 ± 0.02
[b]Obstructed: 300 µg pVAX/hSlo injected (n=10)	47.8 ± 3.7	17.7*,** ± 1.6	6.3 ± 1.1	2.3 ± 0.4	2.2 ± 0.3	0.3 ± 0.2	10.3*,** ± 1.2	5.3 ± 0.6	4.1*,** ± 0.4	0.2*,** ± 0.02
[b]Obstructed: 1000 µg pVAX/hSlo injected (n=12)	57.2 ± 6.2	21.4*,** ± 1.8	5.7 ± 1.1	2.1 ± 0.1	2.0 ± 0.1	0.1 ± 0.04	11.6*,** ± 1.3	5.2 ± 0.3	5.9*,** ± 0.5	0.1*,** ± 0.01

[a] 10, 30, 300, 1000 µg *pVAX/hSlo* in 200 µl PBS-20% sucrose

[b] Control: Obstructed age-matched control animals that received 1000µg of pVAX only, WT: bladder weight (mg), MP: micturition pressure (cm H_2O),

TP: threshold pressure (cm H_2O), BP: basal pressure (cm H_2O), BC: bladder capacity (ml), MV: micturition volume (ml), RV: residual volume (ml),

MIP: mean inter-micturition pressure ((cm H_2O; the mean pressure over the entire inter-micturition interval minus the basal pressure on the same animal);

SA spontaneous activity (MIP-BP); BCOM Bladder compliance (bladder capacity/TP-BP)

* Significantly different from control; $p<0.05$. All pairwise multiple comparison procedures (Holm-Sidak method)

** Significantly different from control; $p<0.05$, One-Way ANOVA.

A rabbit study to evaluate the distribution of different volumes of gene transfer injected into the bladder wall was performed prior to initiation of the clinical trial in women with OAB using direct intravesicular injections. Nine female Adult New Zealand white rabbits weighing an average of 6 pounds were used. The animals were anesthetized and pVAX-*lacz* was to be injected into the detrusor in 0.05, 0.1, and 0.15 ml aliquots into 4, 8, and 10 sites in the bladder wall. An additional set of 3 animals was to be injected with carrier alone at only the highest volume of carrier (4, 8, or 10 sites x 0.15 ml). The plasmids were in solution at a concentration of 4000 µg/ml. One week later the animals were euthanized and the bladders excised and weighed. Areas with blue color were prepared for histological examination and molecular analysis. Molecular analysis of *hslo* expression tissue was done with RNA extraction and real time PCR. In addition, histopathology was performed on the various rabbit tissues.

Table 5. Rabbit Intravesicular Injection Protocol

N=12 rabbits	50-50 mixture of p-VAX*hslo* (ml)	sites/ rabbi	sites/ rabbi	sites/ rabbi
	0.05	4	8	10
	0.1	4	8	10
	0.15	4	8	10

Due to difficulty with direct bladder injections in this animal model, only one rabbit was given the 0.05 ml injection. Six rabbits had 0.1 ml at 4, 8, and 10 sites (3 from inside the bladder; 3 from outside the bladder). Three rabbits had 0.15 ml at 4, 8, and 10 sites. Results indicated that those rabbits with a greater number of injections (8-10 injections) had less expression than some animals with the smallest number of injections (4 injections). The overall conclusion is that the direct injection into the bladder wall results in expression of the gene, however, it seems to work best with wider dispersion of the injections perhaps 1 cm apart. The gene was detected in the blood up until 30 minutes post treatment. There were granulomatous lesions observed due to the sutures (a common artifact in the rabbit model).

1.3 Preclinical Toxicology of *pVAX/hSlo* following Intravesical Administration

Tolerance and biodistribution data were evaluated after intracavernous (i.e., intravenous) injection of 1000 µg of *pVAX/hSlo* in a Sprague-Dawley retired breeder rat model of erectile dysfunction. Importantly, signal was not detected in either the testis or the heart beyond the 24 hour time point. A positive signal in the heart was never detected after the 1-hour time point; a time that surmised to be related to plasmid in the blood, since the kinetics of disappearance of the plasmid from the plasma was previously determined to be less than 0.5 hours for transition from supercoiled to nicked circular, and less than 2 hours for total DNA. Thus, even following an intracavernous administration, which is really equivalent to an intravenous injection, these data support the supposition that the gene transfer would not be expected to elicit any untoward systemic, in particular, cardiac effects.

For the OAB indication it is not technically possible to simulate the same transurethral route of intravesical administration of *pVAX/hSlo* in rats as will be used in the human trials. Therefore, in the toxicology and biodistribution studies evaluating intravesical injection of *pVAX/hSlo*, animals underwent surgical exposure of the bladder and study material was injected directly into the bladder using a needle.

The effect of *pVAX/hSlo* on hematological and chemical parameters were assessed in fifteen 275-300 gm normal female Sprague- Dawley rats. 1000 µg of either *pVAX/hSlo* (8 animals) or pVAX vector (7 animals) was injected directly into the lumen of the bladder following surgical exposure. Blood samples were collected via a heart stick immediately after the animals were euthanized by CO_2 anesthesia at 4, 8, and 24 hours and at 1 week following injection of test material. Samples were analyzed for glucose, urea nitrogen, creatinine, total protein, total

bilirubin, alkaline phosphatase. ALT, AST, cholesterol, sodium, potassium, chloride, A/G ratio, BUN/creatinine ratio, globulin, lipase, amylase, triglycerides, CPK, GTP, magnesium and osmolality. The laboratory parameters were similar between *pVAX/hSlo* and controls at the four timepoints.

The effect of *pVAX/hSlo* on the histopathology in female Sprague-Dawley rats (275 to 300 gm) was evaluated in two studies. In the first study, four rats underwent partial bladder obstruction surgery and 2 weeks later 100 µg *pVAX/hSlo* in 1000 µL PBS-20% sucrose was administered directly into the lumen of the bladder with surgical exposure of the bladder. A single animal was euthanized at 1, 8, and 24 hours, and at one week after injection of *pVAX/hSlo*. The tissues of47 organs were immediately fixed in 10% formalin and processed for routine histopathological examination. Histopathological changes were noted only in the bladder and consisted of serositis, edema, hemorrhage, and fibrosis. These changes were consistent with those expected with partial urethral obstruction and were not considered related to injection of *pVAX/hSlo*.

Because of the histopathological changes in the bladder of rats with PUO administered *pVAX/hSlo*, the effect of *pVAX/hSlo* compared to vector (pVAX) and PBS-20% sucrose on histology of the bladder was evaluated in normal rats. Following surgical exposure, the following test material was injected directly into the bladder lumen: 1) 0.6 ml PBS–20% sucrose, 2) 1000 µg pVAX in 0.6 ml PBS–20% sucrose, or 3) 1000 µg *pVAX/hSlo* in 0.6 ml PBS–20% sucrose. Animals were euthanized with CO_2 72 hours after instillation and the bladders removed and immediately fixed in 10% formalin solution. The 72 hour time point was chosen to limit the mechanical effects of the needle puncture on the bladder wall and minimize any potential effects of inflammation that might be caused by the *pVAX/hSlo* , vector, or diluent.

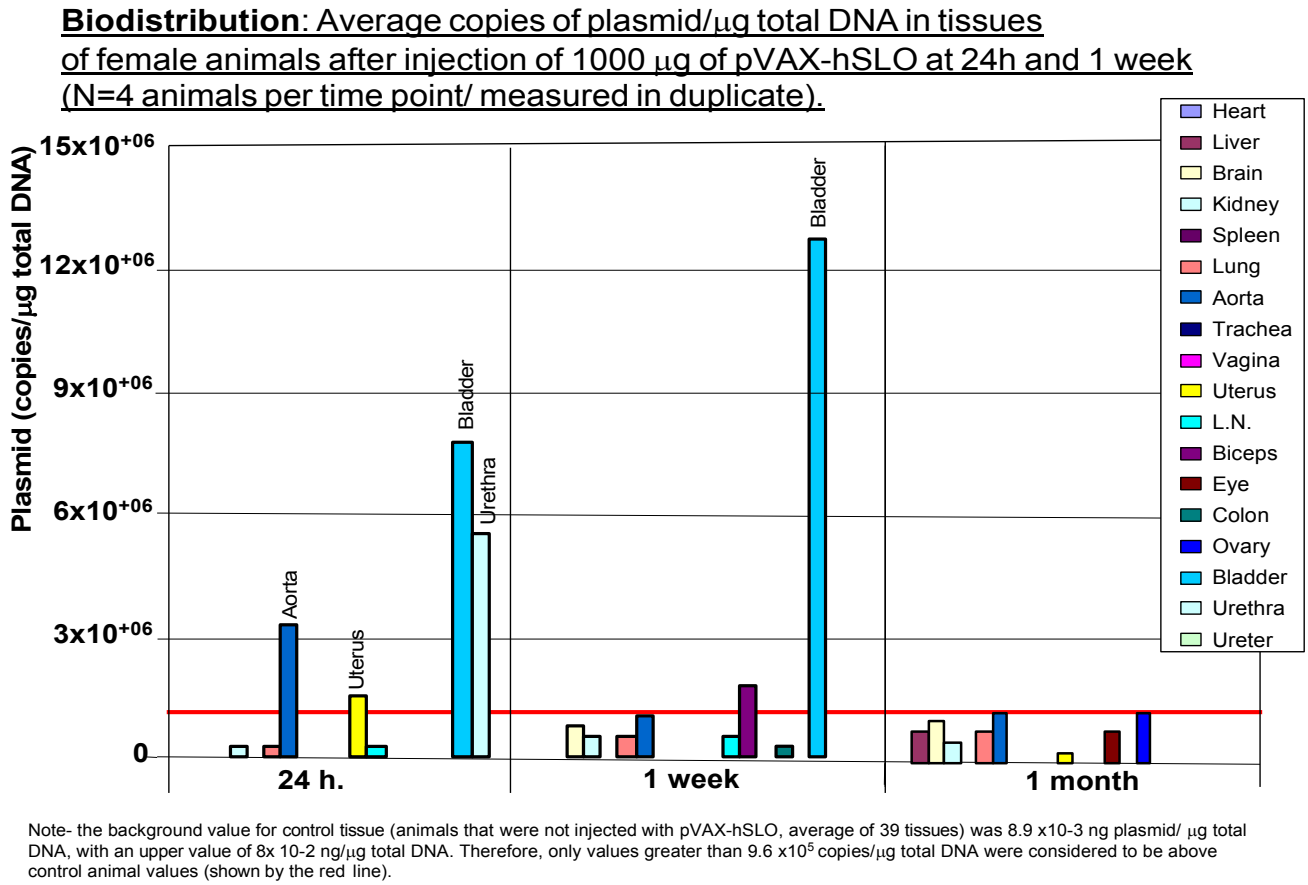
There were no gross findings on examination of the bladder. Overall, there were no treatment-related differences between *pVAX/hSlo* and either the vehicle or pVAX. No treatment-related alterations in the urothelium were noted. The lesions seen on histological examination were consistent with trauma from the needle used for injection since they were focal rather than diffuse or multifocal in distribution.

In the biodistribution study, test material was injected directly into the lumen of exposed bladders in 275-300 g normal Sprague-Dawley rats. 1000 µg *pVAX/hSlo* in 0.6 ml of PBS-20% sucrose was administered to 12 animals and 0.6 ml PBS-20% sucrose administered to 5 animals. Four animals each were sacrificed at 24 hours, 1 week, and 1 month following injection of test material. Tissue samples were collected in the specified order as follows: heart, liver, brain, kidney, spleen, lung, aorta, trachea, lymph node, eye, biceps, colon, vagina, and uterus.

Genomic DNA samples were analyzed for the *kanamycin* gene with a validated QPCR method. The results indicate that after injection of 1000 µg *pVAX/hSlo*, the plasmid could be detected after 24 hours in the aorta, uterus, bladder, and urethra. At 1 week, approximately 13 million copies/µg total DNA were measured in the bladder and *pVAX/hSlo* could also be detected slightly in the biceps. The results are displayed in graphical format in Figure 5 (below).

Although these results differ from findings after intracavernous injection, the detection of 13 million copies/µg total DNA is still lower than the <30 copies plasmid/10^5 host cells that persist at the site of DNA vaccine injections after 60 days in clinical Investigational New Drug (IND)

trials for these vaccines. These DNA vaccine studies have demonstrated that intramuscular, subcutaneous, intradermal, or particle-mediated delivery did not result in long-term persistence of plasmid at ectopic sites.[26] In addition, the procedure to inject *pVAX/hSlo* directly into the surgically exposed bladder in animals may explain the ability to detect plasmid in tissue other than the bladder. In humans, *h*Maxi-K will be instilled directly into the bladder using a using a transurethral catheter and the risk of plasmid distribution due to tissue damage or trauma is obviously markedly reduced.



Note- the background value for control tissue (animals that were not injected with pVAX-hSLO, average of 39 tissues) was 8.9 x10-3 ng plasmid/ μg total DNA, with an upper value of 8x 10-2 ng/μg total DNA. Therefore, only values greater than 9.6 x10^5 copies/μg total DNA were considered to be above control animal values (shown by the red line).

Figure 5. Biodistribution Data

Lastly, the half-life of the plasmid in human urine has been determined to be approximately 3.5 minutes, as compared to about a 30 minute half-life in blood.

1.4 Clinical Data

1.4. 1 Erectile Dysfunction

In humans, the safety and tolerability of *h*Maxi-K administered intracorporally has been assessed in an open-label single-escalating dose study that evaluated the safety and efficacy of doses

ranging from 500 µg up to 7500 µg (Stage 1 of study) and 8000 µg to 16000 µg (Stage 2 of study) in men with recalcitrant ED. Eleven participants received study drug in Stage 1 of the study and 9 participants received study drug in Stage 2 of the study. All participants were followed for 6 months during the conduction of the study and up to an additional 18 months thereafter. All doses were well tolerated. No dose limiting toxicities or significant adverse events occurred to prevent escalation to the next higher dose during this trial as determined by the Data Safety Monitoring Board. In Stage 1 of the study three men received the 500 µg dose, three men received the 1000 µg dose, three men received the 5000µg dose, and two men received the 7500 µg dose. In Stage 2 of the study, three men received 8000 µg, three men received 12000 µg and three men received 16000 µg).

In Stage 1, none of the reported adverse events occurred sooner than 29 days after the gene transfer and none of the events were considered related to the gene transfer product by the investigators. All three participants in the 500 µg dose group reported adverse experiences. One of these subjects had knee arthroscopy. The second participant had atrial flutter with ablation considered unrelated to study drug. This participant had a 25 year history of atrial arrhythmia prior to study entry and had been cardioverted to normal sinus rhythm before receiving *h*Maxi-K. He reverted to atrial flutter approximately 29 days after receiving the *h*Maxi-K. He was successfully cardioverted after this episode. The third participant in the 500 µg dose group had kidney stones removed by lithotripsy and was subsequently hospitalized with an infection following the lithotripsy. These last 2 events were reported as severe and serious adverse events.

One patient given 1000 µg *h*Maxi-K reported acid reflux, sciatic pain and an upper respiratory infection and one patient who received 1000 µg had a parasitic intestinal infection and foot edema. One patient given 5000 µg had bladder stone removal and neither patient given 7500 µg reported any adverse experiences.

