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Gene Therapy for Male Erectile Dysfunction

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Human gene therapy has been described as a promising, but unfulfilled, approach to treat diseases—a virtual “roller coaster” of ups and downs since the first reported gene therapy clinical trial in 1990 [1,2]. The complexities of delivery systems and setbacks, in large part due to the use of viral vectors, have led to some initial successes but many failures. Providentially, smooth muscle disorders of the genitourinary system, including erectile dysfunction (ED), share important differences that make them attractive targets for gene therapy and, in particular, allow the use of naked DNA without requiring viral vectors to effect cell membrane penetration by the gene of interest. The therapeutic goal of therapy in these disorders is modulation of smooth muscle tone, rather than more radical alterations of structure, function, or induction of apoptosis. Additionally, major smooth muscle organs, including the penis, bladder, gut, and lungs, are easily accessible for local, targeted administration of the gene product. Therefore, the need for systemic delivery of gene product with its attendant generalized, nonspecific biodistribution is eliminated. The author’s laboratory has taken advantage of these properties in an attempt to develop a safe, durable treatment for ED based on knowledge of the physiology, pharmacology, and electrical activity of corporal smooth muscle cells [1,3–5]. With publication of preclinical data and, more recently, results from the first human trial of *hMaxi-K* gene transfer in

men who have ED [6,7], we have begun to show that ion channel gene therapy is a viable option to treat smooth muscle cell disorders.

Although the current generation of oral phosphodiesterase-5 (PDE-5) inhibitors for the treatment of ED represents a significant advancement over earlier, more invasive treatments, there are unmet needs among men who have ED. That family of products is least effective in men who have more severe ED, including men who have diabetes, and is contraindicated in association with some families of drugs or medical conditions. The short duration of currently available members of the PDE-5 inhibitor class limits the spontaneity of the sexual act. Many men and their partners are unhappy with the concept of “planned sex.” Current approved oral therapies also have a high incidence of untoward side effects, such as nasal stuffiness and headaches, and the recently described occurrence of nonarteritic ischemic optic neuropathy leading to blindness [8] is troubling to many potential users of the medications.

Several groups have taken diverse approaches to gene therapy for ED. A variety of vectors have been used to insert genes to enhance the production of vascular endothelial growth factor, nitric oxide synthase, preprocalcitonin gene-related peptide, and vasoactive intestinal peptide in the corporal bodies of rodents [9–15]. Although all of these studies demonstrated improved erections and cavernous pressures in response to stimuli in the rat, mostly they relied on viral vectors that insert genes into the host chromosomes. This approach introduces the potential for insertion into unintended targets or outcomes. It is of interest that the plasticity of the erectile apparatus is such that positive outcome has been the case in each of the published reports in animal models;

Portions of this text were published previously in Melman A, Bar-Chama N, McCullough A, et al. *hMaxi-K* gene transfer in males with erectile dysfunction: results of the first human trial. *Hum Gene Ther* 2006;18:1165–76; with permission.

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however, for use in humans, the product must be proven safe and practical to pass the muster of the US Food and Drug Administration (FDA) as well as the target population. Detailed reviews of these varied gene therapy approaches for ED were published recently [3,5,16,17].

Our rationale for ion channel gene therapy that led to the first human trial of *hMaxi-K* for ED is based on the unique ultrastructural features of the smooth muscle of the genitourinary system and the role of ion channel function in the cavernous bodies.

Penis ultrastructural features and ion channels

The penis is an easily accessible external organ, and placement of a tourniquet at the base before administration limits potential biodistribution of the injected gene to other organs. Therefore, the issues related to intravenous injection of gene product are avoided. Smooth muscle cells form a functional syncytium that lines the cavernous bodies of the penis along with endothelial cells, fibroblasts, and collagen [18]. These cells are joined by gap junctions that allow for intracellular communication and transfer of neurotransmitters and second messengers [19]. Flux of ions through these channels governs many of the physiologic properties and activities of muscles in the body. Ion channels for potassium, calcium, and chloride govern many of the contractile properties seen in smooth muscles, including cavernous smooth muscles. The intermittent opening of the Maxi-K channels in response to regional increases of intracellular calcium ion concentration controls the tone of the penile smooth muscle. Proper function of those potassium channels is necessary for the induction of smooth muscle cell relaxation and the induction of a penile erection. Normal erectile function is governed by the limitation of influx of calcium ions into the smooth muscle cells that results in smooth muscle relaxation. The initiating process occurs in response to a sexual stimulus causing production and release of nitric oxide (from nerves and endothelium) diffusing into the cells and causing the production and accumulation of cyclic GMP that facilitates some of the downstream events listed above that cause smooth muscle relaxation.

