A Double-blind, Placebo-controlled, Parallel Design, Randomized, Phase 2A Clinical Trial Evaluating the Potential Activity and Safety of hMaxi-K Gene Transfer in Males with Erectile Dysfunction

Protocol No. ION04-ED

Sponsor

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Protocol Synopsis

Title	A Double-blind, Placebo-controlled, Parallel Design, Randomized Phase 2A Trial Evaluating the Potential Activity and Safety of *h*Maxi-K Gene Transfer in Males with Erectile Dysfunction
Study Number	ION 04-ED
Clinical Study Phase	Phase 2A
Number of Centers	One site
Study Objectives	To evaluate the safety and efficacy of a single intracavernous injection of *h*Maxi K (8000 µg and 16000 µg) or placebo upon penile rigidity or erection in males with erectile dysfunction longer than six months that is attributable to an underlying, stable medical condition.
Study Design	This study is a double-blind, placebo controlled, parallel design, Phase 2A study evaluating the potential activity and safety of a single administration of *h*Maxi-K (8000 or 16000 µg) or placebo (PBS sucrose 20%) injected into the corpus cavernosum of the penis in men who have been unable to tolerate, do not wish to continue, or have had unsuccessful results with, prior therapy for ED.
Study Participant Population	The study population is men with erectile dysfunction attributable to an underlying, stable medical condition but who are otherwise in good health. The target population is men with erectile dysfunction and those who have been unable to tolerate, do not wish to continue, or have had unsuccessful results with, prior therapy for ED and with an erectile function domain score of IIEF >10 and < 21 at screening and baseline.
Study Procedures	Following screening and study drug administration at week 0, eligible participants will be evaluated at weeks 1, 4, 8, 12, and 24. At each study visit, participants will have a physical examination including examination of the penis (all visits), vital signs, electrocardiogram (ECG) (all visits). Laboratory evaluations including chemistry and hematology will be done at V1, 3, 4, 6, and 7. Urinalysis will be done V1, 2 (prior to dosing), 3, 4, 6, and 7. Endocrine parameters and PTT, PT, sed rate and CRP will be evaluated at Visit 1, 3 and 7. The participant will complete the erectile function domain of the IIEF and Sexual Encounter Profile (SEP) at screening/baseline and at V2, (SEP and IIEF at V2 prior to dosing) V3, V4, V5, V6, and V7. In all participants, plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR (V2-V7). These will be kept frozen at -20^{o} C or less at the site for eventual assay by Sponsor.
Primary Study Outcomes	The primary outcome measures will include the Erectile Function (EF) domain of the International Index of Erectile Function (IIEF), Sexual Encounter Profile (SEP), Questions 2 and 3 from SEP. The IIEF EF domain has a 30-point total score, where higher scores reflect better erectile function. SEP is a diary in which participants record each sexual attempt made throughout the study. The two questions from the Sexual Encounter Profile (SEP) deal with the ability to achieve vaginal penetration (SEP2), and the ability to maintain an erection long enough for successful intercourse (SEP3). The erectile function domain category of the IIEF will be used to evaluate the change in erectile status from baseline following

	administration of *h*Maxi-K. Change from baseline on the six questions of the IIEF's Erectile function domain category at every visit after administration of study drug will be calculated and compared among the two dose and one placebo groups.
Secondary Study Outcome	Safety will be assessed by analysis of adverse experiences, and abnormal findings on clinical laboratory tests, electrocardiogram, and physical examinations.
Study Duration	6 months per participant (approximately 1 year to enroll all particpants)
Study Start	TBD
Sample Size	35 participants; N=11 on 8000 µg; N= 11 on 16000 µg; N= 13 on placebo
Statistical Analysis	Both the safety data and data to assess activity will be presented as means and standard deviations or medians and ranges as appropriate for continusous data, and analyzed using either paired t- or Wilcoxon Sign Rank tests for within group changes, and with mixed effects or marginal models to determine differences in trends among the three cohorts over time. Incidence of adverse events will be presented as relative frequencies within groups.

Introduction

1.1 Overview

Erectile Dysfunction (ED) is a disease defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual function. ED is a common and significant problem that afflicts approximately 20 – 30 million men in the United States and 150 million men worldwide.[1-4] Overall, 52% percent of men have some degree of erectile dysfunction and 25% of 75-year old men have complete erectile dysfunction. The disease has a significant negative impact on the quality of life of the patient and their sexual partners and can cause depression and a feeling of inadequacy.

The most common cause of ED is advanced age. Proposed causes of age-induced ED include reduction of NO production, increased contractility of the smooth muscle of the penile corpora, increased myofilament calcium sensitivity due to altered *Rho* kinase expression/activity, and reduction in the relative percentage of smooth muscle fibers and an increase in collagen content thus decreasing the compliance of the cavernous tissue.[5-8] The second most common cause is diminished blood flow to the penis due to cardiovascular disease.

ED can either be reversible or nonreversible. Men over 50 years tend to have permanent causes of ED that are disease-related. The most frequently associated diseases include atherosclerosis, hypertension, anti-hypertensive medications, diabetes mellitus, and pelvic surgery and radiation. Treatment for nonreversible ED include: 1) oral phosphodiesterase inhibitors e.g., sildenafil (Viagra™); 2) vacuum erection devices; 3) intraurethral alprostadil (Muse™); 4) intracavernous injection of prostaglandin E1 (Caverject™ or Edex™) or a combination of PGE1, papaverine and phentolamine; and 5) surgical placement of penile prosthetic implants for physiological disease. Despite the availability of these therapies, the lack of efficacy, their potential side effects, and the loss of spontaneity (the treatment must be planned and taken or used prior to initiating the sex act) have limited therapy for ED to about 10% of the overall patient population with nonreversible ED.

In younger men (ages 20 – 40), psychological causes for ED often predominate and this reversible form of ED can be treated with sex therapy and the drugs listed above. Current therapies for reversible forms of ED include: 1) sex therapy for psychological disease; 2) correction of endocrine abnormality with appropriate hormone therapy in a small percentage of men (<5%); 3) surgical correction for men diagnosed with Peyronie's disease; 4) penile bypass surgery in men who have suffered pelvic or perineal trauma with injury to the penile arteries. ED as a consequence of all other etiologies (more then 90%) is **not** reversible.

There are many reasons why acceptance by physicians and participants for currently available therapies is limited. Sex therapy has limited efficacy and is only suitable for men with a normal penile function and a psychological cause of the ED. Overall, Vacuum Erection Devices (VED) are effective in about 30% of men who use them, phosphodiesterase inhibitors such as Viagra®, (sildenafil), Cialis® (tadenafil), and Levitra® (vardenafil) in about 70%, intracavernous injections in 85%, intraurethral alprostadil in about 40%, and penile implants in over 95%. Medical intervention with drugs is least effective in men with long-standing diabetes because the physiological effects of severe autonomic neuropathy and small vessel atherosclerosis limit the effective action of the medications. Although penile prosthetic implants can be inserted into nearly every man with ED, the negative stigmata of having an operation and a mechanical erection have limited acceptance of the devices by participants since their introduction in the late1960's. Furthermore, the rate of infection in penile prosthetic implants is reported between 3 to 9% and inflatable prostheses have a long-term mechanical failure rate of about 10%. Ion

Channel Innovations has developed hMaxi-K to treat ED using the same principal as drugs that have been used since 1982, i.e., treatment with medicines causing relaxation of the penile smooth muscle resulting in rigid erections. hMaxi-K consists of the gene for the α-pore subunitof 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. This effect on ion exchange across the smooth muscle cells allows the smooth muscle of the corpora to relax; the corporal sinusoids become engorged with blood, and the penis to stay rigid. In preclinical studies, a single administration of hMaxi-K to aging rats with ED has been shown to last at least up to six months.9 Furthermore, results from these preclinical studies suggest that expression of the α-pore subunit for the maxi-K channel following administration of hMaxi-K may have a long-term effect on the erectile mechanism.9 This activity of hMaxi-K may represent a distinct advantage over current drug therapy for ED where the effect of treatment is temporary.

1.2 Physiology of Penile Erection

In order to better understand ED and the mechanism of action for gene transfer using *h*Maxi-K, a description of the normal function of the penis is useful. Normal erection is a neurovascular event that depends upon proper function of two, well-vascularized cylinders of spongy tissue in the penis: the two corpora cavernosa (the corpora). A third channel, the corpus spongiosum, contains the urethra through which either urine or semen passes but is not involved in causing penile rigidity.

Blood flows into the corpora from two pudendal arteries that emerge from the pelvis and separately fork into two smaller branches, the dorsal and cavernous arteries, and enter the penis. The cavernous arteries are the arteries most responsible for erection. Branches of the cavernous artery enter directly into the endothelial-lined, expansible, sinusoidal spaces that comprise the cavernous bodies. Two other branches of the pudendal artery supply the corpus spongiosum and glans penis. When the penis is flaccid the cavernous arteries are only 0.4 millimeter in diameter while during sexual excitement these arteries will double in diameter. These narrow blood vessels are extremely susceptible to blockage in participants with diseases associated with arterial narrowing, e.g., hypertension and atherosclerosis.