There were no statistically or clinically significant changes from normal to abnormal in any blood chemistry, endocrine, hematology or urinalysis values seen at any visit for any subject. No patients reported any discomfort from the injection and no local physical events related to the injections were observed. No clinically significant changes were seen in the general or genitourinary physical examinations during the study. ECGs were monitored at one and three hours immediately following gene transfer and at one, 2, 4, 8, 12, and 24 weeks thereafter. No gene transfer related cardiac events were noted or reported during the study and no significant changes in electrocardiograms as determined by shift analysis (no normal to abnormal occurrences) were observed with the exception of the reoccurrence of atrial flutter in the one patient with the prior history of intermittent spontaneous atrial flutter. As noted above, this event was considered unrelated to treatment. In addition, the two unrelated serious adverse events in this trial were reported at the lowest dose of 500 µg dose. No serious adverse events were reported at the higher doses evaluated (1000, 5000, and 7500 µg) suggesting no increased risk for serious adverse events with increasing doses of *h*Maxi-K administered intracorporally. No residual *hSlo* DNA has been detected in semen.[27]

In Stage 2 of the study only 7 of the 9 men completed the trial.[28] Two participants had early discontinuation; one was a voluntary withdrawal in 8000 µg group and one was for lack of efficacy in the 12000 µg group. In Stage 2 of the study there was only one possibly related adverse event in one man in the 12000 µg dose group who reported tingling in the glans penis.

Other adverse events were considered unrelated to study drug. They included borderline QTc prolongation in one participant in the 8000 µg group, flu like symptoms in one participant in the 8000 µg group, allergic reaction in one participant in the 12000 µg group, right hand pain, penile pain and platelets =570 in one participant in the 16000 µg, and common cold in another participant in the 16000 µg group.

Although the primary objective of the study was evaluation of safety in participants with overall severe recalcitrant ED, efficacy was assessed using change from baseline in the International Index of Erectile Function (IIEF score – Erectile Function [EF] Domain). In this study, positive changes from baseline for most individual subjects were small and did not indicate improvement by IIEF. However descriptive analysis showed the following efficacy conclusions from a very small number of participants at each dose (without placebo control).

- Mean IIEF-EF domain scores for the two lower dose groups (500 and 1000 µg) fluctuated around the baseline score throughout the 24 weeks of the study.
- Improvements in the mean IIEF-EF domain scores observed for the 5000, 7500 and 12000 µg higher dose groups beginning two weeks after transfer.
- Improvements were maintained in the 3 groups throughout the 24 weeks of study.
- Individual subjects given the 5000, 7500 and 12000 µg *h*Maxi-K doses had apparent sustained improvements in IIEF-EF domain over the length of the study which was corroborated by their partner assessments.
- Improvements in the mean IIEF-EF sub domain scores (items 3 and 4) observed for the 5000 and 7500 µg dose groups beginning two weeks after transfer
- Improvements were maintained in the 3 groups through the 24 weeks of study
- Individual subjects given the 5000 and 7500 µg *h*Maxi-K doses had apparent sustained improvements of the IIEF-EF sub-domain over the length of the study
- Partner responses to Items 3 and 4 of the IIEF were consistent and validated the responses of each subject at each visit.
- Rigiscan also improved in one subject given 7500 µg.

1.4. 2 Overactive Bladder

Female patients with moderate OAB and detrusor overactivity (DO) were evaluated for safety following administration of a single intravesical instillation of *h*Maxi-K at three dose levels compared to placebo in a double blind increasing dose tolerance study. In this study *h*Maxi-K 5000 µg/90 ml, 10000 µg/90 ml, and 15000 µg/ 90 ml were to be evaluated, however only the 5000 and 10000 µg/90 ml doses groups were administered. The study was discontinued due to financial issues prior to administration of the 15000 µg/90 ml dose. There were 13 participants per dose level: 10 assigned to active treatment and 3 assigned to placebo (PBS saline = control group). Participants included women ≥ 18 years old of non-child bearing potential with OAB and detrusor overactivity who were otherwise in good health but unable to tolerate, did not wish to continue, or had unsuccessful results with, prior therapy for OAB/DH. They were to have clinical symptoms of overactive bladder of ≥ 6 months duration including at least one of the following:

- Frequent micturition (≥ 8/24hrs)

- Symptoms of urinary urgency
- Complaint of sudden compelling desire to pass urine, which is difficult to defer or nocturia (the complaint of *waking* ≥ 2 x at night to void)
- Urge urinary incontinence (average of 5 per week)
- Urge urinary incontinence is defined as involuntary leakage accompanied by or immediately preceded by urgency
- Residual volume of ≤ 200 ml by transabdominal bladder ultrasound at Screening
- DH [detrusor overactivity] during baseline urodynamic testing of ≥1 uncontrolled contraction(s) of the detrusor of at least 5 cm/ H20.

Participants completed cystometry testing at baseline (prior to dosing) and at Week 4 after dosing, a daily diary at home for 7 days prior to baseline to capture the number of incontinence episodes per week, number of micturitions every 24 hours, volume voided, number of pads used every 24 hours. Micturition diaries were completed daily for 3 days prior to each visit after instillation and quality of life assessments done. Ten participants received the 5000 µg/90 ml dose (7 in this group completed the study; 3 discontinued early seeking other treatment for their OAB), 6 participants received the 10000 µg/90 ml dose and 5 participants received placebo (all participants completed in the 10000 µg/90 ml and placebo groups). There were no serious adverse events reported or a withdrawal for an adverse event. In the 5000 µg/90 ml dose, possibly related adverse events reported in one subject each, included urinary tract infection (UTI); AV block Mobitz type II (possibly related; mild severity, 170 days post treatment); fatigue, headache, shaking chills, and insomnia (all possibly related; mild-moderate severity; 13-76 days post treatment). Unrelated events in one subject each (unless otherwise specified) included headache; rash (2 subjects); fractured left (big) toe; infection left (big) toe; worsening oflabial adipose lipoma; knee tendonitis; right kidney cyst benign; worsening of depression; worsening of GERD; elevated creatinine; diffuse loss of renal parenchyma; hypertensive renal disease; atypical chest pain; CK elevated (possibly secondary to hypertensive renal disease); CK-MB elevated ((possibly secondary to hypertensive renal disease); diarrhea; vomit, hiatal hernia; bacteriuria, asymptomatic; constipation; fatigue; fall; upper lip abrasion secondary to fall; acne on face; reflux; and UTI (2 subjects). In the 10000 µg/90 ml dose group, adverse events were all considered unrelated and included in one subject each, common cold; elevated blood pressure; worsening of urge incontinence; tension headache; low back pain; fall; dysuria; UTI, elevated LFTs; and hepatitis C. In the placebo group heart palpitations were reported in one participant, which were considered possibly related, mild severity, and occurred 6 days post treatment. Unrelated events for placebo that were reported in one participant each included slight irritation at vulva, fractured pelvis (left side) and wrist fracture (left); 4 unrelated UTIs were reported in 2 participants.

The study was designed as a safety study in a moderate to severe OAB patient population; however secondary efficacy endpoints were evaluated in the small number of subjects enrolled per dose. Overall findings showed no significant changes for the mean number of voids or urge incontinence episodes at any time point. However, despite the severity of the OAB population and the small number of subjects, there were some positive findings. There was a trend of mean treatment effect at Week 8 ($p = 0.0812$) with >40 % mean decrease for 5000 µg/90 ml for urge incontinence episodes. In addition, there was a significant mean treatment effect of reduced detrusor contractions at Week 24 ($p<0.0508$) (>80% reduction vs. baseline in contractions in the 5000 µg group at Week 24). There was also a decrease in mean pad weight indicative of less

incontinence in the 10,000 µg/90 ml group vs. placebo which was a significant mean change from baseline (p = 0.0089) at Week 24. Quality of life parameters (King Health Questionnaire) showed some clinically significant mean changes for active treatment vs. placebo and across treatment groups at Week 24; this included the General Health Perception (p = 0.0272, 5000 µg vs. placebo) and Impact on Life (p = 0.0032, 5000 µg vs. placebo).

2.1 Objectives

The primary objective of this study is to evaluate occurrence of adverse events and their relationship to a single treatment of approximately 20 to 30 bladder wall intramuscular injections of *h*Maxi-K compared to placebo (PBS-20% sucrose). Two dose levels (16000 µg and 24000 µg) in females with moderate OAB/DO of ≥ six months duration are initially planned**.** In each dose level, 6 participants will receive *h*Maxi-K and 3 will receive placebo. **Pending safety and efficacy results for the 2 doses that are planned, the protocol may be amended to investigate additional doses in this study.**

The secondary objectives of this study are to evaluate the following safety parameters:

- Clinical laboratory tests- changes from baseline
- Electrocardiogram change from baseline
- Physical Examination- changes from baseline

These changes will be evaluated primarily at the individual level, through clinical assessment of outliers supported by descriptive statistics.

Additional secondary efficacy objectives focus on the potential activity of intramuscular injections into the bladder wall of *h*Maxi-K compared to placebo. Two dose levels (16000 µg, and 24000µg) in female participants with moderate OAB/DO of ≥ six months duration are initially planned. Individual efficacy parameters that will be evaluated as secondary endpoints are:

- Mean number of micturitions per 24 hours –change from baseline
- Mean volume per micturition – change from baseline
- Mean number of Urge Incontinence episodes –change from baseline
- Overall bladder capacity – change from baseline (as defined in section 6.1)

3.1 Study Design

This study is a double blind, placebo-controlled, multicenter, sequential active dose, phase 1 study in females with moderate OAB/DO of ≥ six months duration.

The proposed study period is approximately 6 months following a single administration of study drug per participant. The protocol sample size of this phase I trial is not intended to find statistically significant differences between placebo and treatment for the primary endpoint as outlined in 2.0. Rather, the sample size takes into account clinical safety considerations. However, the study is designed to observe a 30% reduction in the number of voids following gene transfer using the following calculations with NCSS/PASS program (NCSS, Kaysville, Utah, 84037).

Power[29,30,31]	N1	N2	Ratio	Alpha	Beta	Mean1	Mean2	S1	S2
0.80251	9	10	1.111	0.05000	0.19749	10.0	6.6	2.5	2.5

Up to 9 female participants (6 participants on active treatment and 3 on placebo) will be enrolled per dose level "arm". The 2 active doses to be evaluated sequentially are 16000 µg and 24000µg compared to intramuscular injections into the bladder wall of PBS-20% sucrose (control group). Additional participants may be enrolled for evaluating the tolerability to a given dose. Each arm of 9 participants per dose level will be enrolled sequentially, beginning with the lowest dose. Enrollment of the first 5 participants in each cohort will be managed directly by the sponsor (or their designee) with a 2-day waiting period following each participants dosing. The next participant will be enrolled only after the site has contacted the previously dosed participant on day 3 following transfer to determine if a clinically significant adverse event occurred. The site will contact the clinical monitor with the result of that contact. In the event that no event occurred the next participant will be enrolled. The DSMB will review eligibility and all available safety data after the 5th participant has been administered study drug in the first dosing cohort. This first review will occur as soon as possible after the 5th participant's 3-Day Post-visit 2 telephone contact. Following their review of the safety data, the DSMB will recommend whether enrollment into the first dosing cohort may proceed. This process will be repeated through the 5th participant in each cohort before the balance of the cohort (4 additional participants) is enrolled. If a clinically serious sign or symptom is reported the medical monitor will contact all the sites and no further enrollment will be done until the medical monitor or sponsor gives permission. In addition, enrollment into the next cohort or arm in the series (at the next highest dose) will be dependent on safety dose-limiting toxicity assessments as per Section 5.2.

3.2 Duration of study participation

The study will consist of up to a 14-day screening period prior to the single administration *h*Maxi-K or PBS-20% sucrose placebo on Day 0 (V2). A 6 month period for follow-up will complete the study for each participant. The total participation duration will be approximately 184 days; however each participant on active treatment will be required to be followed for an additional 18 months (every 6 months) for safety evaluations only (see Section 7.12).

4.1 Study Population

The study population includes women (of non-childbearing potential) with OAB/DO who are otherwise in good health.

The study population, if they meet the criteria for eligibility, will be selected from the urology practice of 1 to 6 participating sites.

4.2 Inclusion Criteria

1. Healthy women of 18 years of age or older and non-childbearing potential (i.e., prior hysterectomy or bilateral tubal ligation; last menstrual cycle more than 12 months prior to

study enrollment or serum FSH >40 IU/L if woman has not had a bilateral tubal ligation or hysterectomy).

2. Clinical symptoms of overactive bladder for 6 months or longer including at least one of the following:
    a. Frequent micturition ≥8 times per 24 hrs
    b. Symptoms of urinary urgency (the complaint of sudden compelling desire to pass urine, which is difficult to defer) or nocturia (the complaint of *waking* at night two or more times to void)
    c. Urge urinary incontinence five or more incontinence episodes per week based on 7-day voiding diary. Urge urinary incontinence is defined as: the complaint of involuntary leakage accompanied by or immediately preceded by urgency.
3. Detrusor overactivity with ≥1 uncontrolled phasic contraction(s) of the detrusor of at least 5 cm/ H_20 pressure documented on cystometry at Screening Visit 1A (i.e., prior to study drug administration at Visit 2).
4. Bladder scan or direct catheter drainage demonstrating a residual volume of ≤200 ml
5. Non-response or response but poor tolerance to a previous treatment for symptoms of overactive bladder/urinary incontinence (e.g., anti-muscarinic/ anticholinergic agents, or Botox) or do not wish to continue these treatments.
6. Able to understand study requirements (i.e., literate in English), give written informed consent, and comply with all study procedures and requirements.

4.3 Exclusion criteria

1. A woman with a positive serum (HCG) pregnancy test or lactating. (All women of child bearing years who have not had a hysterectomy will have a serum HCG.)
2. History of three or more culture documented recurrent urinary tract infections per year.
3. Current history of bladder outlet obstruction secondary to urethral stenosis, third degree cystocele, or obstruction from prior urethral sling surgery documented by cystoscopy, urothelial cancer, or other significant genitourinary disorders except incontinence (previous vesicle lithiasis or bladder stones that have been successfully removed are not an exclusion).
4. Current history or previous diagnosis of painful bladder syndrome (interstitial cystitis) with pain in the region of the pelvis, perineum, or lower abdomen relieved by voiding.
5. A urine culture (≥ 1000 colonies/ml) of a urinary pathogen from a catheterized urine obtained at screening.
6. Current history of neurological bladder dysfunction.
7. A life expectancy of less than 12 months.
8. Current history of Grade 2 or greater cystocele.

9. Stress urinary incontinence as determined by observation of the participant coughing while standing with a full bladder and/or response of 2 or 3 on the following Stress Urinary Incontinence question:

   Do you experience leakage when laughing, coughing, lifting heavy objects or other types of discreet, moderately intense activities?