At least four types of potassium channels are present in the plasma membranes of human smooth muscle cells [20–24]. With respect to penile erection, these ion channels respond to endogenous intracellular events by opening and

allowing K^+ to flow down its electrochemical gradient out of the smooth muscle cell. The resulting hyperpolarization (ie, increased negativity), in turn, limits calcium entry and promotes relaxation of the corporal and arterial smooth muscle cells (Fig. 1). The Maxi-K is a well-studied potassium channel subtype that is involved in smooth muscle relaxation [22,25,26]. It exhibits little activity in the normal corporal smooth muscle population. When these cells are stimulated, the open time or activity of the Maxi-K channel increases dramatically. This effect represents the convergence point for several upstream signaling pathways [27]. The central role of potassium channels in controlling membrane potentials and excitability makes them an attractive target for gene therapy [21]. Because potassium channels also control ion flux through other channel types, their augmentation modulates the flux of other ions (eg, calcium). The proposed increase in functional membrane potassium channels as a consequence of gene transfer should lead to normalization of smooth muscle cell hyperpolarization and subsequent smooth muscle cell relaxation in the presence of aging or disease (Fig. 2).

A possible mechanism by which Maxi-K gene transfer can improve erectile function

Davies and colleagues [28,29] recently reported from work in the author and colleagues' laboratory, that, in the rat model of aging, there are remarkable changes posttranscriptionally in the expression of Maxi-K protein in corporal smooth muscle cells. In older animals there is a significant decrease in the amount of Maxi-K protein expressed in the membranes and an increase in the amount of Maxi-K retained in the cytosol. The down-regulated expression of the Maxi-K channel in the membranes correlates with a significant increase in the levels of a dominant negative transcript of the Slo gene (SV1) in older animals. As depicted in Fig. 3, Davies and colleagues proposed that the increase in SV1 in older animals traps the Maxi-K protein in the cytoplasm. It was shown that dominant negative mutants trapping Maxi-K in the cytoplasm can lead to an overall decrease in the expression of the Maxi-K protein, probably through protein degradation pathways. Potentially, the physiologic effect of down-regulating the Maxi-K channel activity could lead to heightened tone of the corporal smooth muscle tissue through an increase in intracellular calcium, which

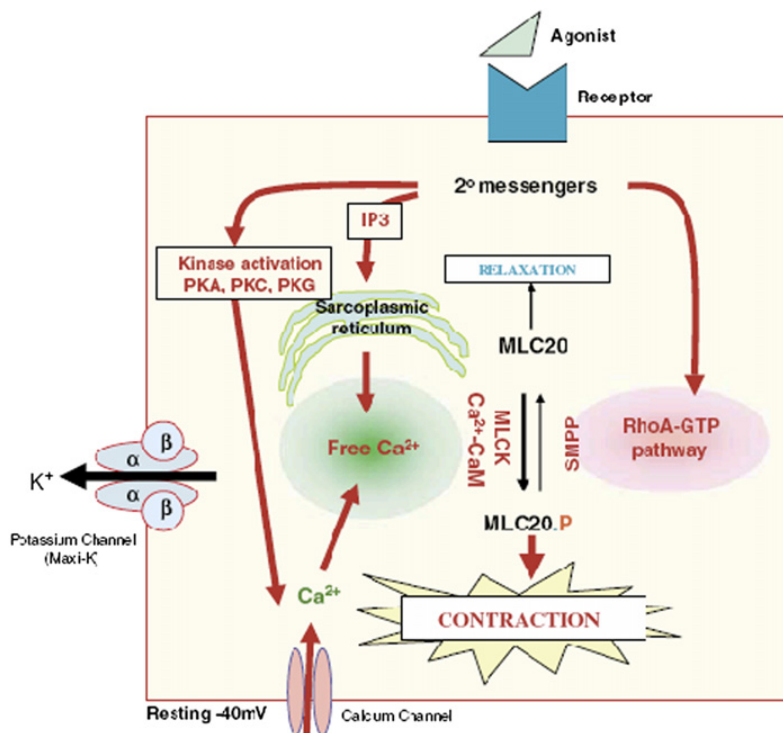


Fig. 1. Smooth muscle cell contraction. The relationship of the smooth muscle cell to the potassium ion channels, intracellular calcium ion, procontractile and relaxant second messengers, and contractile proteins. Because smooth muscle contractility is regulated primarily by intracellular Ca^{2+} , the moment-to-moment control of calcium ion entry by potassium channel activity regulates the tone of the smooth muscle cell. Furthermore, the change to the open state of the channel is in response to an event. Most of the time, the K^+ channels are in the closed state. With the opening of the channel, the membrane potential of the cell becomes more negative (ie, hyperpolarized), and the voltage-sensitive calcium channel closes and prevents influx of calcium ion into the cell. IP3, inositol triphosphate; MLC20, myosin light chain 20; MLCK, myosin light chain kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; SMPP, smooth muscle myosin phosphatase.