During sexual excitement there is a ten-fold increased blood flow to the erectile chambers of the penis. As the sinusoids fill with blood at systemic pressure the smooth muscle cells within the corpora relax, become compressed by the arterial filling pressure and compress the out flowing veins against the thick elastic covering of the corpora, the tunica albuginea, and a rigid erection occurs. Consequently, the penis function as a tone organ, i.e., when tone is high the smooth muscle cells of the corpora are partially contracted and the penis is flaccid. When the tone is low and the myocytes are relaxed, the penis is fully engorged with blood and becomes erect. However, with increased age, diseases (e.g., diabetes mellitus), or states where the smooth muscle cells fail to relax (e.g., anxiety), the occlusion of venous outflow by the corporal smooth muscle is incomplete with a resultant soft, or partial, erection. Regulation of the state of contraction/relaxation of smooth muscle and its effect on the erectile process depends primarily upon the intracellular concentrations of certain ions, in particular potassium and calcium.

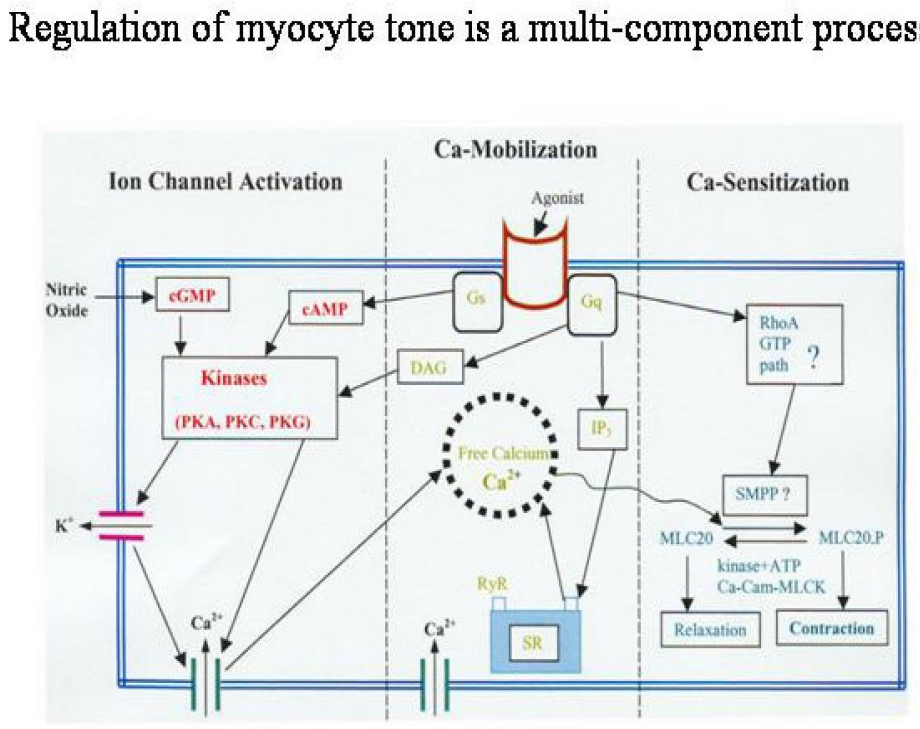
Contraction and relaxation of smooth muscle, is critical to the storage and/or conduit function(s) of hollow organs such as the bladder, gut, blood vessels and penis. K channels play an important role in this process by virtue of their ability to alter the membrane potential and excitability of smooth muscle cells.[10-12] Their primary effect is to modulate Ca^{2+} influx through Ca channels (i.e., L-Type, voltage-dependent). The amount of Ca^{2+} that enters the cell through these channels is a major determinant of the free intracellular calcium levels inside the smooth muscle cell,

which in turn, determine the degree of smooth muscle cell contraction. Overall, regulation of the contractile process is organ-specific and modulated by a variety of K channel subtypes.[13--16] Prominent amongst the K channel subtypes found in smooth muscle is the large conductance, calcium sensitive K channel, referred to as the maxi-K channel. Increased maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. Moreover, alterations in K channel physiology/function are increasingly being recognized as a major contributing factor to the development of the vascular pathologies associated with diabetes,[17,18] as well as urogenital disorders, including erectile dysfunction .[11,14]

Intracellular concentrations of ions and second messengers (e.g., (K^+, Ca^{++},cAMP, cGMP, etc.) are affected by release of neurotransmitters and in turn the changes can be rapidly propagated from cell to cell through specialized intercellular protein pores called gap-junctions.[19-22] The importance of gap junctions to coordinate smooth muscle function cannot be overstated. Even after severe nerve loss such as can occur with diabetes or radical prostatectomy there remains sufficient level of intercellular coupling to allow the corporal tissue to function normally following membrane or receptor stimulation. For example, oral administration or by intracavernous or intraurethral injection of a drug that stimulates the exchange of ions across the cell membranes can allow the penis to become erect. This approach has proven successful with FDA approved intracavernous (Edex^{TM}, Caverject^{TM}) and intraurethral (Muse ^{TM}) injection with prostaglandin E1.

Thus, cellular mechanisms that influence cell membrane potential are of critical importance in regulating the moment-to-moment tone of any organ composed predominantly of smooth muscle whether it is the penis, urinary bladder, blood vessels or gut. The relationship of ions, ion channels, and second messenger interaction in smooth muscle cells is depicted in Figure 1.

Figure 1: Regulation of Myocyte Tone



Inhibitory interaction of K^+ efflux and Ca^{++} influx. **Persistent Ca^{++} influx is necessary to maintain myofilament contraction.**

1.3 Maxi-K Channels

At least four K channel subtypes are present in human corporal smooth muscle cells. These channels include: (1) the voltage and calcium-sensitive maxi-K, (2) ATP dependent K channels (K_{ATP}), (3) inwardly rectifying channels (Kir), and (4) voltage-gated K channels (K_V).[7] The primary function of K channels is to modulate Ca^{++} influx through Ca-channels (i.e., L-Type, voltage-dependent). The amount of Ca^{++} that enters the cell through these channels is a major determinant of the free intracellular calcium levels inside the smooth muscle cell, which in turn, determine the degree of smooth muscle cell contraction.

Overall, regulation of the contractile process is organ-specific and modulated by a variety of K channel subtypes. [16,23-25] The maxi-K channel is the most prominent (numbering approximately 1000 to 1500/cell[22]) and well-studied K-channel subtype involved in corporal smooth muscle relaxation. Increased maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. Moreover, alterations in K channel physiology/function are increasingly being recognized as a major contributing factor to the development of the vascular pathologies associated with diabetes [17,18] as well as urogenital disorders [14] including erectile dysfunction. [10, 11]

The maxi-K channel is composed of α and β subunits.[12] The α, or pore forming, unit of the maxi-K channel is a tetramer of homologous units. Each α subunit is composed of 11 hydrophobic domains. Seven of the domains are transmembrane spanning (S0-S6) and the remaining 4 domains are cytoplasmic (S7-S10). The α subunit has intrinsic sensitivity to Ca^{++} via the cytoplasmic tail region.[25] Even in the absence of the β subunit, expression of the α subunit of the maxi-K channel can form a functional unit; however, expression of the β subunit alone does not form a functional channel.[17] It has been proposed that the role of the β subunit is to modulate the α subunit activity or sensitivity to Ca^{++}. [25]

1.4 Rationale for Development of *h*Maxi-K

Ion Channel Innovations has developed a gene transfer product, *h*Maxi-K, and plans to investigate the effect of increased expression of maxi-K channels in the smooth muscle of the penis in participants with ED. *h*Maxi-K consists of the gene for the α pore of the maxi-K channel, *h*Slo, inserted into a plasmid vector, pVAX. Because heightened smooth muscle tone may be a causative factor of erectile dysfunction, increased numbers of maxi-K channels following gene expression of *h*Maxi-K may effectively correct ED.[9, 26] This approach to treat ED uses the same principal as drugs developed since 1982, i.e., treatment with medicines causing relaxation of the penile smooth muscle results in rigid erections.

How does Maxi-K channel gene transfer work? The rationale for the utility of K channel gene therapy is related to the important contributions that ion channels make to the contraction and relaxation of smooth muscle cells (i.e., myocytes). Ion channels are membrane proteins that provide a selective permeability barrier to the movement of ions across the cell membrane (influx and efflux of ions, i.e., K^+ and Ca^{2+}.) In short, these membrane proteins provide a selective channel through which ions can flow (K^+ flows through K channels, and Ca^{2+} flows through Ca channels, but not vice versa). The opening and closing of these channels is regulated by numerous cellular processes. However, anything that increases the extent that they are open will increase the amount of ion that can move through the channel over any given period of time. The idea behind maxi-K channel gene transfer is to increase the number of maxi-K channels in the cell membrane, so that when the cells are activated by the normal erectile stimulus (i.e., nitric oxide released from nerves), there will be an increase in the efflux of K^+ from the cell.

The role of the maxi-K channel in generation of an erection can be illustrated by its interaction with nitric oxide (NO) as shown in Figure 2.