   0= NONE: No leakage
   1= MILD: Minimal leakage on rare occasions during these types of activities; easily tolerated; do not use pads for this.
   2=MODERATE: Enough leakage that it requires occasional use of pads and may interfere with usual activity & tasks
   3 =SEVERE: Extreme leakage & discomfort that stops all activity &/tasks and requires use of pads on all occasions

10. An indwelling urethral catheter or need for clean intermittent self-catheterization.
11. Any screening laboratory values outside of the normal laboratory range, as defined by the laboratory normal ranges and in the judgment of the investigator is considered clinically significant (hepatic biochemical markers ≤ twice the upper limit of the normal reference range may be accepted with written consent of sponsor).
12. A hemoglobin A1c >7% in a person with diabetes.
13. A history of sickle-cell disease, sickle cell trait, or any other medical condition that, in the judgment of the investigator, would produce significant risk to the patient.
14. In the judgment of the investigator any condition that would interfere with participation in the study (including geographical inaccessibility), that would contraindicate the administration of study medication or interfere with the study evaluations (e.g, potentially poor compliance in completion of diaries).
15. A current malignancy (basal cell carcinoma is not an exclusion).
16. Had within six months prior to enrollment any of the following:
    - Myocardial infarction
    - Cerebrovascular accident
    - Uncontrolled hypertension (systolic >160 or diastolic >100mmHg)
    - Arrhythmia
    - Congestive heart failure (dyspnea on minimal exertion or while supine)
    - Unstable angina (chest pain greater than three times weekly while on therapy)
    - Required treatment with calcium channel, beta-blocker medication, nitrates, or anti-epileptic drugs
17. Latex allergy (unless the facility can treat the participant with all latex free supplies).
18. Allergy to lidocaine, xylocaine or other local anesthetics.
19. Known current drug addiction and/or alcohol abuse.

20. Mental or legal incapacity.
21. Abnormal screening ECG as defined by the over reader (some abnormalities considered benign may be acceptable with the written consent of the Sponsor).

NOTE: Sinus bradycardia of 50-59 bpm is permissible. Other abnormalities that can be normal variants (and considered clinically insignificant) may be permissible. However, participants with such abnormalities cannot be randomized without review of their medical history and prior written approval of the Sponsor (or designee).

22. QTc of ≥ 450 ms (via 12-lead resting ECG using both Fridericia's and Bazett's formula) and/or family history of sudden death or participants with a family history of long-QT Syndrome.
23. Any current treatment or treatment within the last 14 days for OAB including herbal preparations. Botox within the last 6 months.
24. If the participant has used a bladder-training program, she must still fulfill inclusion criteria for symptoms, and exercise/training program must be continued without change.
25. History of urinary retention, or residual bladder volume > 200 ml) (evaluated by bladder scan).
26. Treatment with any compound likely to increase urine production rate, or any compound likely to affect detrusor mechanics or voiding habits (see section 5.4 for full listing of prohibited medication classes). Thiazide diuretics alone or in combination with antihypertensive medications are acceptable if the dose and dose regimen is kept constant throughout the study and symptoms of OAB preceded these treatments.
27. Participation in any other interventional or investigational clinical study during the last two months before inclusion, or during the study period.

5.1 Test Material and Administration

5.2 Study Medication

*h*Maxi-K is a double stranded naked plasmid DNA molecule carrying the human cDNA encoding the gene for the α, or pore forming, subunit of the human smooth muscle Maxi-K channel, *hSlo*. Expression of the *hSlo* gene is under control of the CMV promoter positioned upstream of the transgene and the construct also contains the Bovine Growth Hormone poly A site.

*h*Maxi-K is manufactured by Althea Technologies, Inc under GMP conditions. Sterile borosilicate vials of *h*Maxi-K, 2 ml, will be shipped to Sharp Clinical Services, Inc. (Phoenixville, PA) and stored at -20°C. Identical vials of 2 ml sterile PBS-20% sucrose manufactured by Olympia Compounding Pharmacy (Orlando, FL) will be shipped to Sharp Clinical Services, Inc. and stored at –20°C.

The table below provides information on the 16,000 µg and 24,000 µg doses of *h*Maxi-K. Each 2 mL vial contains 4000 µg per mL, so multiple vials will be used for each dosing event. The sites will store the vials in a locked freezer until use. Participant vials will be retained at the site until

the drug accounting processes have been completed, when they will either be returned to the Sponsor or destroyed with Sponsor approval.

Table 6. Final Dose-*h*Maxi-k

*h*Maxi-K Dose	16,000mcg	PBS-20% sucrose	24,000 mcg	PBS-20% sucrose
Volume	4 mL		6 mL	
Number of Vials	2		3	
Final Volume	4 mL		6 mL	
Number of IM injections	20 injections of 0.2 ml at specified sites in bladder wall approx. 1 cm apart		30 injections of 0.2 ml at specified sites in bladder wall approx. 1 cm apart	

Note: In each dose cohort 6 participants will receive *h*Maxi-K and 3 will receive PBS-20% sucrose (placebo).

Sharp Clinical Services, Inc. will maintain a randomization schedule generated by a Statistical Group (Section 5.3.1). Following notification by the site that an eligible participant is to be randomized, Sharp Clinical Services. Inc. will ship a sequentially labeled study drug participant kit following the central randomization schedule by overnight mail on ice within one to seven days prior to the planned treatment date. The sites will store the participant kit at -20^{o}C in a locked freezer prior to use. Vials can be kept at -20^{o}C for up to 360 days. Participant kits will be retained at the site until study completion when they will either be returned to the sponsor or destroyed.
If there is a single site, then Sharp will be allowed to ship all kits to that site to be distributed by the site's local pharmacy in a sequential fashion. Prior to dosing each participant, the site will confirm which kit number they will be using with the clinical research monitor to minimize errors.

If a kit is damaged, a replacement kit will be made available. Prior to using a replacement kit (which will be shipped to the site from Einstein where will it be stored), the site will contact the clinical research monitor for directive as to which replacement kit they can use as a substitute for a damaged kit. The replacement kit number will be provided by the **unblinded** statistician. If there is an additional site included, then the drug supply may be shipped to Einstein from the single site, where it will be stored and distributed in a sequential and blinded fashion to the sites by overnight mail on ice within one to seven days prior to the planned treatment date.

The individual participants' double blind patient drug kit will contain a two-part (tear-off) label. The duplicate portion of this label will be detached and affixed to a label page (to be retrieved at study close out by Ion Channel Innovations designated monitor) at the time of randomization and test drug administration. There will be central randomization. The sites will be instructed to call the Sponsor or designee for the assigned subject number after each participant's screening visit allowing ample time prior to Visit 2 for shipment of the drug.

The duplicate label from each patient drug kit will be affixed to a separate log in the source documents at Visit 2.

The double-blind patient drug kit is to be given to the participant will be supplied as indicated above and labeled with tear-off labels containing the following information:

- Study ION-03 OAB
- Subject identification number (kit number)

- Contains two or three *2* mL sterile borosilicate vials *(*each vial contains either 4000 µg per mL *h*Maxi-K or PBS-20% sucrose placebo)
- INSTRUCTIONS: Inject 0.2 mL of study drug into the bladder wall (as directed at 20 sites for Cohort 1 and at 30 sites for Cohort 2) approximately 1 cm apart.
- Store kit at -20° C in a locked freezer prior to use. Store at controlled room temperature once thawed: 15º C to 30º C (59º F to 86º F).
- CAUTION: New Drug – Limited by Federal Law (or United States) to investigational use.
  Ion Channel Innovations, LLC
  969 Park Avenue, Suite 1G
  New York, NY 10028

The lot # for *h*Maxi-K used in this study is FIN0400 and the lot # for PBS-20% sucrose is FP-012-092906.

The gene product or PBS-20% sucrose will be prepared and administered in the same manner as Botox® is administered in the office. The participant kit will be taken from the freezer and the vials warmed to room temperature and the cap of the vial will be wiped with alcohol. The 2 or 3 vials in the kit will be administered sequentially. This sequential administration of vials for a total of 4 ml or 6 ml volume maintains the study blind while minimizing the risk for bacterial contamination that could occur if the vials were mixed prior to administration.

To minimize the possibility of dehydration and/or a vasovagal response after required blood collections or the *h*Maxi-K administration, participants will be instructed to eat breakfast and abstain from any alcohol products on that morning.

The investigator (or designee) should administer the drug and must remain blinded. The investigator's pharmacy or a designee will be responsible for dispensing the drug in a timely fashion for use on the day of the participant's Visit 2 and accounting of all drug provided by the sponsor. Records of document control numbers and dates received will be kept on a Drug Inventory Form provided by the sponsor for accounting purposes. Under no circumstances will the investigator supply study drug to other investigators, allow study drug to be used other than directed by this protocol, or destroy or dispose of study drug in any other manner without prior written authorization from the sponsor.

All partially used clinical trial kits will be accounted for and destroyed at the investigative site according to IBC guidance. The investigator or designee, upon completion of the drug accounting process, will send all unused clinical materials back to the Sponsor or designee for disposition.

5.3 Dose Selection and Administration

Two doses of *h*Maxi-K will be evaluated: 16000 µg and 24000 µg.

Up to 9 female participants (6 participants on active treatment and 3 on placebo) will be enrolled per dose level "arm". The 2 active doses to be evaluated sequentially are 16000µg and 24000µg compared to direct intramuscular injection into the bladder wall of PBS-20% sucrose (control group). Additional participants may be enrolled for evaluating the tolerability to a given dose.

Each arm of 9 participants per dose level will be enrolled sequentially, beginning with the lowest dose. Enrollment of the first 4 participants in each cohort will be managed directly by the sponsor (or their designee) with a 2-day waiting period following each participants dosing. The next participant will be enrolled only after the site has contacted the previously dosed participant on day 3 following transfer to determine if a clinically significant adverse event occurred. The site will contact the clinical monitor with the result of that contact. In the event that no event occurred the next participant will be enrolled. The DSMB will review eligibility and all available safety data after the 5th participant has been administered study drug in the first dosing cohort. This first review will occur as soon as possible after the 5th participant's 3-Day Post-visit 2 telephone contact. Following their review of the safety data, the DSMB will recommend whether enrollment into the first dosing cohort may proceed. This process will be repeated through the 5th participant in each cohort before the balance of the cohort (4 additional participants) is enrolled.

If a clinically serious sign or symptom is reported the medical monitor will contact all the sites and no further enrollment will be done until the medical monitor or sponsor gives permission. In addition, enrollment into the next cohort or arm in the series (at the next highest dose) will be dependent on safety dose-limiting toxicity assessments as per Section 5.2 There has been no toxicity observed at any dose level in any of the preclinical or clinical trials at any dose to date, including multiple dosing in the ED rat model. In the completed clinical study evaluating intracorporal administration of up to 16000 µg *h*Maxi-K in participants with ED, there was only one adverse event considered possibly related to *h*Maxi-K ("tingling warmth in the glans").

In the previous OAB trial there were several events considered possibly related: UTI; AV block Mobitz type II (possibly related; mild severity, 170 days post treatment); fatigue, headache, shaking chills, and insomnia (all possibly related; mild-moderate severity; 13-76 days post treatment). In the placebo group heart palpitations were reported (possibly related, mild severity, occurred 6 days post treatment).

The obstructed bladder pre-clinical studies in the rat evaluated single doses of up to 1000 µg based on surface area of the bladder. In the rat ED model, doses of up to 1000 µg were administered by intracavernous injection. In men with ED, doses of up to 16000 µg were administered by injection into the corpus cavernosum (similar to iv). For ease of administration (based upon manufacture and storage of the *h*Maxi-K), during this OAB trial 16,000 and 24,000 µg of *h*Maxi-K have been chosen for bladder wall injection. These concentrations of drug are well within the doses used in the preclinical studies. The doses evaluated in this trial will be equivalent to 240 and 480 µg used in the rat studies.

In addition to preclinical toxicology data, selection of dose was also based upon activity seen in the preclinical pharmacology studies. In the Partial Urethral Obstruction (PUO) studies in female rats, 100 µg in one ml bladder instillation demonstrated highly significant changes. The 100µg dose also corrects detrusor overactivity in the male PUO model. Therefore, based upon preclinical results one would expect to observe an effect in the lowest dose. A rabbit study to evaluate the distribution of different volumes of gene transfer injected into the bladder wall was performed prior to initiation of the clinical trial using direct intravesicular injections in women with OAB. Nine female Adult New Zealand white rabbits weighing an average of 6 pounds were used. The animals were anesthetized and pVAX-*lacz* was to be injected into the detrusor in 0.05, 0.1, and 0.15 ml aliquots into 4, 8, and 10 sites in the bladder wall. An additional set of 3 animals was to be injected with carrier alone at only the highest volume of carrier (4, 8, or 10 sites x 0.15

ml). The plasmids were in solution at a concentration of 4000 microgr/ml. One week later the animals were euthanized and the bladders excised and weighed. Areas with blue color were prepared for histological examination and molecular analysis. Molecular analysis of *hslo* expression tissue was done with RNA extraction and real time PCR.
One rabbit was given the 0.05 ml injection. Six rabbits had 0.1 ml at 4, 8, and 10 sites (3 from inside the bladder; 3 from outside the bladder). Three rabbits had 0.15 ml at 4, 8, and 10 sites. Results indicated that those rabbits with a greater number of injections (8-10 injections) had less expression than some animals with the smallest number of injections (4 injections). The overall conclusion is that the direct injection into the bladder wall results in expression of the gene however, it seems to work best with wider dispersion of the injections perhaps 1 cm apart.

5.2.1 Stopping Rules

No acute toxicity was seen in the preclinical studies in rats with administered intravenous doses up to 1000 μg (approximately 1500 μg/kg in the rat). Potential toxicities would be those that could occur (1) because of enhanced efflux of K^+ with resultant decreased intracellular Ca^{++} concentration, thus altering smooth muscle function in diverse organ systems; (2) reaction to the naked DNA.

In the event that any participant experiences one of the following events, **no** additional participants will be accrued and **no** further doses of hMaxi-K will be administered until the DSMB and FDA have reviewed the case and have provided their recommendation to the sponsor.

1) Urinary retention, i.e., the inability to micturate for more than 12 hours with a urine bladder volume (greater than 400 ml established by bladder scan) that requires placement of a bladder drainage catheter.
2) Any adverse event of Grade 2 that last longer than 96 hours or Grade 3 or higher in the blood chemistry, completed blood count or urine categories of adverse event criteria listed in Appendix A.
3) In the event of a clinically significant sign or symptom reported by the site PI to the medical monitor in any of the first four participants of each cohort unless permission is given by the medical monitor.
4) Bladder pain and /or irritation that persists for four hours or more after gene transfer that is thought by the Principal Investigator not to be related to acute trauma from the cystoscopy, direct bladder muscle injections, and catheterization.

Clinical data regarding the event will be collected, reviewed, and discussed with DSMB and FDA. After review of these data with FDA the clinical study may resume **only** with concurrence of FDA.

5.2.2 Dose Escalation

The DSMB will review participant's eligibility data and all unblinded safety data **at 4 weeks** following administration of study drug for the last participant enrolled into each dose cohort, in

order to assess the need for modifications in safety monitoring and/or dosing. Following their review of the safety data for the last participant in the cohort at the 4th week post-dosing, the DSMB will recommend whether or not enrollment into the next dosing cohort may proceed (Section 11.2).

The DSMB will inform the sponsor or designee of their decision to dose escalate in writing.

The DSMB will also review all safety data following the occurrence of any event throughout the study that would stop the clinical study (section 5.2.1) within a reasonable period of time following the occurrence of the event. Additional meetings may be schedule if requested by the DSMB.

The DSMB will review the unblinded safety data from the participants in all the cohorts at the six month timepoint following the final dose.

5.3 Randomization and description of blinding methods

Sites will be provided blinded treatment code identities. For each participant there is supplied a tear off label which should be kept in the participant's source records, affixed to a study treatment label page. This removable panel contains a scratch off laminate which would serve to unblind the treatment if necessary. The DSMB will receive the blinded codes from unblinded statistician.