results in heightened contractility and ED. These results suggest that if Maxi-K gene transfer were applied to the treatment of ED in an aging patient, the mechanism of action would be to overcome posttranscriptional events that limit the expression of Maxi-K channel activity.

Although the precise mechanism by which the Maxi-K gene transfer works in diabetic animals is not known, it is likely to again involve a change in splicing of the Slo transcript. The author and colleagues recently demonstrated that, in diabetic animals, alternative splicing of the Slo transcript might represent an important compensatory mechanism to increase the ease with which relaxation of corporal tissue may be triggered as a result of a diabetes-related decline in erectile capacity [29]; however, there seems to be a transcriptional upper limit of the Slo gene expression, which when reached, can no longer compensate for the physiologic pressure resulting in ED. We proposed, at this point, that gene transfer of plasmids expressing a functional channel can

supplement endogenous gene transcription, which leads to recovery of erectile function.

Thus, a gene transfer approach that provides the ability to locally overexpress a potassium channel gene in a target tissue theoretically could overcome the age- or disease-related changes in end organ contractility that contribute to ED. Therefore, thus far we have focused on recombinant hSlo, which encodes the α , or pore-forming, subunit of the human Ca^{2+} -activated K^+ potassium channel (Maxi-K).

Vector

Naked DNA was selected as the vector for hMaxi-K gene transfer. Naked DNA has been unpopular as a gene transfer vector because of its reported lack of efficacy and limited duration of effect in systemic trials; however, in an appropriate targeted, local, nonsystemic application, naked DNA is recognized for its lack of chromosomal integration and its lack of toxicity.

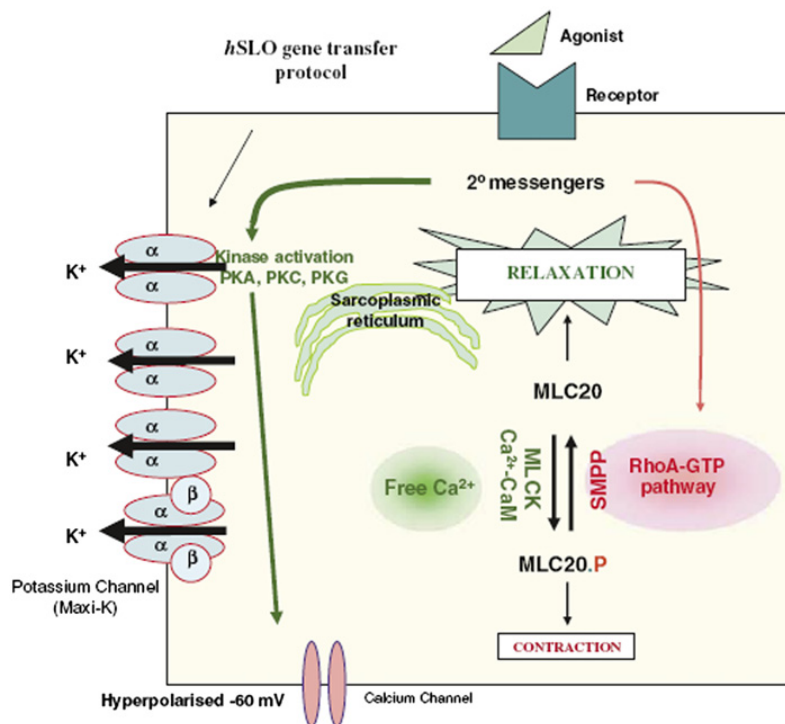


Fig. 2. Insertion of Maxi-K channels into smooth muscle cell. A representative cell into which the α -subunit of the MaxiK channel has been transferred. Three additional MaxiK channels are shown in the cell membrane. Because it is not known if the additional channels expressed by the *h*Maxi-K possess the β -subunit, that unit was not included. In the presence of an appropriate neural or ionic signal, the potassium channels open, hyperpolarize the cell to approximately -60 mV, and inhibit the influx of calcium ion, thus causing the cell to relax. MLC20, myosin light chain 20; MLCK, myosin light chain kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; SMPP, smooth muscle myosin phosphatase.