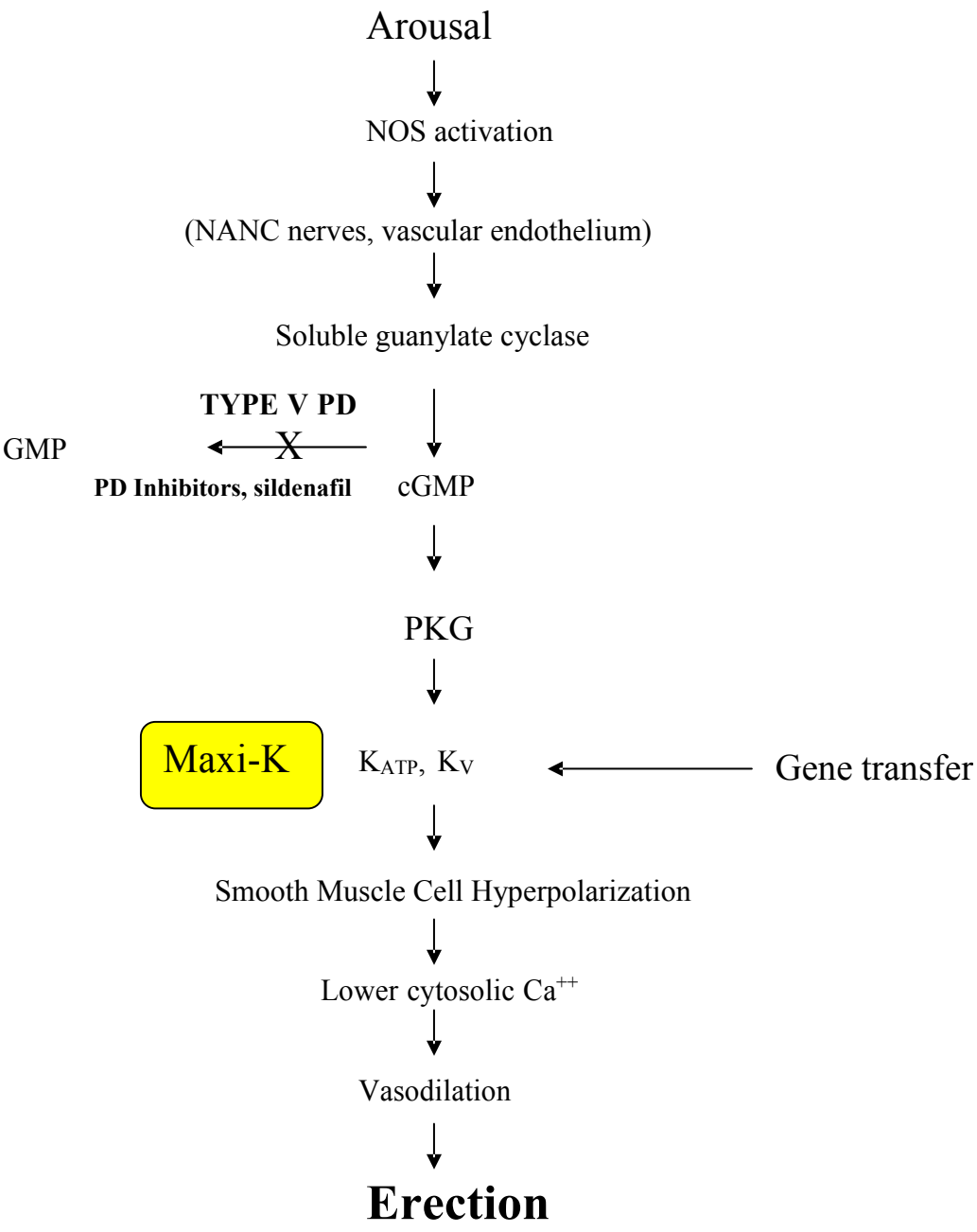


Figure 2. Interaction of Maxi-K channel and nitric oxide. See text for explanation

The production of NO leads to erection through a pathway that involves cGMP-mediated activation of maxi-K channels resulting in hyperpolarization of vascular and cavernosal smooth muscle cells. As described above, hyperpolarization inhibits the influx of the calcium ion and causes the smooth muscle cells of the corpora to relax allowing increased blood flow into the corpora at systolic pressure and cause erection. Note the central role for protein kinase G (PKG) and ion channels in this pathway and the critical position at the end of the pathway to erection that potassium channel activation has in the erectile mechanism. Thus, loss or erectile function in

diseases associated with a reduction in the expression of the maxi-K channel, in NO release, or in PKG production should be reversed with therapies that increase the number and/or expression of the maxi-K channel. (Modified from Archer [11])

1.5 Description of *h*Maxi-K

The gene for the α or pore-forming subunit, *hSlo*, has been cloned and linked to the kanamycin-resistant plasmid vector, pVAX, to construct *h*Maxi-K. The gene, *hSlo*, is transcribed from the CMV promoter.

Transfection and functional activity of the *hSlo* gene using this vector has been demonstrated in several *in vitro* experiments. In two separate experiments, intracellular uptake and expression of the gene was demonstrated on fluorescence, microscopic, and patch clamp electrophysiological examination of transfected Xenopus oocytes and human embryonic kidney cells.[27] Endothelin-1 stimulation causes a transient increase in the intracellular concentration of calcium [28,29] A significant decrease was observed in both the resting Ca^{++} levels and the peak amplitude of the ET-1 – induced intracellular Ca^{++} transient in *hSlo*-transfected human corporal smooth muscle cells. Thus, transfer of plasmid-*hSlo* into the smooth muscle cells of the corpora should result in gene expression that allows hyperpolarization of the cells and smooth muscle relaxation with subsequent erection.

The safety and activity of intracorporal injection of varying doses of the *hSlo* gene has been evaluated in 265 aged rats and 256 streptozotocin-induced diabetic rats. The ability of the plasmid-*hSlo* construct to express physiologically functional maxi-K channels has been demonstrated in at least 3 experiments using these two animal models of ED.[9,26] Animals administered the *hSlo* gene were able to achieve penile rigidity with electrical stimulation of the cavernous nerves.

In order to assay the effect of pcDNA/*h*Slo on the erectile response under conditions that might better mimic natural stimulation for penile erection, two experiments were conducted using either central neural stimulation or apomorphine administration with measurement of the ICP/BP ratio in rats administered a single injection of 100 μg pcDNA/*h*Slo. In those experiments the medial preoptic area (MPOA) and the paraventricular nucleus (PVN) of the hypothalamus are known to be important to erectile function/capacity in humans and rats.[30-33] The drug apomorphine has a higher affinity for D_2-like receptors than other dopamine receptor subtypes (i.e., D1-D5) and it is this receptor subtype that is thought to be the main site for the induction of erections in the paraventricular nucleus of the hypothalamus. Apomorphine is therefore postulated to increase erectile responses by acting as a conditioner in the PVN, increasing the response to sexual stimuli resulting in enhanced erections

Retired breeder Sprague-Dawley rats were injected intracorporally with 100 μg pcDNA/*h*Slo. Following electrostimulation of the MPOA, expression of *h*Slo in the rat corporal tissue was confirmed by RT-PCR. Rats injected intracorporally with pcDNA/*h*Slo had significantly higher ICP response compared to untreated age-matched control animals following central stimulation. There was no significant difference in the ICP/BP between the *h*Slo-treated and young control animals following MPOA electrostimulation.

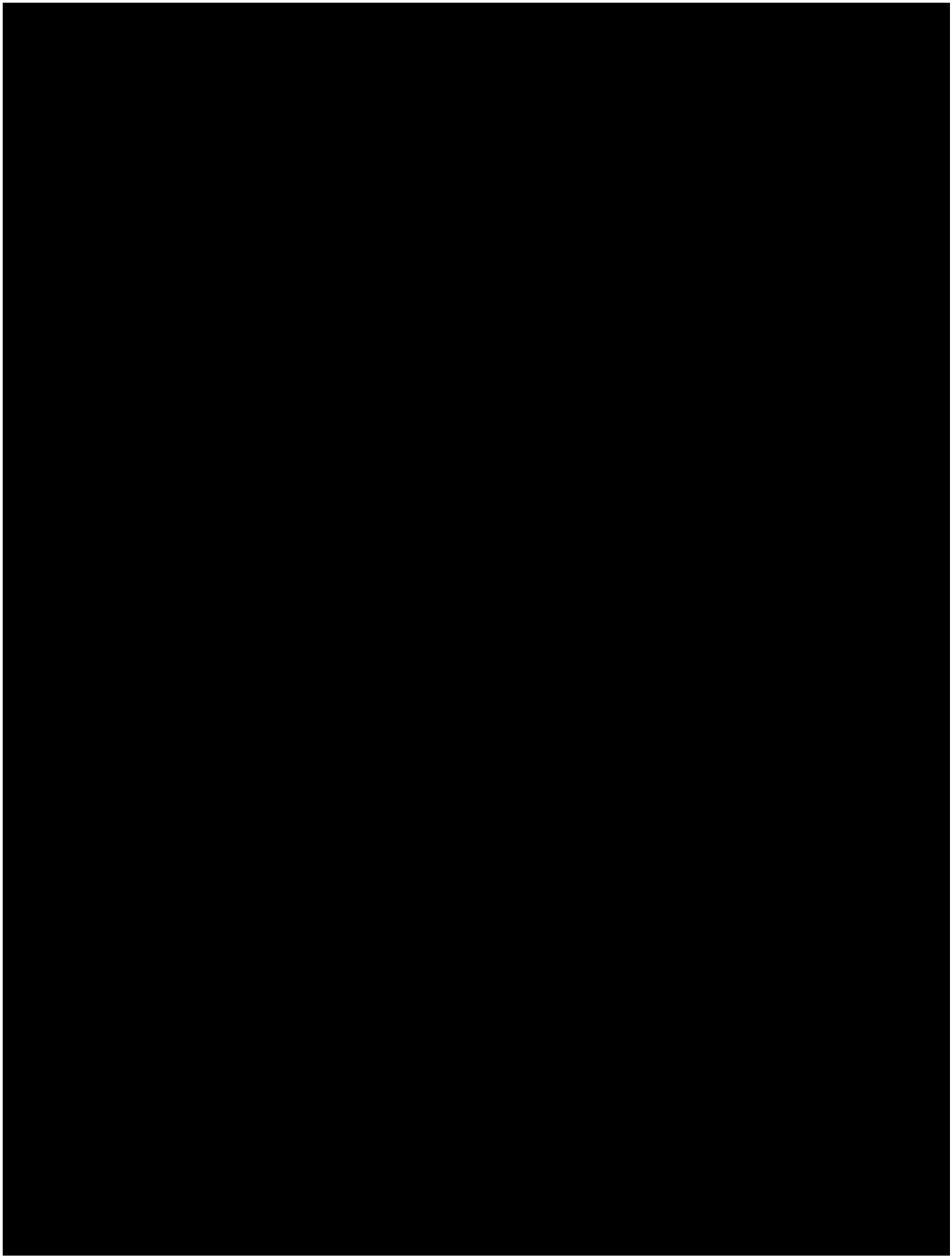
In the second experiment, the hypothalamic region involved in erection was stimulated pharmacologically using apomorphine in the retired breeder rat model of erectile dysfunction. Apomorphine acts in the CNS to elicit erectile responses within several minutes of administration. The *h*Slo-treated retired breeder rats received a single intracavernous injection of 100 μg pcDNA/*h*Slo. Following administration of apomorphine one week later, the *h*Slo-transfected rats had significantly higher ICP/BP ratios than the placebo-treated age-matched