The treatment code must not be broken by the site unless there is an emergency situation where the appropriate management of the participant necessitates knowledge of the treatment allocation. Every attempt must be made to contact appropriate sponsor personnel prior to breaking any participant's treatment code.

5.3.1 Assigning participants to treatment group

The study is a randomized study with an imbalanced-block design (e.g., 6 active and 3 placebo participants per treatment group). As each participant signs a consent form, they will receive a sequential participant number according to their chronological order of screening within each site. This will be their Screening number. The schedule of randomization numbers and sequence will be generated centrally by the CE3 Inc. Statistical group. This code will be provided to the packaging group (Sharp Clinical Services Inc.) who will package the study drug and blind the random number with a corresponding kit number.

When a participant is qualified for randomization and meets all of the inclusion criteria and none of the exclusion criteria, the site requests a shipment of a study kit from the Clinical Supplies Manager who assigns and ships the next available numbered kit as per the assignment schedule to the site. The kit number allocated will be documented in the participant's electronic case report form (E-CRF) and source notes. This will be the participant's assigned kit number which will be their random number provided by CE3 Inc. Statistical group.

5.3.2 Packaging and labeling

Each participant's assigned kit will contain a two-part (tear-off), label. The label will contain appropriate information including the study number, contents, dosing directions, name of sponsor, storage conditions, packaging lot number, treatment number, visit number. The duplicate portion of this label will be detached and affixed to a page in the source documents as outlined in section 5.1.

5.4 Concomitant Medications/Therapies

Participants will continue any medications they are receiving at study entry for underlying medical conditions with the exception of those described below. All medications taken by participants within 2 months of study entry (including over-the-counter preparations) will be recorded on the Concomitant Medication Form. Any changes in concomitant medication, including additions, discontinuations, and dose changes, occurring during the study will be recorded on the concomitant medication form. If participants are receiving hormone replacement therapy, dosing should be stable for at least 2 months prior to enrollment and remain stable throughout the study.

Participants are not allowed to concomitantly use any approved, unapproved, or investigational medications or therapies intended to treat OAB/UUI. Anticholinergic or antimuscarinic agents are not permitted. These medications/therapies include, but are not exclusively limited to Detrol® (tolterodine), Ditropan® (oxybutynin), Enablex® (darifenacin), VESicare® (solifenacin), Sanctura™ (trospium), ddAVP. In addition, Botox® (Botulinium Toxin A) or related products are not permitted. Use of Myrbetriq® (mirebegron) is also an exclusion. Agents known to affect urine production output or detrusor function should also be avoided or kept constant throughout the study (if permitted by Sponsor), including: adrenoreceptor (alpha1a subtype) antagonists; adrenoreceptor agonists (beta2 and beta3 subtypes); phosphodiesterase-1 inhibitors; adenyl cyclase stimulators; neurokinin receptor antagonists (NK-1, NK-2 subtypes); potassium channel activators; calcium channel blockers; serotonin reuptake inhibitors (serotonin-noradrenalin uptake inhibitors); cyclooxygenase-2 inhibitors. Thiazide diuretics alone or in combination with antihypertensive medications are acceptable if the dose and dose regimen is kept constant throughout the study. All other diuretics will be excluded, unless approved by the Sponsor or their designee prior to the participant's entry into the study. The investigator should consult the Trial Monitor for any specific questions related to concomitant medication initiation or change. Participants should not take any antiplatelet including over the counter NSAIDs and/or anticoagulant drugs for at least 10 days prior to the treatment visit.

6.1 Outcome Assessments and Procedures

6.2 OAB / DO Objective Assessment: Cystometry

Clinical assessment of the efficacy hypothesis that *h*Maxi-K will reduce detrusor overactivity. Detrusor overactivity will be assessed with two standardized, sitting position, saline cystometries followed by pressure flow studies (see **Appendix D**). Every attempt should be made to perform the studies at the same time of day and in the same way for each participant. Studies are to be completed at V1A (screening), V5 (4 weeks post dosing with study drug) and V8 (or Final Visit).

An over-reader will interpret the results as well as the investigator. If there is a discrepancy between the reads, then the central over reader's interpretation will take priority.

Table 7: Cystometry Parameters and Definitions

Parameter	Description	Definition
DO; Yes/No	Detrusor overactivity ≥ 5 cm H_2O	Any involuntary contraction that has a pressure >/= 5 cm H_2O **(INCLUSION CRITERIA)**
$V_{First\ des}$ (mL)	Volume at first desire to void	The volume attained during filling cystometry that would lead the patient to pass urine at the next convenient moment but voiding can be delayed if necessary *(ICS definition)*.
$P_{det.\ open}$ (cm H_2O)	Detrusor pressure at beginning of voiding (prior to onset of first contraction (either volitional or involuntary)	Detrusor pressure at onset of the first detrusor contraction (either volitional or involuntary).
V_{1st} (mL)	Volume at first involuntary contraction; if detrusor overactivity (DO) present. Not applicable if no DO present.	Volume infused at the point of maximum detrusor pressure during the 1st contraction. [1]
Det_{frq}	Total number of detrusor contractions during procedure (voluntary **AND** involuntary)	Total number of detrusor contractions including voluntary **AND** involuntary
Leak (Yes/no)	Was there a leak with overactivity	Involuntary detrusor contractions resulting in leak (NOT counting voluntary voids).
DO $_{Leak\ freq}$	Number of involuntary detrusor contractions resulting in leak (if DO present)	Number of involuntary detrusor contractions resulting in leak (if DO present).[1]
V_{leak} (mL)	If yes, Volume of each leak	Volume of each leak for each episode of leak[2]
$V_{leak\ cum}$ (mL)	If yes, cumulative volume of ALL leaks during procedure	Cumulative volume of leaks (calculated by computer from individual volumes)[2]
DO $_{No\ Leak\ freq}$	Number of involuntary detrusor contractions NOT resulting in leak.	Total number of involuntary detrusor contractions that do not have an associated leak. [1]
$P_{det\ Max}$ (cm H_2O)	Maximum detrusor pressure at involuntary contraction	Maximum amplitude of detrusor contraction during an involuntary contraction (if present) [3]
$P_{det\ 1st}$ (cm H_2O)	Maximum detrusor pressure at FIRST contraction	Max detrusor pressure at **FIRST** contraction (voluntary or involuntary). [1]
$P_{Det\ Any}$ (cm H_2O)	Maximum detrusor pressure at ANY contraction	MAXIMUM detrusor pressure attained during the entire study (voluntary or involuntary). [4]
Cys Cap (mL)	Cystometric Capacity: Volume at strong urge to void	The volume at which the patient feels that he/she can no longer delay micturition (has a strong desire to void). *(ICS definition)*
Q_{max} (mL / sec)	Peak flow rate during voiding (for both leaks and voluntary voids)	The Maximum rate of flow during any void (for both involuntary and voluntary voids)
V_{voided} (mL)	Total voided volume (leaks and voluntary)	Total volume voided for leak and volitional voids during the procedure
PVR (mL)	Post Void Residual Volume (PVR) (from catheterization)	Volume left in bladder after voiding at the conclusion of the study. This is measured by urodynamic catheterization.
($V_{total\ bladder}$) (mL)	Total Bladder Volume (Total voided volume +catheterized PVR)	Post void residual volume + total voided volume (will be *calculated*)

DO_{dur} (seconds)	Duration of detrusor overactivity	Duration of MAXIMUM detrusor activity. [2]	

$P_{Det,}$ Q_{Max} (cm H_2O)	Detrusor pressure at maximum flow during an involuntary contraction	Maximum detrusor pressure during terminal void (voluntary or involuntary). [2]
$V_{bladder\ DO}$ (mL)	Bladder volume at each involuntary detrusor contraction	Infused bladder volume at the peak pressure of each involuntary contraction minus the amount leaked at each point for each involuntary contraction; Total will be calculated by the computer. [2]
P_{abd} (cm H_2O)	Maximum abdominal pressure during any contraction	MAXIMUM abdominal pressure during **ANY** contraction. This will assess the abdominal straining component to voiding. [2]
1. This is done by overreader, but not the sites 2. Done by site only 3. Detrusor pressure at first involuntary contraction is called "**Detrusor Pressure**" for sites on eCRF. 4. Maximum detrusor pressure at ANY contraction is called "**Maximum Detrusor Pressure**" for sites on eCRF.		

Digitized recordings should be captured and stored for each patient. Waveforms from filling cystometries and pressure flow studies will be interpreted by a trained professional for each parameter noted above in table. Printed waveforms should be kept with the patient's source records, with items such as artifacts and peak pressure measurements clearly identified (see details in **Appendix D**).

Table 7. Procedure Flow Guidance on Cystometry Visit Days (and Cystoscopy)

Review diaries, pads, con meds, and AEs; ECG, physical exam, vital signs, QoL assessments, questionnaires	Prior to cystometry
Bladder Scan	Bladder scan at V8 to be done before catheterization for urine culture.
Urinalysis	Prior to cystometry (by Dipstick at Visit 1A)
Urine culture	Prior to cystometry by catheterization and repeat prior to discharge by clean void
Urine/blood for *hSlo* cDNA detection	Prior to cystometry (not at Visit 1A)
Phlebotomy	Prior to cystometry (not at Visit 1A)
Cystometry	Should be done exactly the same way at each visit
Cystoscopy: Visit 2 Only	

Table 7. Procedure Flow Guidance on Cystometry Visit Days (and Cystoscopy)

Review diaries, pads, con meds, and AEs; ECG, physical exam, vital signs, QoL assessments, questionnaires	Prior to cystoscopy and drug administration
Urine/blood for *hSlo* cDNA detection	Prior to cystoscopy and drug administration
Urinalysis	Prior to cystoscopy (by Dipstick at Visit 2 prior to drug administration)
Urine culture	Prior to cystoscopy by catheterization and repeat prior to discharge by clean void (first clean void after study drug administered)
Phlebotomy	Prior to cystoscopy
Administer the local anesthesia through a catheter	At Visit 2 only; after all required Baseline procedures completed
Cystoscopy	At Visit 2 only, insert cystoscope 10-20 minutes after local anesthesia given
Study drug administration through a cystoscope	At Visit 2 only, 20-30 injections in bladder wall depending on dose
Blood pressure	BP will be taken every 15 minutes for 2 hour post administration of study drug.
Urine/blood for *hSlo* cDNA detection; ECG	2-hours post-dosing

6.3 Clinical Evaluation of OAB / DO

6.3.1 Voiding Diary

Clinical signs and symptoms of OAB / DO will be determined by a daily voiding diary. The participants must complete the diaries for the 7 days prior to the Baseline visit (visit 2), and for 7 days prior to visits 3-8. The participants will also complete the diaries prior to visit 1A to test for compliance and evaluate for some inclusion criteria. The site will contact the participant 7-8 days prior to the scheduled visit (V2-8) to remind the participant to complete the diary. The daily voiding diary will collect standard bladder function and frequency parameters includingfrequency of micturitions, volume voided and urgency episodes. The urgency questionnaire is a 4 point scale. **See Appendix F**.

6.3.2 Pad Test

Each participant will record their use of any pads/panty liners (e.g., Poise® pads or Depends®, etc.) in their diary. The participant will bring in pads/diapers worn prior to Visit 1A and for the **three days** prior to Visit 2 (as well as a new pad/diaper to use as a baseline) and for the 3 days prior to all subsequent visits (V3 to V8). Used pads/diapers will be stored in a plastic bag

provided by the site and weighed upon receipt and compared to the clean pad. All pads/diapers used by the participants for the 7 days prior to each visit, will be counted by the participants and entered in the voiding diary by themselves.

6.3.3 King Health Questionnaire and Assessment of Disease State/Treatment

Patients will also complete a disease-specific quality of life tool, the King's Health Questionnaire (KHQ) at Baseline (V2 prior to dosing), V5, V6, V7, and end of study (V8). See **Appendix B**. Patients will also be queried on subjective assessment of their disease state at Baseline (V2), and subsequent visits. Participants will also be asked to evaluate their response to treatment at Visits 3 to 8. Participants will rate their perceived bladder condition severity at Baseline (V2) and Visits 3 to 8 using a 6-validated point rating scale (1, no problems; 2, very minor problems; 3, minor problems; 4, moderate problems; 5, severe problems; and 6, many severe problems). Participants' assessment of their response to treatment will be measured by asking "Has the treatment been of benefit to you?" with possible responses of no benefit; yes, a little benefit; and yes, very much benefit. **See Appendix C**.

6.3.4 International Consultation on Incontinence Questionnaire-Short From

The International Consultation on Incontinence Questionnaire- Short Form will be completed in this study by all participants at V2, 5, 6, 7, and 8. Please refer to **Appendix E**.

6.2. 5 SF-12

The SF-12 which includes 12 questions evaluating general quality of life will be completed in this study by all participants at V2, 5, 6, 7, and 8. Please refer to **Appendix G**.

6.4 Bladder Scan

Residual volume will be measured using a scan of the bladder. This examination will be carried out at the Screening visit (V1) and V4, 6, and 8. At Visit 1 and Visit 8 urine cultures by catheterization should be done after bladder scans are performed. Copies of the bladder scan figures supplied by the device or a report of the examination must be appended to the patients' source documents.

6.5 Physical Evaluation

6.5.1 Physical Examination

A complete physical examination, including a complete urogenital exam, and review of body systems will be performed at V1 to V8 (excluding V1A). Vital signs (pulse, respiratory rate, oral body temperature, and blood pressure) *will be performed at all visits as below (except V1A)*. Height will be obtained at Screening V1 only. Weight will be obtained at Screening V1 and V8. Any untoward clinically significant change from Screening will be recorded on the E-CRF as an adverse event.

6.5.2 ECG

A 12-lead ECG will be performed at Screening (V1) Baseline (V2 -prior to treatment and 2 hours post treatment), V3, V5 and V8. These ECGs obtained at the study centers, will be forwarded to a central reader for final interpretation by a board-certified cardiologist or physician board certified in ECG interpretation. The over reader will return the results to the study center within 48 hours. This does not negate the responsibility of the staff conducting the study to initially review the ECG and to take appropriate clinical actions. The following parameters will be assessed: heart rate, rhythm, PR interval, RR interval, QT interval, QTcF, QTcB, QRS duration, and overall evaluation. For the Visit 2 pre-dose ECG, if abnormalities or clinically significant changes are noted from V1 screening ECG, then the investigator in conversation with the Sponsor and/or designee will determine the participant's eligibility to proceed with dosing.

6.5.3 Vital Signs

Vital signs will be performed at all visits as above (excluding V1A). Vital signs should include an apical pulse measurement and brachial blood pressure both taken after 5 minutes resting in the sitting position (standard sphygmomanometry). For all blood pressure measurements, the same arm should be used throughout the study and whether the right or left arm is used should be specified. O*ral body temperature will be obtained at all visits (except V1A).* Any clinically significant changes from screening will be recorded on the E-CRF as an adverse event. Following administration of study drug at V2, BP and pulse will be measured and recorded every 15 minutes for 2 hours.

6.6 Laboratory Safety Tests

Laboratory safety tests will be performed at Screening (V1), Baseline (V2) and 1 (V3), 2 (V4), 4 (V5), 12 (V7), and 24 (V8) weeks post dosing with study drug.

The laboratory safety tests include:

- Hematology: V1, V2-V5, V7-V8; Complete blood count (CBC) with differential, platelet count, sedimentation rate (sed. rate), PT and PTT (no PT and PTT at V2 and V4).
- Fasting chemistry: V1,V3-V5, V7-V8; Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Mg^{++} , Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, CPK, total bilirubin, total protein, C-reactive protein (CRP), antinuclear antibody (ANA), and glucose. V4 chemistry testing: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+), C-reactive protein (CRP), glucose, and ANA only.
- At screening, participants of child bearing years must have a serum pregnancy test for beta- HCG if they have not had a hysterectomy. In addition, they must have either a serum FSH >40 IU/L or a history of no menses for more than 12 months to confirm post-menopausal status if a hysterectomy or bilateral tubal ligation has not been done.
- At screening only, participants must have a serum hemoglobin A1c performed

- Urine analysis; Conducted at V1, V1A (by dipstick), V2 (by dipstick), V3-V5, V7-V8; microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity. Dipstick will measure blood, leukocyte esterase and nitrites.

- Urine culture: At Screening Visit 1 and 1A (collect by catheterization) and at V3. At Visit 1A, Visit 5 and Visit 8, prior to cystometry (collect using urodynamic catheter) and repeated with clean void prior to discharge. At Visit 2, collect using urodynamic catheter prior to cystoscopy and first clean voided urine after study drug dosing. If V1A cystometry performed at V1 then urine cultures pre-and post-cystometry should be done using urodynamic catheter before cystometry and repeated with clean void prior to discharge.

6.7 Concomitant Medication

At baseline, a history of concomitant medications taken within the past 2 months with special attention given to treatment with anticholinergics, antispasmodics or antimuscarinic medications (including mirebegron and Botox) will be completed. At subsequent visits, assessments of any changes in medication for all prescription or nonprescription medications will be performed.

6.8 Pharmacokinetics

Urine and blood specimens will be obtained at Visit 2 (pre-dosing and 2-hour post dosing) and Visits 3-8 for analysis of *hSlo* cDNA with PCR. If DSMB states specimens are still positive after 6 months, then the participant will be required to return monthly until 2 consecutive specimens are negative and this may unblind this participant.

Instructions for collection of urine and blood for DNA testing:

Blood

- Collect specimen in heparinized tube
- Centrifuge for 10 minutes at 3000 rpm
- Pipette plasma, freeze immediately and store in labeled plastic vial at -20 (or colder)

Urine

- Collect urine sample in sterile container, label and freeze immediately at -20 (or colder)

6.9 Laboratory Evaluations

A local safety laboratory (for example Quest) will perform analysis of all blood and urine specimens. The blood samples for the safety laboratories evaluations should be taken when the participant is fasting if possible. In addition, the participant should be asked to not perform any strenuous exercise or activities prior to blood sampling.

The urine analysis done immediately prior to cystometry and drug administration will be performed by the PI with a Dipstick™ to see if the Dipstick™ is positive for leucocytes and

nitrites which may be a sign of infection. A spun microscopic evaluation may be required for further evaluation,

Urine and blood specimens that are collected from the participants will have real time PCR testing done for detection of *hSlo* cDNA in the Urology laboratory at the Albert Einstein College of Medicine. It is anticipated that the following amounts of blood will be collected for the following procedures over the 6 month study participation:

Table 8. Blood collection (minimum amount of blood that should be taken)

Procedure	Visit number	Blood volume drawn (mL)	Total blood volume drawn (mL)
Chemistry	1, 3, 4, 5, 7 and 8	15	90
Hematology	1, 2, 3, 4, 5, 7 and 8	3.0	21
Blood for analysis of *hSlo* cDNA)	2 (pre- and 2 hr post-dosing), 3, 4, 5, 6, 7 and 8	0.5	4
Minimum volume of blood drawn for study over 6 months:			115 ml

Table 9. Summary of Laboratory Tests By Visit								
	Chemistry Fasting	**Hematology**	**βHCG**	**HA1c**	**FSH**	**Urine Cultures**	***hSlo Blood/ urine***	**Urinalysis**
Visit 1	♦	♦	♦	♦	♦	1 (one from catheter drainage) – after bladder scan		♦
Visit 1A Cystometry						2 (one from catheter drainage pre-cystoscopy and 1 from first clean void post-cystoscopy)		via dipstick
Visit 2 (Treatment by Cystoscopy)		♦ No PT/PTT				2 (one from catheter drainage pre-treatment and 1 from first void post-treatment)	♦ (blood and urine: pre-dosing and 2 hours post-dosing)	via dipstick (prior to treatment)
Visit 3	♦	♦				1 (clean void)	♦	♦
Visit 4	♦	♦ No PT/PTT					♦	♦
Visit 5 Cystometry	♦	♦				2 (one from catheter drainage pre and 1 from first clean void post)	♦	♦
Visit 6							♦	
Visit 7	♦	♦					♦	♦
Visit 8 Cystometry	♦	♦				2 (one from catheter drainage pre and 1 from first clean void post-treatment); Bladder scan prior to catheterization	♦	♦

7.1 Schedule of Evaluations

The schedule of evaluations is summarized in Table 1 and described in detail below.

7.2 Visit 1 (Study Week - 2: Screening)

The following evaluations and tests will be performed:

- Preliminary Screening:
  - Obtain written informed consent process for study participant;
  - A sequential participant enrollment Screening number will be assigned to each patient who signs an informed consent;
  - Review inclusion/exclusion criteria, medical history including history of OAB/DO and prior/concomitant medications to assess eligibility for study treatment;
  - Determine proof of surgical sterilization or menopause;
  - Physical examination including urogenital examination and vital signs (with height and weight);
  - Complete screening if entry criteria are met on preliminary screening:
  - Bladder scan for residual volume and bladder capacity (prior to catheterization for urine culture);
  - Obtain urine for routine urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity;
  - Obtain a catheterized urine specimen for culture (after bladder scan);
  - Electrocardiogram;
  - Blood specimens for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate), PTT and PT.
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, CPK, total bilirubin, total protein, C-reactive protein (CRP), antinuclear antibody (ANA), and glucose
    - Serum pregnancy test; FSH > 40 IU/L if last menstrual cycle not > 12 months prior to study enrollment and no hysterectomy or bilateral tubal ligation documented.
    - Blood hemoglobin A1c
  - Provide patient with diary materials;
  - Instruct the participant to complete daily voiding diaries for 7 days prior to next visit (if Visit 1A is scheduled within less than 1 week, then these diaries can still be used to review compliance, answer any questions participants have about diary completion, and

review inclusion criteria such as incontinence) and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as baseline);

- Inform the participants that they should not take any antiplatelet drugs including over the counter NSAIDS and/or anticoagulant drugs, for at least 10 consecutive days prior to Visit 2.
- Schedule next study visit for Screening cystometry when Screening laboratory results are available (within at least 1 week).
- Depending on the screening results some participants may be allowed to rescreen, if they are unable to be dosed in a timely fashion due to some unforeseen factors (e.g., hospitalization for fracture, etc.). In this circumstance the same participant will be assigned a different screening number.
- If a participant meets all inclusion/exclusion criteria, but has a positive urine culture at screening, then all screening evaluations performed at V1 (and V1A) may be accepted after the participant is treated with appropriate antibiotics and the urine cultures come back negative. The maximum time frame would be approximately up to 4 weeks after screening V1A for acceptance of these screening evaluations after discussion with the sponsor or designee.
- If necessary due to scheduling issues, Visit 1A (i.e., screening cystoscopy) can overlap with Visit 1. In this case Visit 1A procedures not already performed at Visit 1 will be completed at Visit 1 (pads and diaries will not be available in this case). If V1A coincides with V1, then pad collection and diaries will not be completed prior to V1A and these must be checked for compliance at V2.

7.3 Visit 1A (Study Day - 14 to -8): Screening Cystometry

- Review medical history and results of ECG and lab tests for eligibility and for any events which occurred **after** informed consent was signed at the screening visit. These events should be recorded on Medical History page;
- Review concomitant meds since last visit;
- Review voiding diaries and check compliance with completion **(do not enroll participant who is not compliant)** and for inclusion criteria such as incontinence;
- Collect and weigh pads used for 72 hours (**check for participant compliance**);
- Collect urine for urine culture prior to (using urodynamic catheter) and post cystometry (clean void). Also prior to cystometry, test sample of this urine by Dipstick™ in office to ensure there is no increase in leucocytes and nitrites;
- Have participant empty bladder and then perform baseline cystometry using preferably 7 or 8 French catheter (detrusor overactivity with ≥1 uncontrolled contraction(s) of the detrusor of at least 5 cm/ H_20 pressure must be documented during this Screening urodynamic testing) (see Appendix D);
- If participant is eligible, schedule Visit 2 at least 8 days (+2 days) after Visit 1A (they must complete 7 consecutive days of diaries prior to V2).

- Remind the participants that they should not take any antiplatelet/anticoagulant drugs including over the counter NSAIDS prior to the next visit.

7.4 Visit 2 (Study Week 0: Baseline - Administration of study drug)

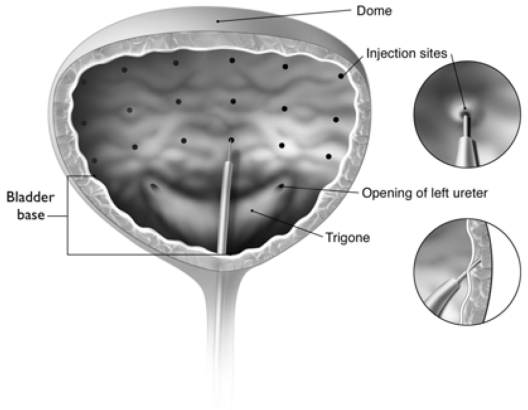
Based on the participant fulfilling all eligibility criteria following the screening procedures (Visit 1 and Visit 1A) the participant will be considered eligible to receive study drug. If a participant is unable to receive test material because a clinically significant condition has evolved which, in the investigator's opinion, represents a potential safety risk, the participant will be considered a screen failure.

The following tests and evaluations will be performed on eligible participants:

- Review medical history, results of ECG, cystometry and lab tests for eligibility and for any events which occurred **after** informed consent was signed at the screening visits until prior to dosing at this visit. These events should be recorded on Medical History page. New events or changes reported **after** dosing must be recorded on the Adverse Event log;
- Review concomitant meds since last visit;
- Review voiding diaries and check compliance with completion **(do not enroll participant who is not compliant)** and inclusion criteria;
- Participants complete KHQ, SF-12 (QoL questionnaires) and global assessment of disease state **prior** to cystoscopy and study drug administration (Baseline questionnaires);
- Participants complete the ICIQ-SF (Baseline) **prior** to cystoscopy and study drug administration;
- Physical examination including urogenital exam and vital signs for changes since the screening visit **prior** to cystoscopy and study drug administration;
- ECG **prior** to cystoscopy and study drug administration (and 2 hours post administration of study drug). If abnormalities or clinically significant changes are noted from V1 screening ECG, then the investigator in conversation the Sponsor or designee will determine the participant's eligibility to proceed with dosing.
- Blood specimens for the following laboratory determinations **prior** to cystoscopy and study drug administration:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate). No chemistry testing required this visit.
- Obtain freshly voided urine specimen for culture **prior** to study drug administration using urodynamic catheter. Also test sample of this urine by Dipstick™ in office to ensure there is no increase in leucocytes and nitrites;
- Obtain urine and blood specimen for analysis of *hSlo* cDNA with PCR prior to cystoscopy and study drug administration (pre-dosing baseline) and 2 hour post-dosing;
- Collect and weigh pads used for 72 hours;

- Request (kit should be at the site at least 24 hours prior to drug administration) and Assign participant kit number;
- Drain the participant's bladder through the transurethral catheter. Administer local anesthesia through the 7 or 8 French catheter using an instillation of 40 ml of 2% lidocaine and 2 % xylocaine gel in the urethra before insertion of the cystoscope. Once the catheter is removed administer the assigned randomized dose of study drug by sterile aseptic technique as follows. Approximately 10- 20 minutes after the local anesthetic has been administered the cystoscopic device can be inserted. Inject intravesically into the detrusor muscle, under cystoscopic control, 4 ml (for 16000 µg group) or 6 ml (for 24000 µg group) of randomized selected drug. The randomized drug is injected into the detrusor muscle via a rigid cystoscope (preferably 18 to 21 French), avoiding the trigone (see Figure 6 below). The bladder should be instilled with enough saline to achieve adequate visualization for the injections, but over-distension should be avoided. The injection needle should be filled (primed) with approximately 1 mL of study drug prior to the start of injections (depending on the needle length) to remove any air. The needle should be inserted approximately 2 mm into the detrusor, and 20 injections of either 0.2 ml (for 16000 ug dose) or 30 injections of 0.2 mL (for the 24000 µg dose) each (total volume of 4 or 6 mL, respectively) should be spaced approximately 1 cm apart (see Figure 6, below). For the final 5 injections of each dose, PBS 20% sucrose should be used in the injection syringe so that the product in the needle is delivered. After the injections are given, the bladder should be drained.

Figure 6. Bladder Injection Sites



- The time of voiding following drug administration will be recorded. The participant will be observed in the clinic for two hours after the bladder injections are completed. During that time blood pressure and pulse will be measured and recorded by the nurse every 15 minutes. The participant will be asked if there is any persistent pain/irritation in the bladder/urethra and will be treated symptomatically, if needed. The participant will be asked if they have experienced any new symptoms or problems they want to report to the investigator or study personnel. Emergency equipment for resuscitation will be immediately available in the event of an unexpected serious reaction to the study drug;
- Participants are required to stay at the study site for at least 2 to 3 hours after dosing. They may be asked stay longer if the investigator believes it is necessary.

- Collect first voided urine (clean void) after drug administration for culture prior to discharge;
- Provide participant with diary materials;
- Provide participant with prophylactic antibiotics prior to discharge (to take immediately and for the 2 days following Visit 2)
- Instruct the participant to complete daily voiding diaries for 7 days prior to next visit and return with results at next visit, including pads used for 72 hours prior to that visit (and clean pad to be used as baseline).

7.5 1-Day and 3-Day Post Visit 2 Telephone Contact

One day and three days (3 days ± 1 day) following Visit 2 (when the participant received the bladder injections) she should be contacted to assess for any problems she may be experiencing. The patient must be asked in a general fashion if she has had any complaints since her last visit. All adverse events should be documented on the adverse event E-CRF. The patient may be brought back to the clinic sooner than Visit 3 if required.