control rats. [34] Overall, these experiments evaluating the effect of central neural stimulation on the ICP/BP ratio in *h*Slo-treated rats, suggest that expression of *h*Slo and increased maxi-K channels in the membrane of corporal smooth muscle of penis can be responsive to normal physiological mechanisms involved in erection.

Although this phase 1 study is designed to evaluate a single administration of *h*Maxi-K, the safety of *h*Maxi-K following multiple administrations has also been investigated in preclinical studies. Doses of *h*Maxi-K evaluated were 10, 30, 100, 300, and 1000 μg. Histopathological examination of the major organs (heart, lung, liver, spleen, kidney, brain, and testes) demonstrated normal tissues among all treatment groups. Importantly, no cardiac toxicity was detected in any animal at any dose of *h*Maxi-K. Laboratory parameters were within normal limits at all timepoints for all doses tested. No adverse clinical effects were seen in cardiovascular parameters.

Extensive biodistribution studies at the ten-copy level of *h*Maxi-K have been conducted in rats administered 1000 μg of intracavernous *h*Maxi-K. Fourteen organs were examined at 1, 4, 8, and 24 hours and 1, 2, and 4 weeks after transfer. Importantly, no signal of gene transfer was detected at any time point in either cardiac myocytes or testis tissue following administration of *h*Maxi-K. In a separate study, the half-life of *h*Maxi-K in human blood was found to be less then 30 minutes. Given the results of the biodistribution studies, the known half-life of *h*Maxi-K, and the proposed use of a penile tourniquet, the amount of *h*Maxi-K able to access the systemic circulation is expected to be negligible.

1.6 Clinical Data



[redacted]

2.0 Objectives

The primary objective of this study is to evaluate thesafety of a single intracavernous injection of *h*Maxi-K (either 8, 0000 or 16,000 µg) compared to placebo in males with erectile dysfunction longer than six months that was attributable to an underlying, stable medical condition upon penile rigidity or erection in males with erectile dysfunction longer than six months that is attributable to underlying, stable medical condition.

The secondary objective of this study is to evaluate the efficacy of a single intracavernous injection of *h*Maxi-K, either 8,000 or16,000 µg, compared to placebo.

3.0 Study Design

This study is double-blind, placebo controlled parallel design Phase 2A trial evaluating the potential activity and safety of two different doses of *h*Maxi-K (16000 µg, and 8000µg) given in a single administration relative to placebo injected into the corpus cavernosum of the penis. The proposed study period is approximately 6 months following the single injection of study drug
per participant. This study will enroll 11 participants at *h*Maxi-K doses of 8000 µg, 11 participants at *h*Maxi-K doses of 16000 µg and 13 on placebo. A maximum of 35 participants will be enrolled.

4.0 Study Population

The study population includes men with erectile dysfunction attributable to an underlying, stable medical condition but who are otherwise in good health. The most common stable medical diseases that cause erectile dysfunction include hypertension and atherosclerosis, antihypertensive medication, type I and type II diabetes mellitus, pelvic surgery and pelvic radiation, cerebrovascular accidents (stroke), multiple sclerosis, and Parkinson's disease. Men with erectile dysfunction secondary to aging as a solitary cause will not be included in the trial. . The target population is men with erectile dysfunction who have been
unable to tolerate, do not wish to continue, or have had unsuccessful results with, prior therapy for ED.

Only participants considered eligible according to the protocol-specified eligibility criteria will be able to receive study drug.

4.1 Inclusion Criteria

Eligible participants must meet the following inclusion criteria:

a) Signed Informed Consent

b) Be adult males over 18 years of age diagnosed with erectile dysfunction and whose ED is attributable to an underlying, stable medical condition such as hypertension and

atherosclerosis, antihypertensive medication, type I and type II diabetes mellitus, pelvic surgery and pelvic radiation, cerebrovascular accidents (stroke), multiple sclerosis, and Parkinson's disease;

c) Participants must have been unable to have successful sexual intercourse for 3 months prior to study entry without specific ED therapy such as Vacuum Erection device (VED), Viagra™ (sildenafil), Cialis ™ (tadalafil), Muse™ (alprostadil), or intracavernous injection therapy with an erectile function domain score of IIEF >10 and < 21 at screening and baseline;

d) Have been unable to tolerate, do not wish to continue, or have had unsuccessful results with, prior therapy for ED, e.g., Viagra™, intracavernous injection therapy, Muse™, or VED;

e) If diabetic, documentation of HgA_{1c} less than or equal to 8.5 % prior to enrollment;

f) If receiving medication for hypertension, documentation of blood pressure has been stable on the same medication for at least 2 months prior to enrollment;

g) Be heterosexual and in a stable, monogamous relationship of at least six months duration;

h) Agree to attempt intercourse with their partner at least four times per month while participating in the study;

i) Agree not to use other treatments for ED while participating in this study;

k) Have screening laboratory values and ECG that are within the normal range. See exclusion criterion below.

l) Have a normal physical examination of the penis;

m) If participant had a radical prostatectomy a PSA <0.4 for at least one year documented by 2 measurements during the preceding year;

n) Be literate, able to give written informed consent, and comply with all study procedures and requirements.

4.2 Exclusion Criteria

Participants will be excluded who have:

a) A history of sickle-cell disease, sickle cell trait, or any other medical condition that, in the judgment of the investigator, would contraindicate the administration of study medication or interfere with the study evaluations;

b) 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.

c) 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 medicationnitrates, or anti-epileptic drugs for less than 6 months at the time of randomization

d) Poorly controlled diabetes mellitus as defined by $HgA_{1c} > 8.5$ mg% at time of enrollment;

e) Change in medication for diabetes or hypertension within 2 months of study enrollment;

f) Gonadal failure (testosterone < 200 ng/dl) not treated with hormone replacement;

g) History of malignancy except non-melanomatous skin cancers

h) A life expectancy of less than 12 months;

i) An indwelling urethral catheter;

j) A prior penile prosthetic implant

l) Received an investigational drug, investigational therapy, or other form of ED therapy, including approved treatments, within the past 30 days;

m) Peyronie's disease;

n) Any screening laboratory values outside of the normal laboratory range as defined by the central laboratory normal ranges and in the judgment of the investigator is considered clinically significant (hepatic biochemical markers [AST, ALT, GGT, alkaline phosphatase, and bilirubin] > twice the upper limit of the normal reference range may be accepted with written consent of the sponsor).

o) Any clinically significant ECG abnormality

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).

5.0 Test Material and Administration

5.1 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.

Table A below provides information on the dosing of h Maxi-K dosing 8000 µg, 16000 µg and placebo. Each 2 mL vial contains 4000 µg per mL. The sites will store the participant vials at -20°C in a locked freezer prior to use. Vials can be kept at -20°C for up to 360 days. Participant vials will be retained at the site until study completion and after the drug accounting processes have been completed, when they will either be returned to the Sponsor or destroyed with Sponsor approval.

Table 1. - Final Dose – *h*Maxi-K

	8000 μg	16000 μg	PBS-20% sucrose
*h*Maxi-K	2 ml	4 ml	0 ml
PBS-20% sucrose	2 ml	0 ml	4 ml
Number of Vials*	2	2	2

*Each vial contains 4000 μg per mL.