7.6 Visit 3 (Study Week 1 +2 days)

The following tests and evaluations will be performed:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;
- Review voiding diaries;
- Participants complete global assessment of disease state and response to treatment;
- Physical examination including urogenital examination and vital signs
- Electrocardiogram;
- Obtain blood for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate), PT and PTT.
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, CPK, total bilirubin, total protein, C-reactive protein (CRP), antinuclear antibody (ANA), and glucose;
- Obtain urine for routine urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, and specific gravity, pH;
- Collect and weigh pads used for 72 hours;
- Obtain urine and blood specimen for analysis of *hSlo* cDNA with PCR;
- Obtain urine culture by clean void;

- Provide patient with diary materials;
- Instruct the participant to complete daily voiding diaries for 7 days prior to next visit and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as a baseline). The site must call participants at least 7-8 days prior to next scheduled visit to remind patient to complete the diaries for 7 days prior to next visit;

7.7 Visit 4 (Study Week 2 +2 days)

Two weeks after administration of study drug the participant will return to the clinic. The following tests and evaluations will be performed on the participants:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;
- Review voiding diaries
- Collect and weigh pads used for 72 hours
- Participants complete global assessment of disease state and response to treatment;
- Physical examination including urogenital examination and vital signs
- Bladder scan for residual volume and bladder capacity (prior to catheterization);
- Blood specimens for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate).
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+), C-reactive protein (CRP), glucose, and ANA.
- Obtain urine for routine urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity;
- Obtain urine and blood specimen for analysis of *hSlo* cDNA with PCR;
- Provide patient with diary materials;
- Instruct the participant to complete daily voiding diaries and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as a baseline) The site must call participants at least 7-8 days prior to next scheduled visit to remind patient to complete the diaries for 7 days prior to next visit;

7.8 Visit 5 (Study Week 4)

The following tests and evaluations will be performed:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;

- Review voiding diaries;
- Collect and weigh pads used for 72 hours
- Participants complete KHQ and SF-12 (QoL), global assessment of disease state, and response to treatment prior to cystometry;
- Participants complete ICIQ-SF prior to cystometry;
- Physical examination including urogenital examination and vital signs prior to cystometry
- Blood specimens for the following laboratory determinations prior to cystometry:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate), PT and PTT.
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, total bilirubin, total protein, C-reactive protein (CRP), CPK, antinuclear antibody (ANA), and glucose.
    - Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity;
- Electrocardiogram prior to cystometry;
- Collect urine for culture **prior to cystometry** using urodynamic catheter
- Obtain urine and blood specimen for analysis of *hSlo* cDNA with PCR prior to cystometry;
- Perform cystometry;
- Collect first voided urine (clean void) for culture prior to discharge;
- Provide patient with diary materials;
- Instruct the participant to complete daily voiding diaries and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as a baseline). The site must call participants at least 7-8 days prior to next scheduled visit to remind patient to complete the diaries for 7 days prior to next visit;

7.9 Visit 6 (Study Week 8)

The following tests and evaluations will be performed:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;
- Review voiding diaries;
- Collect and weigh pads used for 72 hours
- Participants complete KHQ and SF-12 (QoL), global assessment of disease state and response to treatment;

- Participants complete ICIQ-SF;
- Physical examination including urogenital examination and vital signs;
- Obtain urine and blood specimen for analysis of *hSlo* cDNA with PCR;
- Bladder scan for residual volume and bladder capacity;
- Provide patient with diary materials;
- Instruct the participant to complete daily voiding diaries and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as a baseline). The site must call participants at least 7-8 days prior to next scheduled visit to remind patient to complete the diaries for 7 days prior to next visit;

7.10 Visit 7 (Study Week 12)

The following tests and evaluations will be performed:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;
- Review voiding diaries;
- Participants complete KHQ and SF-12 (QoL), global assessment of disease state and response to treatment;
- Participants complete ICIQ-SF;
- Physical examination including urogenital examination and vital signs;
- Obtain urine and blood specimens for analysis of *hSlo* cDNA with PCR;
- Obtain blood for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate), PTT and PT.
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, total bilirubin, total protein, C-reactive protein (CRP), CPK, antinuclear antibody (ANA), and glucose.
- Obtain urine for routine urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity;
- Collect and weigh pads used for 72 hours;
- Provide patient with diary materials;
- Instruct the participant to complete daily voiding diaries and return with results at next visit including pads used for 72 hours prior to that visit (and clean pad to be used as a baseline). The site must call participants at least 7-8 days prior to next scheduled visit to remind patient to complete the diaries for 7 days prior to next visit.

7.11 Visit 8 (Study Week 24) or Exit Visit:

The following tests and evaluations will be performed:

- Review medical history for events since last visit;
- Review concomitant meds since last visit;
- Review voiding diaries;
- Participants complete KHQ and SF-12 (QoL), global assessment of disease state and response to treatment;
- Participants complete ICIQ-SF;
- Physical examination including urogenital examination and vital signs (and weight);
- Electrocardiogram;
- Bladder scan for residual volume and bladder capacity (prior to catheterization);
- Perform cystometry;
- Obtain blood for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, sedimentation rate (sed rate), PTT and PT.
    - Fasting chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Ca^{++}, Mg^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, GGT, LDH, CPK, total bilirubin, total protein, C-reactive protein (CRP), antinuclear antibody (ANA), and glucose.
- Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites, pH, and specific gravity (prior to ultrasound and cystometry);
- Collect and weigh pads used for 72 hours
- Collect urine for culture prior to cystometry procedures using urodynamic catheter;
- Collect first clean voided urine for culture prior to discharge after cystometry;
- Obtain urine and blood specimens for analysis of *hSlo* cDNA. If DSMB informs the site that *hSlo* was detectible in either of two preceding specimens, participants will be required to return monthly to the clinic post study until there are two negative specimens.

7.12 Visit schedule and Visit Windows

In this outpatient study, every attempt must be made to adhere to the structured visit schedule. The Screening period (Visit 1 and 1A) should not exceed 14 days (±2 days) unless approved by Sponsor or designee (e.g., UTI found during Screening). All other visits should occur with respect to previous visit: V2, at least 8 days after V1A + 2 days; V3 +2 days; V4 +2 days; V5 ±2 days; V6± 3 days; V7± 5 days; V8± 5 days. In addition, a 1-day and 3-day (±1 day) post Visit 2 telephone contact is required to evaluate the subject for any complaints after receiving the study

drug injections. If it is necessary to bring the participant into the clinic for evaluation earlier than required, this may be considered an unscheduled visit, unless the participant has completed the necessary diary pages and the Sponsor or its designee agrees to the window deviation.

7.13 Definition of source data

Source data is defined as all information in original records of clinical findings, observations, or other activities of the clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents are the original documents, data, and records. Source documents include, but are not limited to hospital records, clinical and office charts, laboratory notes and reports, radiological films and reports, memoranda, participant's quality of life assessments, participants voiding diaries and pharmacy dispensing records.

Study data that should be included in the source documents:

- Participant's identity
- History of the disease
- Medical history, associated diseases (dates of onset)
- History of surgical sterilization, if applicable
- Date and time of administration of the study drug
- Previous and concomitant treatments
- Dates of participation in the study
- A statement on the participant's chart that the informed consent form was signed by the participant
- Dates of study visits / telephone contacts
- Examinations or assessments carried out during the study:
- Laboratory results including cultures
- Vital signs: blood pressure, heart rate, biological exams
- ECGs
- Cystometry readings
- Adverse events (+ follow-up)
- Date of drop-out and reason

Study data that will be considered as raw data:

- Efficacy data entered by diary
- Laboratory test results
- Electronic cystometry results
- ECG tracings
- QoL data

7.14 Access to the randomization code during the study

In case of a Serious Adverse Event, the code must be broken **only** in exceptional circumstances when knowledge of the study medication is essential for treating the participant. If possible, a

contact should be initiated with the Monitoring Team before breaking the code. The data will be reviewed by the DSMB.

For each participant there is a supplied tear off label which should be kept in the participant's source records, affixed to a study treatment label page. This removable panel contains a scratch off laminate which would serve to unblind the treatment if necessary.

7.15 Long-term follow-up

If urine and/or blood samples are still positive for *hSlo* DNA assay at week 24 the participant will be asked to return to the site to give additional urine and/or blood specimens until 2 consecutive specimens are negative.

All participants upon completion or exit from the study will be presented with written instructions on how to contact the sponsor if they experience any serious adverse event that they consider possibly related to study treatment or study participation. All participants receiving bladder injections of *h*Maxi-K will continue to be followed after the completion of all study related procedures (Visit 8 or last study visit) for an additional 18 months. These participants will be contacted at 6 month intervals to evaluate for any safety concerns.

8.1 Participant Withdrawal

While participants are encouraged to complete all study evaluations, they may withdraw from the study at any time and for any reason. Participation is voluntary, and refusal to participate will involve no penalty, or loss of benefits to which the participant is otherwise entitled. The participant will be encouraged to maintain contact with the investigator and report any serious adverse events experienced for the 6 months study period. Follow-up following the study period is described in Section 7.13.

An **evaluable** participant will be any participant who receives at least one dose of test material and has at least one post dose assessment.

Screen failures will include any participant who consented and entered into the screening process appropriately, but subsequently did not meet the entry criteria and was not administered study drug. Participants who fail screening will not be followed for safety or activity of study drug, and no other study procedures will be performed.

Up to a maximum of 3 participants per dose group may be replaced if participants drop out prior to completion of week 4 post study drug.

8.2 List of withdrawal criteria

WITHDRAWAL OF A PARTICIPANT DUE TO ANY SERIOUS ADVERSE EVENT REQUIRES THAT THE SPONSOR BE INFORMED **WITHIN ONE WORKING DAY AFTER THE INVESTIGATOR IS MADE AWARE OF THE EVENT.** PLEASE REFER TO SECTION 9, ADVERSE EVENT REPORTING.

Those participants who do not complete the study for any reason will be considered a premature termination. The procedures for the Visit 8 should be completed when a participant discontinues the study.

- **Adverse Events:**

  Participants with adverse events, severe enough to necessitate discontinuation of study drug administration as judged by the investigator.

  The treating physician should take appropriate clinical action. The condition should be treated according to routines of the clinic. The participant should be followed up regarding the safety measurements stated in the protocol.

- **Consent Withdrawn:** participant decided to withdraw from the study for non-medical reasons that the investigator/patient deemed sufficient to warrant premature termination.
- **Lost to Follow-up:** participant failed to return for required visits and cannot be contacted. Reasonable effort should be made by the investigator to contact any patient who fails to return to the clinic for a scheduled visit in order to complete assessments and retrieve any outstanding data. All such efforts should be documented in the source notes.
- **Sponsor/Investigator Decision:** A participant may be withdrawn at any time at the discretion of the investigator.

If a participant discontinues from the study, she should always be contacted in order to give the investigator information about the reason(s) for discontinuation and any adverse events. Whenever possible, the patient should return for a clinic visit at the time of or soon after discontinuation. Any outstanding data or study medication should be collected. Adverse events should be followed up.

8.3 Reasons for withdrawal

The participants may withdraw from the study if they decide to do so, at any time and irrespective of the reason, or at the Investigator's decision. Participants who have been withdrawn from the study cannot be re-included in the study. Their inclusion and treatment number must not be re-used.

8.4 Replacement of participants

Up to 3 replacements per dose will be allowed for participants who received study drug and discontinue without follow-up data of at least one-month post study drug administration.

8.5 Withdrawal follow-up procedure

For participants considered lost to follow-up, the E-CRF must be filled in up to the last visit.

9.1 Adverse Events Definitions and Monitoring

9.2 Adverse Event

An adverse event is any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen.

9.3 Serious Adverse Event

A serious adverse event (experience) is defined (21 CFR 312.32) as any adverse experience that suggests a significant hazard, contraindication, side effect, or untoward medical occurrence that:

- Results in death,
- Is life threatening,

Note: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires (or prolongs) hospitalization, including emergency room care,
- Causes persistent or significant disability/incapacity,
- Results in congenital anomalies or birth defects, or
- Other conditions which in the judgment of the investigators represent significant hazards.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above.

These should also usually be considered serious.

Note: Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, convulsions or asymptomatic ALT increase > 10 ULN that do not result in hospitalization, or development of drug dependency or drug abuse.

9.4 Assessment of Causality

The relatedness of an adverse event to the study drug is the best estimate of the principal investigator at the time of reporting of the causal relationship between an experimental intervention and an adverse event.

The following study drug relationships will be used for this clinical trial:

Unrelated*:* There is no temporal relationship between the event and the administration of the study drug or the event is clearly due to the participant's medical condition, other therapies, or accident.

Possibly Related*:* There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the participant's medical condition or other therapies.

Probably Related*:* The temporal relationship between the event and the administration of the study drug is compelling, and the participant's medical condition or other therapies cannot explain the event.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication or follows a known or suspected response pattern to the medication.

The categories of "Certainly", "Probably" and "Possibly Related" are considered study drug related.

9.5 Severity of Adverse Event

Adverse events included in the toxicity table (Appendix A) will be graded according to the definitions provided. For adverse events not listed in the toxicity table, assignment of grade based on intensity of symptoms, degree of limitation of usual daily activities, or level of abnormality of objective clinical signs or laboratory parameters will be according to severity using the following criteria:

- Grade 1: transient or minimal symptoms; not interfering in function or ability to perform activity of daily living or require a medication change. No medical interventionrequired.

- Grade 2: symptoms interfering in function but not with activities of daily living. Minimal or no medical intervention required.

- Grade 3: incapacitating symptoms that interfere with function and activities of daily living; required bed rest and/or resulted in loss of work or cancellation of social activities. Medical intervention required. Hospitalization possible.

- Grade 4: Medical intervention is required to prevent permanent impairment or death; OR Permanently Disabling: bed-ridden or disabling, significant medical intervention/therapy required, hospitalization or hospice care possible.

9.6 Monitoring of Adverse Events

The investigator will monitor participants for the occurrence of adverse events during the course of the study and record all observed adverse events in the electronic case report form.

All Adverse Events regardless of seriousness or relationship to study drug, including those occurring during the Screening period (where applicable), are to be recorded on the corresponding page(s) in the E-CRF. Whenever possible, symptoms should be grouped as a single syndrome or diagnosis. The Investigator should specify the date of onset, maximal intensity, corrective therapy given, outcome, and his/her opinion as to whether there is a reasonable possibility that the Adverse Event was caused by the study drug.

The investigator must provide the sponsor appropriate information concerning any findings suggesting significant hazards, contraindications, side effects or precautions pertinent to the safety of the study drug. The investigator will instruct participants prior to administration of study drug to report any physical changes or new symptoms that they notice during the course of the study. The investigator must report all serious adverse events (defined above) within 24 hours of their onset or following the investigators awareness of the event to the Sponsor's designee:

Dr Sharon Jacobs
212-308-0948
Cell: 646-361-6161
skrashes@aol.com

Or

Dr Arnold Melman
Cell: 347-782-1734
arnold.melman@gmail.com

The Sponsor or designee will report all serious adverse events to the IRB, FDA, OBA, and NIH according to regulatory requirements. Any adverse event that is not resolved by the end of study (or early termination) visit and considered to be potentially related to study drug, or was the cause for participant withdrawal will be followed as clinically indicated until its resolution, or if non-resolving, until considered stable.

In the case of a Serious Adverse Event the Investigator must immediately:

- SEND (within 1 working day if possible) the signed and dated corresponding page(s) in the SAE Report Form to the Sponsor (as above) or designee. The date of receipt of this form by the Sponsor will be the date used as "Day 1" for expedited reporting to the regulatory agencies as is required.

- ATTACH the photocopy of all examinations carried out and the dates on which these examinations were performed. For laboratory results, include the laboratory normal ranges.

- All additional data or corrections are to be reported on a follow-up SAE form.

- A blinded code must be broken only in exceptional circumstances when knowledge of the study medication is essential for treating the participant.

Follow-up Procedures

- The Investigator should take all appropriate measures to ensure the safety of the participants, notably he/she should follow-up the outcome of any Adverse Events (clinical signs, laboratory values or other, etc.) until the return to normal or until stabilization of the participant condition.
- In the case of any Serious Adverse Event, the participant must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized.
- In case of any Serious Adverse Event brought to attention of the Sponsor.

ECG Monitoring

In case of prolongation of $QT_cF \geq 500$ ms, or an increase in $QT_cF \geq 60$ ms from baseline, a blood sample should be drawn for potassium, calcium, magnesium and a plasma assay for *hSlo* cDNA with PCR. The hSlo analysis will be done by the under the direction of Kelvin Davies, PhD. of the Department of Urology Research Laboratory, Albert Einstein College of Medicine.

A cardiologist should be consulted, and the participant should continue to have his or her ECG monitored until the ECG abnormality resolves or becomes stable and part of the patient's new medical history.

10.1 Assessment of Endpoints

10.2 Safety

Safety and tolerability of the study drug will be evaluated by analysis of adverse experiences, clinical laboratory tests, electrocardiogram, and physical examinations.

10.3 Efficacy

The sample size has been estimated assuming a standard deviation of 2.5 and a two sample t-test to have 80% power.

The protocol is designed to observe a 30% reduction in the number of voids compared to placebo following gene transfer using the following calculations with NCSS/PASS program (NCSS, Kaysville, Utah, 84037-3233).

Table 10;. Powering

Power	N1	N2	Ratio	Alph	Beta	Mean	Mean2	S1	S2
0.80251	9	10	1.111	0.050	0.19749	10.0	6.6	2.5	2.5

The mean number of micturitions per 24 hours will be evaluated at all visits and changes compared to baseline and placebo.

The other secondary outcome measures will be the change in the mean number of urge incontinence episodes per 24 hours; urgency episodes per 24 hours; mean volume voided per micturition, diminished urgency perception; change in weight of 72 hour pad test; and change in

detrusor overactivity (DO) including peak amplitude, volume at which DO appears and presence or absence of DO. The participants' perception of their bladder condition and response to treatment will be assessed at baseline (Week 0) and after 1, 2, 4, 8, 12, and 24 weeks. Improvement will be defined as a decrease of 1 point or more from baseline. Quality of Life (QoL) will be evaluated using the Kings Health Questionnaire (see Appendix B for algorithm). Change in maximum bladder capacity (difference of pre-treatment to post-treatment volume) as determined by cystometry of *h*Maxi-K versus pool of placebo participants from each treatment level.

Participants will be assessed for changes from baseline in urinary symptoms following administration of *h*Maxi-K using the questions of the ICIQ-SF form and the urgency rating scale.

Participants will also be pooled across treatment groups (all active vs. all placebo).

10.4 Analysis of Endpoints

The methods described in this section will be updated during the course of the study. The final version of the statistical analysis plan (SAP) will be issued before the treatment code is broken.

This double blind, phase 1 study is designed to evaluate the safety of a single bladder injections of 2 different doses of *h*Maxi-K in individual participants. Both the safety data and data to assess efficacy will be analyzed using summary descriptive statistics for the two cohorts and the total study population. Associations between study therapy and the outcomes will be assessed using-repeated measures analyses, and explored in multivariate models, as needed.

11.1 Monitoring of Participant Safety

11.2 Informed Consent

The investigator will be responsible for obtaining from every participant prior to the participant's participation in the study a written Informed Consent signed and dated in accordance with U.S. federal regulations (21 CFR 50 and 21 CFR 312.60). The written Informed Consent will be obtained after the investigator has provided a full explanation, both verbally and in writing, of the purpose, risks and discomforts involved and potential benefits of the study to the participant. The original signed and dated copy of the Informed Consent must be maintained in the institution's records. The names of the participants enrolled during this study will be considered confidential.

11.3 Data Safety Monitoring Board (DSMB)

In order to monitor the safety of participants participating in this study, the sponsor will establish an independent Data Safety Monitoring Board (DSMB). The members will have training in medicine and/or gene therapy. All members will be independent of the study conduct.

The DSMB will review eligibility and all available safety data after the 5th participant has been administered study drug in the first dosing cohort. This first review will occur as soon as possible after the 5th participant's 3-Day Post-visit 2 telephone contact. Following their review of the

safety data, the DSMB will recommend whether enrollment into the first dosing cohort may proceed.

The DSMB will review participant's eligibility data and all safety data at 4 weeks following administration of study drug for the last participant enrolled into each dose cohort, in order to assess the need for modifications in safety monitoring and/or dosing. Following their review of the safety data for the last participant in the cohort at the 4th week post-dosing, the DSMB will recommend whether or not enrollment into the next dosing cohort may proceed (Section 5.2). The DSMB will also review the clinical safety data from the participants in all the dosing cohorts at the six month timepoint following the final dose.

12.1 Regulatory Standards

12.2 Electronic case report forms

An electronic case report form (E-CRF) will be completed for every participant who signed a written Informed Consent form and receives study drug. Any correction of data recorded onto the E-CRF will be entered in to the E-CRF which will create an electronic audit trail of the corrections and electronic signature of the study personnel who made the changes. For the long term follow-up period paper CRFs will be used.

The principal investigator must sign and date the certification form of each case report upon completion. This signature will indicate that thorough inspection of the data has been made and will thereby certify the contents of the electronic case report forms.

The investigator or institution will retain all original source documentation (e.g., laboratory results, treatment records, audit queries, etc.) unless specified otherwise by the protocol. The results as they become available will be entered on the appropriate electronic case report forms.

Electronic case report forms will be reviewed at the study site by a clinical monitor who will make a decision as to their acceptability in regard to completeness and accuracy of the data. Audit queries will be generated for omissions, corrections, and clarifications.

12.3 Clinical and Regulatory Monitoring

The study will be monitored in compliance with the relevant parts of 21 CFR and according to the ICH GCP Guidelines.

12.3.1 Study Conduct (Site)

Ion Channel Innovations will have an independent monitor, knowledgeable in GCP guidelines and regulations, monitor the clinical study. This representative of the sponsor (CE^3 Inc.) will visit the institution prior to initiating the study and periodically thereafter to monitor acceptability of facilities, the agreement between E-CRF entries and original source documentation, adherence to the protocol, Good Clinical Practice (GCP) and to applicable FDA regulations and the maintenance of adequate clinical records. The monitor will have access to participant records, medication sheets, laboratory data, and other source documentation.

In addition to the initiation visit, the clinical sites will be audited after enrollment into each cohort has been completed. Once the study has been completed or terminated, a close-out or termination visit will be made. The Investigator and/or Study Coordinator will receive reasonable notification before each monitoring visit during the course of the study. At each visit, the Investigator will cooperate with the Sponsor's representative(s) for the review and verification of all E-CRFs, drug supply and inventory records, and any additional records requested for review. The monitor will ensure that all safety reports are submitted to the sponsor who is responsible for reporting required safety reports to the IRB, FDA, and OBA (see Section 12.2.2).

All information contained in a participant's E-CRF must have corresponding source documentation. This source documentation includes, but is not limited to, notes taken at participant visits recording the date of the visit, vital signs, physical findings, adverse events, or concomitant medications; laboratory reports; hospital records; and clinic records. Any correction of errors in the E-CRF will be first reviewed with the investigator prior to correction. The reason for any correction to the E-CRF will be noted along with the date and electronic signature of the person making the correction.

The records of the study may be participant to audit by Sponsor representatives (Clinical Quality Assurance or designee) or by government regulatory authorities (e.g. U.S. Food and Drug Administration). The Investigator agrees to allow access to the required participant records inthe event of such an audit.

12.3.2 Adherence to Reporting Requirements

Ion Channel Innovations will have an independent monitor, knowledgeable in GCP guidelines and regulations, monitor adherence to the reporting requirements. This designee will ensure that the sponsor submitted any modifications to the protocol to the IRB and FDA. They will also review all safety reports submitted to the sponsor and ensure that those safety events requiring expedited reporting are submitted to the IRB, FDA, and OBA[2] within the required timeframes.

Within a reasonable time following completion of the study, a final study report will be written, reviewed by the sponsor's designee/CRO, and submitted to FDA.

12.4 Participant Confidentiality

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties, other than those cited below, is prohibited. Participant confidentiality will be further ensured by utilizing participant identification code numbers for all reports.

In compliance with regulatory guidelines regarding the monitoring of clinical studies and in fulfillment of the Investigator's obligations to Ion Channel Innovations, it is required that data generated as a result of the study be available for inspection, on request, by personnel from Ion Channel Innovations and regulatory agencies. These shall include all study-relevant documentation, including medical histories to verify eligibility, laboratory test results to verify

[2] OBA is the Office of Biotechnical Activities of the National Institutes of Health (NIH) which includes NIH Genetic Modification Clinical Research Information System (GeMCRIS); OHSR is the Office of Human Subject Research,NIH.

transcription accuracy, treatment and diagnostic reports, admission/discharge summaries for hospital admissions occurring while the participant is on-study, and autopsy reports (if available) for deaths occurring during or in temporal proximity to the study.

As part of the required content of the informed consent, participants must be informed that their records will be reviewed by Ion Channel Innovations and regulatory agencies. Should access to medical record require a separate waiver or authorization, it is the Investigator's responsibility to obtain such permission from the participant in writing before the participant is entered into the study.

12.5 Records

The Food and Drug Administration requires that an investigator retain records for a period of two (2) years following the date a New Drug Application or Product License Application is approved for the drug for the indication for which it is being investigated; or, if no application or license is to be filed or, if the application or license is not approved for such indication, until two (2) years after the investigation is discontinued (21 CFR 312.62).

The investigator should ensure that the following records are maintained:

Participant files containing copies of completed case reports and supporting documentation and a copy of the signed, Informed Consent form.

Investigator files containing copies of the documents required for the initiation of the study (executed form FDA 1572, signed Investigator's Agreement, Curricula Vitae for the principal investigator, copy of the IRB approval of the protocol and Informed Consent forms), copies of correspondence received from and sent to Ion Channel.

Pharmacy files containing copies of the record of use for the Investigational Drug, instructions for completion of these records, and the Investigator's Brochure.

13.0 References

(bolded references are included in Section 10 of the IND; others available upon request)

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APPENDIX A Grading of Adverse Events

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
HEMATOLOGY				
Hemoglobin	10.0 g/dL – 11.0 g/dL **OR** any decrease ≥ 2.5 g/dL	9.0 g/dL – 9.9 g/dL **OR** any decrease ≥ 3.5 g/dL	7.0 g/dL – 8.9 g/dL **OR** any decrease ≥ 4.5 g/dL	< 7.0 g/dL
WBC—Elevated	13,000 – 14,999/mm^3	15,000 – 19,999/mm^3	20,000 – 24,999/mm^3	> 25,000/mm^3
WBC—Decreased	2001 – 2499/mm^3	1501 – 2000/mm^3	1000 – 1500/mm^3	< 1000/mm^3
Platelets—Decreased	100,000 – 124,999/mm^3	50,000 – 99,999/mm^3	25,000 – 49,999/mm^3	< 25,000/mm^3
Platelets—Elevated	NA	550,000 – 600,000/mm^3	> 600,000/mm^3	NA
PT	1.1-1.24 X ULN	1.25 – 1.49 X ULN	1.5 – 3.0 X ULN	>3.0 XULN
PTT	>1 – 1.5 X ULN	>1.5 – 2.0 X ULN	>2 X ULN	-
CHEMISTRIES				
BUN	25 - 30 mg/dL	31 - 40 mg/dL	41 – 50 mg/dL	>50 mg/dL
LDH	1.5 – 2.5 X ULN	2.6 – 3.5 X ULN	3.6 – 5.0 X ULN	>5.0 X ULN
Hyponatremia	<LLN – 130 mEq/L	123 – <130 mEq/L	116 – <123 mEq/L	<116 mEq/L
Hypernatremia	>ULN – 150 mEq/L	>150 – 155 mEq/L	>155 – 160 mEq/L	>160 mEq/L
Hyperkalemia	>ULN – 5.5 mEq/L	>5.5 – 6.0 mEq/L	>6.0 – 7.0 mEq/L	>7.0 mEq/L
Hypokalemia	<LLN – 3.2 mEq/L	3.0 – <3.2 mEq/L	2.5 – <3.0 mEq/L	<2.5 mEq/L
Bicarbonate (serum)	<LLN – 16 mEq/L	10 – <16 mEq/L	8 – < 10 mEq/L	<8 mEq/L
Phosphate	<LLN – 2.5 mg/dL	≥ 2.0 – <2.5 mg/dL	≥ 1.0 – <2.0 mg/dL	<1.0 mg/dL
Hypocalcemia	<LLN – 8.0 mg/dL	7.0 – <8.0 mg/dL	6.0 – <7.0 mg/dL	<6.0 mg/dL
Hypercalcemia	>ULN – 11.5 mg/dL	>11.5 – 12.5 mg/dL	>12.5 – 13.5 mg/dL	>13.5 mg/dL
Magnesium	<LLN - 1.2 mEq/L	0.9 – <1.2 mEq/L	0.7 – <0.9 mEq/L	<0.7 mEq/L
Total bilirubin	>ULN – 1.5 X ULN	>1.5 – 3.0 X ULN	>3.0 – 10.0 X ULN	>10.0 X ULN
Hypoglycemia	<LLN - 55 mg/dL	40 - <55 mg/dL	30 – <40 mg/dL	<30 mg/dL
Hyperglycemia (nonfasting & no history of diabetes)	>ULN – 160 mg/dL	>160 – 250 mg/dL	>250 – 500 mg/dL	>500 mg/dL
CPK	>ULN – 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 - 10.0 X ULN	>10 X ULN
Creatinine	>1.0 – 1.5 X ULN	>1.5 – 3.0 X ULN	>3.0 – 6.0 X ULN	>6.0 X ULN
AST (SGOT)	ULN – 2.5 X ULN	>2.5– 5.0 X ULN	>5.0 – 20.0 X ULN	> 20.0 X ULN
ALT (SGPT)	>ULN – 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 – 20.0 X ULN	> 20.0 X ULN
GGT	>ULN– 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 – 20.0 X ULN	>20.0 X ULN
Alkaline Phosphatase	>ULN – 2.5 X ULN	>2.5 – 5.0 X ULN	>5.0 – 20.0 X ULN	>20.0 X ULN

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
URINALYSIS				
Proteinuria (random urine sample)	1+	2-3+	4+	-
Microscopic RBC (exception is urine sample post-catherization)	6 – 10 RBC/hpf	>10 RBC/hpf	Gross hematuria	-
CARDIOVASCULAR				
Hypertension	Asymptomatic, transient (<24 H) increase by >20 mmHg (diastolic) or >150/100 if previously WNL; not requiring treatment	Recurrent or persistent (>24H) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 if previously WNL; may require monotherapy	Requiring more than one drug or more intensive therapy than previously	Life-threatening consequences (e.g., hypertensive crisis)
Hypotension	Transient orthostatic hypotension, intervention not indicated	Symptoms corrected with oral fluid replacement	IV fluid required; hospitalization not required	Hospitalization required
Conduction abnormality/ atrioventricular heart block	Asymptomatic, no intervention indicated	Non-urgent medical intervention indicated	Symptomatic & incompletely controlled medically or controlled with device (e.g., pacemaker)	Life-threatening
Prolonged QTc interval	Asymptomatic, QTc interval 0.45 – 0.48 sec	Asymptomatic, QTc interval >0.48 sec	Symptomatic	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
Supraventricular & nodal arrhythmia	Asymptomatic, no intervention indicated	Symptomatic, but not requiring treatment	Symptomatic & incompletely controlled medically or controlled with device (e.g., pacemaker)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock
Ventricular arrhythmia	Asymptomatic, intervention not indicated	Symptomatic, but not requiring treatment	Symptomatic & incompletely controlled medically or controlled with device (e.g., pacemaker)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope shock
Cardiac Arrhythmia – Other	Asymptomatic, not requiring treatment	Symptomatic, but not requiring treatment	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
Cardiac ischemia/infarction	Asymptomatic arterial narrowing without ischemia	Asymptomatic and testing suggests ischemia	Asymptomatic ischemia (angina) or testing consistent with ischemia, intervention indicated	Acute myocardial infarction