5.1.1 Packaging and Labeling

Each assigned vial will have a double blinded 2 part detachable label (tear off part to be placed in the participant's source documents). Each vial label will contain the following information:

- Study ION 04-ED
- Participant identification number
- INSTRUCTIONS: Inject contents of both vials into corpora cavernosa
- Store at controlled room temperature once constituted: [redacted]C to [redacted]C [redacted] F to [redacted] F).
- CAUTION: New Drug – Limited to investigational use
- Manufactured for Ion Channel Innovations, LLC by Althea Technologies, Inc., San Diego, CA 92121

Note: In each dosing arm, 11 participants will receive *h*Maxi-K and 13 will receive PBS-20% sucrose (placebo).

Sharp Clinical Services, Inc. will prepare 40 kits of product using randomization schedule generated by a Statistical Group. The kits will be shipped to the central pharmacy at Dasman and stored at -20C. Following notification by the site that an eligible participant is to be giventhe study medication the pharmacy will send the blinded kit to the investigaro for use.
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 site 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.

5.2 Dose Selection and Administration

Two doses of *h*Maxi-K will be evaluated: 8,000 μg and 16,000 μg compared to PBS-20% sucrose (control group).
The gene product or PBS-20% sucrose will be administered in the office. The blinded participant kit will be taken from the freezer and the vials warmed to room temperature. The rubber cap of the vial will be wiped with alcohol. The 8 ml contents of the 2 vials (4 ml each) in the kit will be drawn into an 10 ml syringe and administered by the investigator through a 25 gauge needle into the corpora of each participant.

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.

The investigator or designee will administer study drug and be responsible for the 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 or designee. 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 authorization from the sponsor. In the event that study drug is only partially administered (e.g., an adverse event occurs during injection), then any partially used vials must be retained for drug accountability. After the Sponsor's designee has completed the drug accountability and approval for drug disposal, study drug may be disposed using the site's standard operating procedure for biohazard waste.

If a clinically serious sign or symptom is reported the medical monitor will contact the sponsor and no further enrollment will be done until the medical monitor or sponsor gives permission. In the previously completed clinical study evaluating intracorporal administration of up to 16000 µg *h*Maxi-K in 20 participants with ED, there was only one adverse event considered possibly related to *h*Maxi-K ("tingling warmth in the glans").

To minimize the possibility of dehydration and/or a vasovagal response following injection of the *h*Maxi-K, participants will be instructed to eat breakfast and drink fluids on that morning.

5.2.1 Dose Limiting Toxicity

A DLT is defined as the occurrence within the first 4 weeks following a single administration of *h*Maxi-K of an unacceptable toxicity, as described below. Adverse events for cardiovascular, blood chemistry, complete blood count, or urine categories of adverse events will be graded according to the criteria provided in Appendix A.

No acute toxicity was seen in the preclinical studies with administered doses up to 1000 µg (approximately 1500 µg/kg) or in men and women gien doses up to 16000 µg. Potential toxicities would be those that could occur due to enhanced efflux of K^+ with resultant decreased intracellular Ca^{++} concentration thus altering smooth muscle function in diverse organ systems.

The following criteria will be used to define:
DLT:

1) Any adverse events of Grade 2 or higher in the cardiovascular category of adverse event criteria listed in Appendix A;

2) Any adverse events of Grade 2 or higher in the blood chemistry, complete blood count, or

urine categories of adverse event criteria listed in Appendix A.

3) Penile pain and/or erection that persists for more than four hours after gene transfer;
4) Change in random hormone levels greater than 2 times the upper or lower limits of normal at any time post dosing..

Stopping Rule

If within 4 weeks following the single administration of *h*Maxi-K, if any participant experiences a grade 3 or higher dose limiting toxicity, no additional participants will be accrued and no further doses of *h*Maxi-K (or placebo) will be administered. Clinical data regarding the event will be collected, reviewed, and discussed with the Ethics Committee (EC) and Kuwait Ministry of Health (MOH). After review of these data with EC and MOH the clinical study may resume only with concurrence of EC and MOH.

5.3 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 two weeks of study entry (including over-the-counter preparations) will be recorded on the Medical History Form. Any changes in concomitant medication, including additions, discontinuations, and dose changes, occurring during the study will be recorded on the concomitant medication form.

Participants are not allowed to concomitantly use any approved, unapproved, or investigational medications or therapies intended to treat erectile dysfunction. These medications/therapies include, but are not exclusively: Viagra™, Cialis™, Levitra™, Muse™, prostaglandin E1 as Caverject™ or Edex™, Trimix, yohimbine, apomorphine (Uprima™), vacuum erection devices, or penile prosthetic implants.

6.0 Outcome Assessments and Procedures

6.1 International Index of Erectile Function (IIEF)

The International Index of Erectile Function (IIEF) [35] is a validated, self-administered questionnaire that has been shown to be a cross-culturally and psychometrically valid measure of male erectile dysfunction.

The test contains 15 questions in five domains: (a) erectile duration (six items); (b) orgasmic function (two items); (c) sexual desire (two items); (d) intercourse satisfaction (three items); and overall sexual satisfaction (two items). The questionnaire has been accepted by the FDA and peer-reviewed scientific journals and is used currently as the clinical endpoint in all pivotal studies evaluating new drugs for erectile function.

The erectile function domain, questions 1-5, and 15 of the IIEF has been validated to assess erectile changes only.[36, 37] The EF domain of the IIEF will be completed at Visit 1 and V2-V7.

6.2 Sexual encounter profile (SEP)

The SEP is a questionnaire composed of 5 questions assessing sexual function. SEP 2 evaluates the penetrability of the erection and SEP 3 the maintenance of the erection. This questionnaire will be completed at V2-V7. See Appendix C.

6.3 Physical Examination

6.3.1 Physical Examination

A complete physical examination, including a complete urogenital exam (see below), and review

of body systems will be performed at all visits. Vital signs (heart rate and blood pressure) and an examination of the penis (see below) will be performed at all visits. Height will be obtained at Screening only. Weight and oral temperature will be obtained at Screening and V7. Any untoward clinically significant change from Screening will be recorded on the CRF as an adverse event.

6.3.2 Physical Examination of the Penis

Physical examination of the penis will be done at each visit and will include inspection, and palpation.

6.4 Electrocardiograms

A 12-lead ECG will be performed at all visits: Screening (V1) Baseline (V2 -prior to treatment and at 1 and 2 hours post treatment), V3, V4, V5, V6, and V7. These ECGs obtained at the study centers, will be forwarded to a central reader for final interpretation. The over reader will return the results to the study center within 48 hours. This does not negate the responsibility of the staffconducting the study to initially review the ECG and to take appropriate clinical actions. The following parameters will be assessed: heart rate, rhythm, PR interval, QT interval, QTcF, QTcB, QRS duration, and overall evaluation.

6.5 Vital Signs

Vital signs will be performed at all visits. Vital signs should include an apical heart rate and brachial blood pressure (BP) both taken after 5 minutes resting in the sitting position (standard sphygmomanometry). Any clinically significant changes from screening will be recorded on the CRF as an adverse event. Following administration of study drug at V2, BP and heart rate will be measured and recorded every 15 minutes for 2 hours.

6.6 Laboratory Safety Tests and Pharmacokinetics Evaluations

An accredited laboratory will function as the central laboratory for all plasma and urine specimens.

Laboratory safety tests will be performed as follows. The laboratory safety tests include:

- Hematology: Complete blood count (CBC) with differential: Screening (V1), Week 1 (V 3), Week 4 (V4), 12 weeks (V6) and 24 weeks (V7) post dosing with study drug.
- Platelet count, PT, PTT, sedimentation rate (sed. rate), CRP only at Visits 1, 3 and 7 only.
- Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, LDH, GGT, total bilirubin, total protein, glucose at Screening (V1), Week 1 (V3) , Week 4 (V4), 12 weeks (V7) and 24 weeks (V7) post dosing with study drug.
- Urinalysis: Microscopic RBC and WBC, protein, glucose, nitrites, and specific gravity at Screening (V1), Week 1 (V 3), Week 4 (V4), 12 weeks (V6) and 24 weeks (V7) post dosing with study drug.
- Endocrine: Total testosterone, random cortisol, TSH, T4 (V1, V3, and V7 only);
- At screening visit only, participants must have a hemoglobin A1c
- At V2 –V7, plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20^o C or less at the site for eventual assay

by Sponsor. At Visit 2 specimens should be collected both pre-dosing (to serve as a baseline) and 2 hours post-dosing

6.7 Pharmacokinetics

Blood specimens will be obtained at Visit 2 (predose) and Visits V3-7 for analysis of *hSlo* cDNA with PCR.

Instructions for collection of 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)

7.0 Schedule of Evaluations

The schedule of evaluations is summarized in Appendix B and described in detail below.

7.1 Visit 1 (Study Week (-) 2 - Screening)

The following evaluations and tests will be performed:

- Preliminary Screening:
  - Obtain written informed consent process for study participant ;
  - Medical history including history of erectile dysfunction to assess eligibility for study treatment;
  - Physical examination including physical examination of penis;
  - Vital signs (BP, heart rate, oral temperature, weight and height)
  - Participant completes the IIEF (screening)
  - Complete screening if entry criteria are met on preliminary screening:
  - Obtain urine for routine urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific gravity;
  - Electrocardiogram;
  - Fasting blood specimens for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, Partial Thromboplastin (PTT), Prothrombin Time (PT), sedimentation rate (sed rate), C-reactive protein (CRP).
    - Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, LDH, GGT, total bilirubin, total protein, glucose;
    - Hemoglobin A1c (diabetic participants);

- Endocrine: total testosterone, random cortisol, TSH, T4

- Schedule next study visit when screening laboratory results are available.