APPENDIX B. King's Health Questionnaire

Site No.	**Subject No.**	**Subject Initials**	**Date** _____

I - How would you describe your health al present?
- D Very good (I)
- D Good (2)
- D Fair (3)
- D Poor (4)
- D Very poor (5)

2- How much do you think your bladder problem affects your life?
- D Not at all (J)
- D A lillle (2)
- D Moderately (3)
- D A lol (4)

3a- To what extent does your bladder problem affect your household tasks (e.g. cleaning, shopping, etc.)?
- D Not at all (J)
- D A lillle (2)
- D Moderately (3)
- D A lol (4)

3b- Does your bladder problem affect yourjob, or your normal daily activities out side the home?
- D Not at all (J)
- D A lillle (2)
- D Moderately (3)
- D A lol (4)

4a- Does your bladder problem affect your physical activities (e.g. going for a walk, run, sport, gym, etc.)?
- D Not at all (J)
- D A lillle (2)
- D Moderately (3)
- D A lol (4)

4b- Does your bladder problem affect your ability lo travel?
- D Not at all (J)
- D A lillle (2)
- D Moderately (3)
- D A lol (4)

4c- Does your bladder problem limit your social life?
- D Not at all (I)
- D A liHle (2)
- D Moderately (3)
- D A lot (4)

4d - Does your bladder problem limit your ability to see/visit fiiends?
- D Not at all (I)
- D A liHle (2)
- D Moderately (3)
- D A lot (4)

Sa- Does your bladder problem affect your relationship with your partner?
- D Not applicable (0)
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D A lot (4)

Sb- Does your bladder problem affect your sex life?
- D Not applicable (0)
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D A lot (4)

Sc- Does your bladder problem affect your family life?
- D Not applicable (0)
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D A lot (4)

6a- Does your bladder problem make you feel depressed?
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D Very much (4)

6b- Does your bladder problem make you feel anxious or nervous?
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D Very much (4)

6c- Does your bladder problem make you feel bad about yourself?
- D Not at all (I)
- D Slighlly (2)
- D Moderately (3)
- D Very much (4)

7a- Does your bladder problem affect your sleep?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

7b- Do you feel worn oul/tired?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

8a - Wear pads lo keep dry?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

8b- Be careful how much fluid you drink?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

8c- Change your underclothes when they gel wet?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

8d- Worry in case you smell?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

8e- Gel embarrassed because of your bladder problem?
- D Never (I)
- D Sometimes (2)
- D Often (3)
- D All the lime (4)

Sign with Participant Initials Date Fonn Completed :

Calculation of KHQ–QoL domain scores

KHQ–QoL domain scores were calculated using the following formulae.

1. **General health perceptions:**
Score= ((score to Q1 - 1)/4) x 100
2. **Impact on life:**
Score = ((score to Q2 - 1)/3) x 100
3. **Role limitations:**
Score= (((score to Q3a + Q3b) - 2)/6) x 100
4. **Physical limitations:**
Score = (((score to Q4a + Q4b) - 2 /6) x 100
5. **Social limitations:**
if score Q5c $\geq$ 1,
Score = (((score to Q4c + Q4d + Q5c) - 3)/9) x 100
if score Q5c = 0,
Score= (((score to Q4c + Q4d) - 2)/6) x 100
6. **Personal Realtionships:**
if score Q5a + Q5b $\geq$ 2,
Score = (((score to Q5a + Q5b) - 2)/6) x 100
if score Q5a +Q5b = 1,
Score = (((score to Q5a + Q5b) - 1)/3) x 100
if score Q5a + Q5b = 0, treat as missing value (not applicable),
7. **Emotions:**
Score = (((score to Q6a + Q6b + Q6c) - 3)/9) x 100
8. **Sleep/energy:**
Score = (((score to Q7a + Q7b) - 2)/6) x 100
9. **Incontinence severity measure:**
Score = (((score to Q8a + Q8b + Q8c + Q8d +Q8e) - 5)/15) x 100

APPENDIX C. Participant Assessment of Disease and Response to Treatment

Site No. _____ Subject No. _____ Subject Initials _____ Date _____

1. Participant Assessment of Disease

Participants will rate their perceived bladder condition severity as follows:

Using validated 6-point rating scale participants will be asked the following.

"How bothersome do you consider your bladder problems? "

1- No problems

2 - Very minor problems

3 - Minor problems

4 - Moderate problems

5 - Severe problems

6 - Many severe problems

Improvement will be defined as a decrease of 1 point or more from baseline.

2. Participant Assessment of Response to Treatment

Participants' assessment of their response to treatment will be measured by asking:

"Has the treatment been of benefit to you?"

1. No benefit
2. Yes, a little benefit
3. Yes, very much benefit

Improvement will be defined as a decrease of 1 point or more from baseline.

Sign with Participant Initials: _____ Date Form Completed: _____

APPENDIX D. Cystometry Procedure Guidance

The importance of performing well-controlled cystometries cannot be over-emphasized. The methodology advocated by the International Continence Society in Neurourology and Urodynamics 21:261-274, 2002 should be followed.

Minimally, patients should be positioned in the sitting position. Using sterile aseptic techniques, a 7 or 8 Fr double-lumen transurethral catheter will be inserted into the urethra and a 9 Fr balloon catheter will be placed in the rectum to measure abdominal pressure. Room-temperature saline will be used at a filling rate of 50 ml/min. Either fluid filled catheters connected to pressure transducers or solid-state catheters should be used to help minimize artifact. The same system must be used for pre and post transfer studies on each patient. Participants should be instructed to neither void nor to try to inhibit micturition, but to simply report their sensations to the examiner. Participants should be instructed to indicate first desire to void, strong urge to void, and/or when they can longer tolerate infusion (i.e. no longer delay micturition). Bladder filling should be discontinued as soon as the participant experiences a strong desire to void, uncomfortable fullness or pain.

Digitized recordings should be captured and stored for each participant. Waveforms from filling cystometries and pressure flow studies should be interpreted by a trained professional for each parameter noted in the following Table A. A cystometry overreader as well as the investigator will be required to interpret the results. The central overreader's interpretation will take priority. Printed waveforms should be kept with the patient's source records, with items such as artifacts and peak pressure measurements clearly identified. Digitized pressure recordings will be analyzed for the following parameters:

Table A: Cystometry Parameters and Definitions		
Parameter	**Description**	**Definition**
DO; Yes/No	Detrusor overactivity ≥ 5 cm H_2O	Any involuntary contraction that has a pressure >/= 5 cm H_2O **(INCLUSION CRITERIA)**
$V_{First\ des}$ (mL)	Volume at first desire to void	The volume attained during filling cystometry that would lead the patient to pass urine at the next convenient moment but voiding can be delayed if necessary *(ICS definition)*.
$P_{det.\ open}$ (cm H_2O)	Detrusor pressure at beginning of voiding (prior to onset of first contraction (either volitional or involuntary)	Detrusor pressure at onset of the first detrusor contraction (either volitional or involuntary).
V_{1st} (mL)	Volume at first involuntary contraction; if detrusor overactivity (DO) present. Not applicable if no DO present.	Volume infused at the point of maximum detrusor pressure during the 1st contraction. [1]
Det_{frq}	Total number of detrusor contractions during procedure (voluntary **AND** involuntary)	Total number of detrusor contractions including voluntary **AND** involuntary
Leak (Yes/no)	Was there a leak with overactivity	Involuntary detrusor contractions resulting in leak (NOT counting voluntary voids).
DO $_{Leak\ freq}$	Number of involuntary detrusor contractions resulting in leak (if DO present)	Number of involuntary detrusor contractions resulting in leak (if DO present).[1]
V_{leak} (mL)	If yes, Volume of each leak	Volume of each leak for each episode of leak[2]
$V_{leak\ cum}$ (mL)	If yes, cumulative volume of ALL leaks during procedure	Cumulative volume of leaks (calculated by computer from individual volumes)[2]
DO $_{No\ Leak\ freq}$	Number of involuntary detrusor contractions NOT resulting in leak.	Total number of involuntary detrusor contractions that do not have an associated leak. [1]
$P_{det\ Max}$ (cm H_2O)	Maximum detrusor pressure at involuntary contraction	Maximum amplitude of detrusor contraction during an involuntary contraction (if present) [3]
$P_{det\ 1st}$ (cm H_2O)	Maximum detrusor pressure at FIRST contraction	Max detrusor pressure at **FIRST** contraction (voluntary or involuntary). [1]
$P_{Det\ Any}$ (cm H_2O)	Maximum detrusor pressure at ANY contraction	MAXIMUM detrusor pressure attained during the entire study (voluntary or involuntary). [4]
Cys Cap (mL)	Cystometric Capacity: Volume at strong urge to void	The volume at which the patient feels that he/she can no longer delay micturition (has a strong desire to void). *(ICS definition*)
Q_{max} (mL / sec)	Peak flow rate during voiding (for both leaks and voluntary voids)	The Maximum rate of flow during any void (for both involuntary and voluntary voids)
V_{voided} (mL)	Total voided volume (leaks and voluntary)	Total volume voided for leak and volitional voids during the procedure
PVR (mL)	Post Void Residual Volume (PVR) (from catheterization)	Volume left in bladder after voiding at the conclusion of the study. This is measured by urodynamic catheterization.
($V_{total\ bladder}$) (mL)	Total Bladder Volume (Total voided volume +catheterized PVR)	Post void residual volume + total voided volume (will be *calculated*)

DO_{dur} (seconds)	Duration of detrusor overactivity	Duration of MAXIMUM detrusor activity. [2]
$P_{Det, Q Max}$ (cm H_2O)	Detrusor pressure at maximum flow during an involuntary contraction	Maximum detrusor pressure during terminal void (voluntary or involuntary). [2]
$V_{bladder\ DO}$ (mL)	Bladder volume at each involuntary detrusor contraction	Infused bladder volume at the peak pressure of each involuntary contraction minus the amount leaked at each point for each involuntary contraction; Total will be calculated by the computer. [2]
P_{abd} (cm H_2O)	Maximum abdominal pressure during any contraction	MAXIMUM abdominal pressure during **ANY** contraction. This will assess the abdominal straining component to voiding. [2]

5. This is done by overreader, but not the sites
6. Done by site only
7. Detrusor pressure at first involuntary contraction is called "**Detrusor Pressure**" for sites on eCRF.
8. Maximum detrusor pressure at ANY contraction is called "**Maximum Detrusor Pressure**" for sites on eCRF.

APPENDIX E. International Consultation on Incontinence Questionnaire: Short Form[1].

Site No. Subject No. Subject Initials _____ Date _____

How often do you leak urine?

Never	0
About once a week or less ofien	I
Two to three limes a week	2
About once a day	3
Several times a day	4
All oflhe lime	5

We would like to know how much urine you think leaks. How much urine do you usually leak (whether you wear protection or not)?

None	0
A small amount	2
A moderate amount	4
A large amount	6

Overall how much does leaking urine interfere with your everyday life?
Please circle a number between O (not at all) and 10 (a great deal).

0	1	2	3	4	5	6	7	8	9	10
Not all										A great deal

When does urine leak? (Please check all that apply)

Never - urine does not leak	D
Leaks before you can get to the toilet	D
Leaks when you cough or sneeze	D
Leaks when you are **asleep**	D
Leaks when you are **physically active/exercising**	D
Leaks when you have finished urinating and are **dressed**	D
Leaks for no obvious reason	D
Leaks all the time	D

Sign with Participant Initials:_____ Date Form Completed:_____

[3] Hajebrahimi S, Corcos J, Lemieux MC. International consultation on incontinence questionnaire short form: comparison of physician versus patient completion and immediate and delayed self-administration. Urology. 2004 Jun;63(6):1076-8.

• Avery K, Donovan J, Alrams P. Validation of a new questionnaire for incontinence: the International Consultation on InC(HJtincncc Questionnaire (ICIQ). abstract n• 86 of the International Continence Society 31st annual meetin Seoul, Korea. *Neurourol Urodynamics* 2001;20:510-1.

APPENDIX F. Participant Daily Diary

Please complete the information below **every day for the 7 days prior to your next scheduled visit**.

DAILY DIARY

Site #	Subject #	Subject initials

Start Date:____/___/___to End Date:_____/___/___

#	Time of Urination (circle AM or PM)	Urgency (Scale 0-3) *	Was this an accident? Y/N	Volume (mL)**	#	Time of Urination (circle AM or PM)	Urgency (Scale 0-3) *	Was this an accident? Y/N	Volume (mL)**
1	: AM PM				11	: AM PM			
2	: AM PM				12	: AM PM			
3	: AM PM				13	: AM PM			
4	: AM PM				14	: AM PM			
5	: AM PM				15	: AM PM			
6	: AM PM				16	: AM PM			
7	: AM PM				17	: AM PM			
8	: AM PM				18	: AM PM			
9	: AM PM				19	: AM PM			
10	: AM PM				20	: AM PM			

****Urgency Scale**
0=None: No Urgency
1=Mild: Awareness of urgency or need to go to the bathroom ***but easily tolerated***
2= Moderate: Enough urgency discomfort that it interferes with usual activities and tasks
3=Severe: Extreme urgency and discomfort that stops all activities and tasks

**** Urinate into supplied cylinder and measure the volume**

Number of pads used on this day:_____(Daily pad count includes all pads newly applied during a calendar day- dates as above). Be sure to seal used pads **for 3 days prior to next visit** in the provided zip lock bag to bring to clinic at next visit.). Also bring one new clean pad for comparison.

To be completed at Study Center
Total number of urinations on this day _____
Mean volume per urination on this day_____

Sign with Participant Initials: _____ Date Form Completed: _____

APPENDIX G. Components and Guidelines for Health Status Questionnaire (SF-12)

SF-12 HEALTH SURVEY (STANDARD)[5]

Site No. **Subject No. _____** **Subject Initials _____** **Date _____**

INSTRUCTIONS: This questionnaire asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Please answer every question by marking one box. If you are unsure about how to answer, please give the best answer you can.

In general, would you say your health is:

☐	☐	☐	☐	☐
Excellent	**Very good**	**Good**	**Fair**	**Poor**

The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, Limited A Lot	**Yes, Limited A Little**	**No, Not Limited At All**
2. **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling or playing golf	☐	☐	☐
3. Climbing **several** flights of stairs	☐	☐	☐

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	Yes	**No**
4. **Accomplished less** than you would like	☐	☐
5. Were limited in the **kind** of work or other activities	☐	☐

[5] Ware JE, Kosinski M, Keller, SD. SF-12: How to score the SF-12 Physical and Mental Health Summary Scales. Lincoln, RI: Quality Metric Incorporated, third Edition, 1998

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	No
6. **Accomplished less** than you would like	☐	☐
7. Didn't do work or other activities as **carefully** as usual	☐	☐

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

☐	☐	☐	☐	☐
Not at all	**A little bit**	**Moderately**	**Quite a bit**	**Extremely**

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
9. Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
10. Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
11. Have you felt down-hearted and blue?	☐	☐	☐	☐	☐	☐

12. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

☐	☐	\☐	☐	☐
All of the time	**Most of the time**	**Some of of the time**	**A little of the time**	**None of the time**

Sign with Participant Initials: _____ Date Form Completed: _____