7.2 Visit 2 (Study Week 0 - Administration of study drug)

Based on the participant fulfilling all eligibility criteria following the screening procedures (Visit 1), 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 patient will be considered a screen failure.

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

- Adverse events since screening visit;
- Physical examination including examination of the penis for changes since the screening visit;
- Vital signs (BP, heart rate);
- ECG prior to administration of study drug and at 1 and 2 hours post study drug;
- Participant completes SEP and IIEF Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific gravity;
- Plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20^{o} C or less at the site for eventual assay by Sponsor. Samples will be collected pre-dosing and at 2 hours post dosing.
- Administer study drug. The participant will be observed in the clinic for two hours after the injection. During that time blood pressure and heart rate will be measured and recorded by the nurse every 15 minutes. ECG will be done at one and two hours after administration of study drug and reviewed by a physician at those times. The penis will be inspected for ecchymosis or persistent erection. The participant will be asked if there is any persistent pain at the injection site and will be treated symptomatically, if needed. The participant will be asked if symptoms of malaise, vertigo, light-headedness, and nausea are present. Emergency equipment for resuscitation will be immediately available in the event of an unexpected serious reaction to the study drug.

7.3 Visit 3 (Study Week 1)

The following tests and evaluations will be performed:

- Adverse events and concomitant medications since the last visit;
- Physical examination including examination of penis;
- Vital signs (BP, heart rate);
- Electrocardiogram;
- Obtain plasma for the following laboratory determinations:
    - Hematology: Complete Blood Count (CBC) with differential, platelet count, Partial Thromboplastin (PTT), Prothrombin time (PT), sedimentation rate, C-reactive protein;
    - Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^{+}, K^{+}, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, , LDH, GGT, ALT, AST, total bilirubin, total protein, glucose;
    - Endocrine: total testosterone, random cortisol, TSH, T4;

- Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific gravity;
- Plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20^{o} C or less at the site for eventual assay by Sponsor.
- Participant completes the IIEF and SEP.
- Schedule next study visit.

7.4 Visit 4 (Study Week 4)

The following tests and evaluations will be performed:

- Adverse events and concomitant medications since last study visit;
- Physical examination including examination of penis
- Vital signs (BP, heart rate);
- Electrocardiogram;
- Obtain plasma for the following laboratory determinations:
- Hematology: Complete Blood Count (CBC) with differential, platelet count;
- Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^{+}, K^{+}, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, , LDH, GGT, ALT, AST, total bilirubin, total protein, glucose;
- Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific
- gravity;
- Blood specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20^{o} C or less at the site for eventual assay by Sponsor.
- Participant completes the IIEF and SEP;
- Schedule next study visit.

7.5 Visit 5 (Study Week 8)

The following tests and evaluations will be performed:

- Adverse events and concomitant medications since last study visit;
- Physical examination including examination of penis;
- Vital signs (BP, heart rate);
- Blasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20^{o} C or less at the site for eventual assay by Sponsor.
- Electrocardiogram;
- Participant completes the IIEF and SEP;
- Schedule next study visit.

7.6 Visit 6 (Study Week 12)

The following tests and evaluations will be performed:

- Adverse events and concomitant medications since last study visit;
- Physical examination including examination of penis;
- Vital signs (BP, heart rate);
- Electrocardiogram;
- Obtain plasma for the following laboratory determinations:
- Hematology: Complete Blood Count (CBC) with differential, platelet count,
- Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, , LDH, GGT, ALT, AST, total bilirubin, total protein, glucose;
- Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific gravity;
- Plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20° C or less at the site for eventual assay by Sponsor.
- Participant completes the IIEF and SEP;
- Schedule next study visit.

7.7 Visit 7 (Study Week 24) or Exit Visit:

The following tests and evaluations will be performed:

- Adverse events and concomitant medications since last study visit;
- Physical examination including examination of penis;
- Vital signs (BP, heart rate, oral temperature, weight);
- Electrocardiogram;
- Obtain plasma for the following laboratory determinations:
- Hematology: Complete Blood Count (CBC) with differential, platelet count, Partial Thromboplastin (PTT), Prothrombin time (PT), sedimentation rate, C-reactive protein;
- Chemistry: Blood Urea Nitrogen (BUN), creatinine, electrolytes (Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl), albumin, alkaline phosphatase, ALT, AST, LDH, GGT,total bilirubin, total protein, glucose;
- Endocrine: total testosterone, random cortisol, TSH, T4;
- Urine analysis: microscopic RBC and WBC, protein, glucose, nitrites and specific gravity;
- Plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. These will be kept frozen at -20° C or less at the site for eventual assay by Sponsor.

- Participant completes the IIEF and SEP.

7.8 Long-term follow-up

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 an injection of *h*Maxi-K will continue to be followed after the completion of all study related procedures (Visit 7 or last study visit) for an additional 18 months. These subjects will be contacted by the site at 6 month intervals to evaluate for any safety concerns.

8.0 Participant Withdrawal

While participants are encouraged to complete all study evaluations, they may withdraw from the study at any time and for any reason 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.

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 7.9.

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.

Those participants who do not complete the study for any reason will be considered a premature termination. (eg. Adverse Event and/or lack of therapeutic effect and/or participants desire to discontinue study procedures/testing but allows for safety follow-up). The procedures for the Visit 7 should be completed when a participant discontinues the study.

- **Adverse Events:** Subjects 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 his consent to participate in the study for any reason. In this event the participant refuses to participate in any additional evaluations and is withdrawing their consent for the site to utilize their data going forward. In this event the participant's data post the consent withdrawn will not be utilized in the clinical trial summary in accordance with enactment of 1996 (HIPAA) and subsequent Privacy Rule, required as of April 14, 2003.
- **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

participant who fails to return to the clinic for a scheduled visit in order to complete assessments and retrieve any outstanding data or supplies of study medication. All such efforts should be documented in the source notes. Up to three participants "lost to Follow-up" can be replaced in each dosage cohort for participants that received gene transfer but failed to complete the Visit 4 (week 4) evaluations.

- **Sponsor/Investigator Decision:** A participant may be withdrawn at any time at the discretion of the Sponsor/investigator.
- **Lack of Therapeutic Effect:** A participant may choose to withdraw at any time if they feel that they want to pursue alternative treatment for their ED based on their perceived lack of therapeutic effect.

9.0 Adverse Events Definitions and Monitoring

9.1 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.

A treatment emergent adverse event (TEAE) will be considered an event that is temporally associated with the use of the study drug, whether or not considered related to the study drug.

Examples of AEs are as follows:

- Changes in the general condition of the participant
- Subjective symptoms offered by or elicited from the participant
- Objective signs observed by the Investigator or other study personnel
- All concurrent diseases that occur after the start of the study, including any change in severity or frequency of preexisting disease
- All clinically relevant abnormalities in laboratory values or clinically relevant physical findings that occur during the study.

9.2 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,
- 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.

9.3 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 patient'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 patient'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 patient's medical condition or other therapies cannot explain the event.

Certainly 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.

9.4 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 intervention required.
- 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: Considered potentially life threatening (see definition under section 9.2)

A serious adverse event (SAE) requires medical intervention to prevent permanent impairment or death, permanently disability: (bed-ridden or disabling AE), required significant medical intervention/therapy, hospitalization or hospice care or in the judgment of the investigator was a medically significant event. Serious adverse events may be mild, moderate or severe in intensity (e.g., mild stroke).

9.5 Monitoring of Adverse events

The investigator(s) will monitor their participants for the occurrence of adverse events during the course of the study and record all observed adverse events in the case report form. Adverse Events regardless of seriousness or relationship to study drug, including those

occurring during the Screening period (where applicable), are to be recorded in the 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.

Any adverse events that meet the 4 criteria under Stopping Rules (Section 5.2.1) must be communicated to the sponsor (or designee) within 24 hours of first knowledge of such event

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 to Ion Channel Innovations, LLC.:

[redacted] M.D.

969 park Avenue, Suite 1G

New York, NY 10028

Fax 212 249 5140

Telephone 212 639 1561

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 measurement of serum potassium, calcium, magnesium, and *hSlo* cDNA.

A cardiologist should be consulted, and the participant should continue to have his ECG monitored until the ECG abnormality resolves or becomes stable.

10.0 Assessment of Endpoints

10.1 Safety

Safety and tolerability of the study drug will be evaluated by analysis of adverse experiences, clinical laboratory tests, electrocardiogram, and physical examinations. It will be conducted in all subjects who received treatment or there was an attempt to provide treatment.

10.2 Efficacy

The erectile function domain category of the IIEF and the SEP will be used to evaluate the change in erectile status from baseline following administration of *h*Maxi-K.[35-37] It will be done in those who received treatment.

10.3 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 2A study is designed to evaluate the safety and efficacy of a single injection of *h*Maxi-K (8000 and 16000 μg) compared to placebo 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.

10.3.1 Safety

Safety and tolerability of the study drug will be evaluated by tabulating incidence of adverse experiences, clinical laboratory tests, electrocardiogram, and physical examinations in the safety population.

10.3.2 Efficacy

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

Table 2. Statistical Power

Power	N1	N2	Placebo	Ratio	Alpha`	$Mean_1$	$Mean_2$	SD_1	SD_2
80%	11	11	13	1.111	0.0167	10.0	6.6	2.5	2.5

The mean change in IIEF and SEP and participant questionnaire (unmedicated and active phase) questions will be evaluated at all visits and changes compared to baseline and placebo.

Alpha' is the alpha level adjusted for three multiple comparisons (the two doses compared with each other, and each dose compared with the placebo). Given the three comparisons that incorporated a Bonferroni multiple comparison procedure, the overall alpha level is preserved at .05.

A 10% attrition rate over the 24 weeks of followup is anticipated, which inflates the sample size to approximately 40 subjects in total.

Descriptive statistics will be presented as means and standard deviations for normally distributed data at each timepoint by group, and medians and ranges otherwise. Incidence of adverse events will be presented as relative frequencies. All estimates of differences will be presented with 95% confidence limits. The mean change in IIEF and SEP and participant questionnaire (unmedicated and active phase) questions will be evaluated at all visits and changes compared to baseline and placebo using either paired t-tests for normally distributed changes, or Wilcoxon Sign Rank tests, otherwise. A mixed effects or marginal model will be used to assess trends in changes over time among treatment groups. With regard to missing data, the assumption of missing at random will be assessed by looking for patterns over time, and by comparing those with missing values to all

other subjects with regard to baseline characteristics. Chi-square or Exact tests will be used to compare incidence rates among the treatment groups. All tests of significance with regard to efficacy will be one-tailed, and performed at an overall alpha level of .05 using SAS Version 9.3 or higher, Cary, NC.

11.0 Monitoring of Participant Safety

11.1 Informed Consent

The investigator will be responsible for obtaining from every participant 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 Consents 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 both the participant and his partner. The original signed and dated copy of the Informed Consents must be maintained in the institution's records. The names of the participants enrolled
during this study will be considered confidential.

A copy of the informed consent for both the participant and his partner is provided in Attachment 2.

12.0 Regulatory Standards

12.1 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.

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.

12.2 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.

12.2.1 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 will visit the institution prior to initiating the study and periodically thereafter to monitor acceptability of facilities, the agreement between 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.

Independent designee audits site.	Reports to [redacted] MD, Directing Member, Ion Channel Innovations

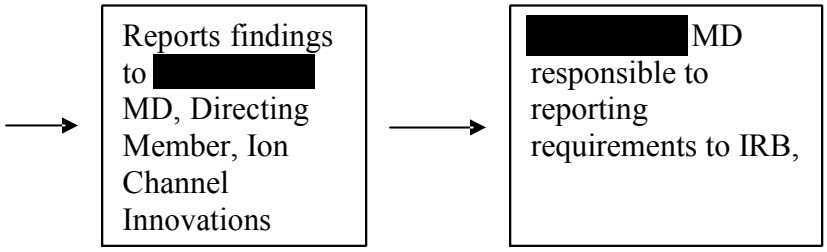
In addition to the initiation visit, the clinical site will be audited after enrollment into each cohort has been completed. Once the study has been completed or terminated, a close-out or termination visit will be made. The Investigator and/or Study Coordinator will receive reasonable notification before 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 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 eCRF 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 eCRF will be first reviewed with the investigator prior to correction. The reason for any correction to the eCRF will be noted along with the date and initials 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. The Investigator agrees to allow access to the required participant records in the event of such an audit.

12.2.2 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 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 (CE3 Inc), and submitted to FDA.

12.3 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 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.

12.4 Records

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

1. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 151: 54-61, 1994.

2. Melman A, Gingell JC. The epidemiology and pathophysiology of erectile dysfunction. Urol 161: 5-11, 1999.

3. Feldman, H.A., Goldstein, I., Hatzichristou, D.G., et al. Impotence and its medical and psychosocial correlates: results of the Massachusetts male aging study. J. Urol 151: 54-61, 1994.

4. Benet, A.E., Melman, A. The epidemiology of erectile dysfunction. Urol Clin. N Am 22:699-707, 1995

5. Saenz de Tejada, I., Goldstein,I., Azadzoi,K., Krane, R.J., Cohen, R.A.: Impaired neurogenic and endothelium- mediated relaxation of penile smooth from diabetic men with impotence. N Engl J Med 320:1025-1030, 1989.

6. Christ, G. J., Stone, B., Melman, A.: Age-dependent alterations in the efficacy of phenylephrine-induced contractions in vascular smooth muscle isolated from the corpus cavernosum of impotent men. Can J Physiol Pharmacol, 69: 909-913, 1991.

7. Mills, T.M., Chitaley, K., Wingard, C.J., Lewis, R.W.,,Webb, R.C. Effect of Rho-kinase inhibition on vasoconstriction in the penile circulation. J Appl Physiol 91:1269-1273, 2001.

8. Wespes, E.: Erectile dysfunction in the ageing man. Curr Opin Urol, 10: 625-629, 2000.

9. Melman, A., Zhao, W., Davies, K.P., Bakal, R., Christ, G.J. The successful Long-term treatment of age-related erectile dysfunction with hSlo cDNA in rats *in vivo*. J Urol 170:285-290, 2003.

10. Christ, G.J.: K channels as molecular targets for the treatment of erectile dysfunction. J. Androl. 23: S10-9, 2002.

11. Archer, S.L. Potassium channels and erectile dysfunction. Vasc Pharm 38:61-71, 2002

12. Karicheti, V., and Christ, G.J. Physiological roles for K^+ channels and gap junctions in Urogenital Smooth muscle: Implication for Improved understanding of Urogenital Function, Disease and therapy. Current Drug Targets 2:1-20, 2001.

13. Nelson, MY,Quayle, JM. Physiological roles and properties of K channels in arterial smooth muscle. Am J Physiol 264:C799-822, 1995.

14. Lawson, K., Dunne, MJ.: Peripheral channelopathies as target for potassium channel openers. Expert Opinion on Investigational Drugs. 10: 1345-1259, 2001.

15. Somlyo, AP, Wu, X, Walker, LA, and Somlyo, AV.: Pharmacomechanical coupling: the role of calcium, G- proteins kinases and phosphatases. Rev Physiol Biochem.& Pharmacol 134:202-234,1999.

16. Cole, W.C., Clement-Chomienne, O. Properties, Regulation, and role of potassium channels of smooth muscle. In A functional view of Smooth muscle , p247-318. Eds Barr,L., and Christ, G.J. JAI Press, Stanford, CT. 2000.

17. Korovkina VP, England SK.: Detection and implications of potassium channel alterations.Vascul Pharmacol. 38:3-12, 2002.

18. Sobey CG. Potassium channel function in vascular disease. Arterioscler Thromb Vasc Biol.21:28-38., 2001.

19. Christ GJ, Brink PR, Melman A, Spray DC. The role of gap junctions and ion channels in the modulation of electrical and chemical signals in human corpus cavernosum smooth muscle. Int J Impot Res 5: 77, 1993.

20. Christ GJ. The penis as a vascular organ. The importance of corporal smooth muscle tone in the control of erection. Urol Clin N Am: 727-745, 1995.

21. Christ GJ, Moreno AP, Parker ME, Gondre CM, Valcic M, Melman A, Spray DC. Intercellular communication through gap junctions: potential role in pharmacomechanical coupling and syncytial tissue contraction in vascular smooth muscle isolated from the human corpus cavernosum. Life Sci 49:PL195, 1991.

22. Melman, A, Christ,GJ. Integrative erectile biology. The effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. Urol Clin N Am 28:217-231, 2001.

23. Fan, S.F., Brink, P.R., Melman, A., and Christ, G.J. An analysis of the maxi-K+ (K_{Ca})

channel in cultured human corporal smooth muscle cells. J Urology 153:818-825, 1995.

24. Spektor, M., Rodriquez, Rosenbaum, R.S., Wang, H.Z., Melman, A., and Christ, G.J. Potassium channels and human corporeal smooth muscle cells tone: further evidence for the physiological relevance of the Max-K channel subtype to the regulation of human corporeal smooth muscle tone in vitro. J Urol 167: 2628-2635, 2002.

25. Tanaguchi, J., Furakawa, K.I., Shigekawa, M. Maxi-K^+ channels are stimulated by cyclic guanosine monophosphate dependent protein kinase in canine coronary artery smooth muscle cells. Pfluegers. Arch 423:167-172, 1993.

26. Christ, G.J., Rehman, J., Day, N., Salkoff, L., Valcic, M., Melman, A., and Geliebter, J. Intracorporal injection of *hSlo* cDNA in rats produces physiologically relevant alterations in penile function. Am J Physiol 275: H600-608, 1998.

27. Shipston, M.J., Duncan, R.R., Clark, A.G., Antoni, F.A., Tian, L. Molecular components of large conductance calcium-activated potassium (BK) channels in mouse pituitary corticotropes. Mol Endocrinol 13:1728-1737, 1999.

28. Kim, D.C., Gondre, C.M, Christ, G.J.: Endothelin-1-induced modulation of contractile responses elicited by an α_1-adrenergic agonist on human corpus cavernosum smooth muscle. Int. J. Impotence Res., 8:17-24, 1996.

29. Zhao, W. & Christ, G.J.: Endothelin-1 as a putative modulator of erectile dysfunction. II. Calcium mobilization in cultured human corporal smooth muscle cells. J. Urol., 154:1571-1579, 1995.

30. Ariolas, Hedlund, H. The pharmacology and clinical pharmacokinetics of apomorphine SL. . BJU. Int 88 suppl 3, 18-21, 2001.

31. Chen, K.K., Chan, J.Y., Chang, L.S. Dopaminergic neurotransmission at the paraventricular nucleus of hypothalamus in central regulation of penile erection in the rat. J Urol. 162: 237-242, 1999.

32. Andersson, KE. Pharmacology of erectile function and dysfunction. Urol Clin N Am 28: 233-247, 2001.

33. Rampin, O. Mode of action of a new oral treatment for erectile dysfunction:apomorphine SL. BJU Int Suppl 3, 22-24, 2001.

34. Sato, Y., Day, N., Valcic, M., Melman, A., Christ, G.J. Gene therapy with maxi-K channels restores diminished erectile response in older animals during electrical stimulation of the medial pre-optic area (MPOA). J Urol 165: 220 (A), 2001.

35. Rosen, C.R., Riley, A., Wagner, G., Osterloh, I.H., Kirkpatrick, J., Mishra, A. The International Index of Erectile Function (IIEF): A Multidimensional Scale for Assessment of Erectile Dysfunction.. Urology 49:822-830, 1997.

36. Cappelleri, J.C., Rosen, R.C., Smith, M.D., Mishra, A., Osterloh, I.H. Diagnostic Evaluation of the Erectile Function Domain of the International Index of Erectile Function. Urology 54:346-351, 1999.

37. Cappelleri, J.C., Siegel, R.L., Osterloh, I.H., Rosen, R. Relationship Between Patient Self-Assessment of Erectile Function and the Erectile Function Domain of the International Index

of Erectile Function. Urology 56:477-481, 2000.

38. Melman, A., Bar-Chama, N., McCullough, A., Cavies, K., Christ, G.: Results of a phase I HMAXI-K gene transfer trial for erectile dysfunction. Eur Urol Suppl5 (2):140, 2006.

Appendix A. Grading of Averse 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 0.43 – 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. Schedule of Events

Test or Procedure	Visit 1 -2 Week Screening	Visit 2 Week 0	Visit 3 Week 1 ± 2 days	Visit 4 Week 4 ± 3 days	Visit 5 Week 8 ± 3 days	Visit 6 Week 12 ± 3 days	Visit 7 Week 24 ± 3 days
Informed Consent Process	X						
Inclusion and Exclusion Criteria	X						
Demography	X						
Medical History	X	X[5]	X	X	X	X	X
Physical Examination	X	X[5]	X	X	X	X	X
Physical Examination of Penis[1]	X	X[5]	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Blood Tests (Chemistry and Hematology)[2]	X[4]		X	X		X	X
Urine analysis[3]	X	X[5]	X	X		X	X
ECG[4]	X	X[4]	X	X	X	X	X
IIEF[5]	X	X[5]	X	X	X	X	X
SEP[5]		X[5]	X	X	X	X	X
Study drug administration		X					
Conmomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X
Assay for *h*Slo DNA[6]	X[6]	X	X	X	X	X	X

[1]PE of penis includes: inspection, palpation and neurosensory testing

[2]Blood tests include: Hematology (CBC with differential, platelet count, PTT, PT, sedimentation rate, CRP), Chemistry (BUN, Cr, Na^+, K^+, Mg^{++}, Ca^{++}, CO_2, Cl, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein; Endocrine (testosterone, random cortisol, TSH, T4). Hemoglobin A1c at Screening (V1) only.

[3]Urine analysis includes: microscopic RBC and WBC, protein, glucose, and specific gravity.

[4]ECG at V2 will be done prior to administration of study drug and at 1 and 2 hours following administration of study drug

[5]Test will be done prior to administration of study drug.

[6] In all participants, plasma specimens will be collected to assay for the presence of *h*Slo DNA by PCR. At Visit 2 collect pre- dosing and 2 post dosing.

Appendix C. Sexual Encounter Profile (SEP)

Sexual Encounter Profile DIARY

Questions to be addressed after each attempt at intercourse	___/___/___ MM/DD/YY	___/___/___ MM/DD/YY	___/___/___ MM/DD/YY	___/___/___ MM/DD/YY
1. Were you able to achieve at least some erection?	**N Y**	**N Y**	**N Y**	**N Y**
2. Were you able to insert your penis into your partner's vagina?	**N Y**	**N Y**	**N Y**	**N Y**
3. Did your erection last long enough for you to have successful intercourse?	**N Y**	**N Y**	**N Y**	**N Y**
4. Were you satisfied with the hardness of your erection?	**N Y**	**N Y**	**N Y**	**N Y**
5. Overall, were you satisfied with the sexual experience?	**N Y**	**N Y**	**N Y**	**N Y**

Appendix D. International Index Erectile Function

INTERNATIONAL INDEX OF ERECTILE FUNCTION PAGE 1 OF 2

PARTICIPANT

(check the appropriate box – In the last month…….)

1. How often were you able to get an erection during sexual activity?
   0. No sexual activity…….☐
   1. Almost never / never……..... ☐
   2. A few times (much less than half the time)…… ..... ☐
   3. Sometimes (about half the time)….. ☐
   4. Most times (much more than half the time)…… ☐
   5. Almost always/always…… ... ☐

2. When you had erections with sexual simulation, how often were your erections hard enough for penetration?
   0. No sexual activity…….☐
   1. Almost never / never……..... ☐
   2. A few times (much less than half the time)…… ..... ☐
   3. Sometimes (about half the time)….. ☐
   4. Most times (much more than half the time)…… ☐
   5. Almost always/always…… ... ☐

3. When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner?
   0. No sexual activity…….☐
   1. Almost never / never……..... ☐
   2. A few times (much less than half the time)…… ..... ☐
   3. Sometimes (about half the time)….. ☐
   4. Most times (much more than half the time)…… ☐
   5. Almost always/always…… ... ☐

4. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?
   0. Did not attempt intercourse……☐
   1. Almost never / never……...☐
   2. A few times (much less than half the time)……..... ☐
   3. Sometimes (about half the time)..☐
   4. Most times (much more than half the time)…… ☐
   5. Almost always/always……..... ☐

5. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?
   0. Did not attempt intercourse……..☐
   1. Extremely difficult……....☐
   2. Very difficult……..... ☐
   3. Difficult……☐
   4. Slightly difficult……☐
   5. Not difficult……...☐

6. How many times have you attempted sexual intercourse?
   0. No attempts……..☐
   1. One to two times……..☐
   2. Three to four times……..☐
   3. Five to six times…….....☐
   4. Seven to ten times……...☐
   5. Eleven + times…….☐

7. When you attempted sexual intercourse, how often was it satisfying for you?
   0. Did not attempt intercourse……..☐
   1. Almost never / never…..……..... ☐
   2. A few times (much less than half the time)…….. ..... ☐
   3. Sometimes (about half the time).… ☐
   4. Most times (much more than half the time)…….…… ☐
   5. Almost always/always……..... ☐

INTERNATIONAL INDEX OF ERECTILE FUNCTION PAGE 2 OF 2

8. How much have you enjoyed sexual intercourse?
    0. No intercourse…….....☐
    1. No enjoyment…….....☐
    2. Not very enjoyable……...☐
    3. Fairly enjoyable……....☐
    4. Highly enjoyable…..…….....☐
    5. Very enjoyable……..…..☐
9. When you had sexual stimulation or intercourse, how often did you ejaculate?
    0. No sexual stimulation/intercourse ☐
    1. Almost never/never……..☐
    2. A few time (much less than half the time)……..……... ☐
    3. Sometimes(about half the time)…☐
    4. Most times (much more than half the time)……..☐
    5. Almost always/always…….....☐
10. When you had sexual stimulation or intercourse, how often did you have the feeling or orgasm or climax?
    0. No sexual stimulation/intercourse……..…☐
    1. Almost never/never……...☐
    2. A few time (much less than half the time)…….... ☐
    3. Sometimes(about half the time)…☐
    4. Most times (much more than half the time)……..☐
    5. Almost always/always…….....☐
11. How often have you felt sexual desire?
    1. Almost never/never…….☐
    2. A few times (much less than half the time)……☐
    3. Sometimes (about half the time).☐
    4. Most times (much more than half the time)……..☐
    5. Almost always/always……☐
12. How would you rate your level of sexual desire?
    1. Very low/none at all……..…☐
    2. Low……..☐
    3. Moderate……..☐
    4. High…….☐
    5. Very high…….☐
13. How satisfied have you been with your overall sex life?
    1. Very dissatisfied……..…….....☐
    2. Moderately dissatisfied……...☐
    3. About equally satisfied and dissatisfied…….☐
    4. Moderately satisfied……...☐
    5. Very satisfied……..☐
14. How satisfied have you been with your sexual relationship with your partner?
    1. Very dissatisfied…….☐
    2. Moderately dissatisfied……...☐
    3. About equally satisfied and dissatisfied……..☐
    4. Moderately satisfied……....☐
    5. Very satisfied……..☐
15. How do you rate your confidence that you could get and keep an erection?
    1. Very low……☐
    2. Low……☐
    3. Moderate……☐
    4. High……...☐
    5. Very high……...☐