Most previous gene therapy protocols used retroviral vectors to integrate DNA into the target (Fig. 4). This results in integration of the DNA into the host nucleus. With naked plasmid DNA transfer, the plasmid is incorporated into the cell and then translocates into the nucleus, but is not integrated [22]. Theoretically, at least, plasmid DNA could integrate into the nuclear DNA; however, experimentally this has not been seen at a sensitivity of one copy per $1 \mu\text{g}$ of DNA, or three orders of magnitude below the spontaneous mutation frequency. The *h*Slo plasmid construct (*h*Maxi-K) illustrated in Fig. 5 has demonstrated excellent uptake into cavernosal smooth muscle cells in rats [4,30,31]. Experiments have demonstrated excellent uptake of the plasmid vector into cells and excellent functional results in terms of restoring intracavernosal pressures in response to stimuli (Fig. 6). Overall, the unique properties of the genitourinary smooth muscle cells seem to overcome the perceived disadvantages of limited efficacy and duration of the naked DNA plasmid.

The extensive preclinical evidence showing the safety, effectiveness, and long duration of action

of *h*Maxi-K led to the design and approval to implement the first human trial of gene transfer therapy for ED.

Phase 1 human safety trial of *h*Maxi-K: initiating a human gene transfer trial for a nonfatal indication

All gene transfer trials are conducted under the auspices of the Center for Biologics Evaluation and Research of the FDA. Those trials must be reviewed first by the Recombinant DNA Advisory Committee (RAC) of the Office of Biotechnology of the National Institutes of Health (NIH). The output of the RAC is advisory to the FDA; however, by law, the FDA cannot act on an Investigational New Drug (IND) application unless that review is undertaken. The RAC must review and make recommendations for the proposed clinical trial before an Institutional Review Board and the Institutional Biosafety Committee of private and institutional based trials can allow a study to proceed. The RAC reviews the

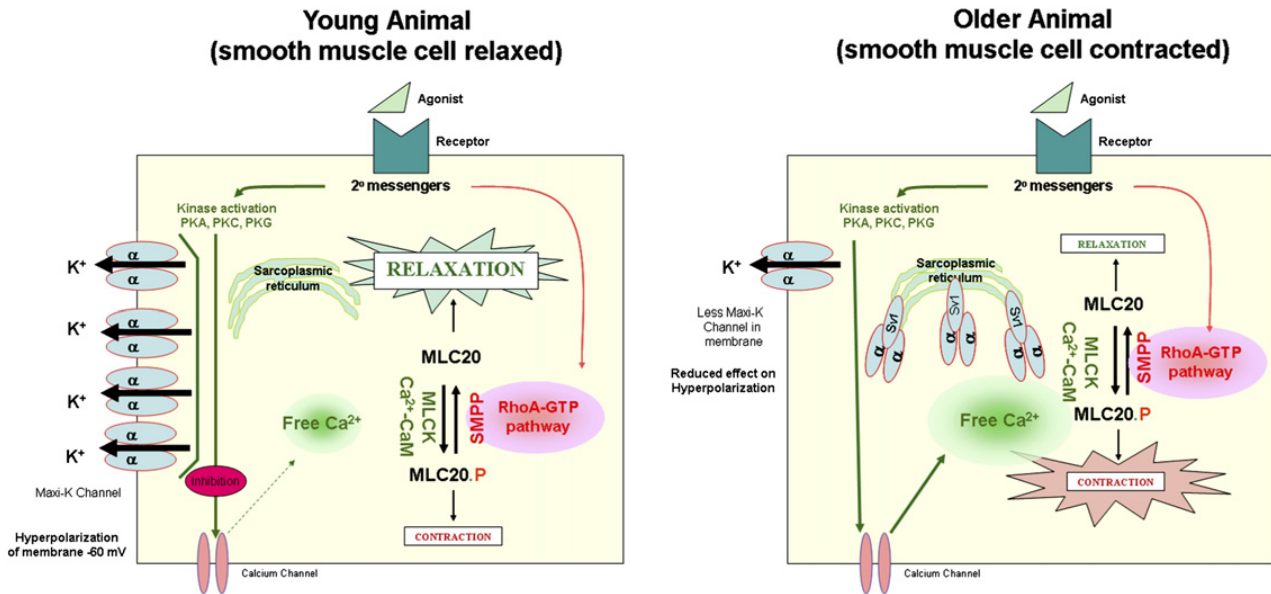
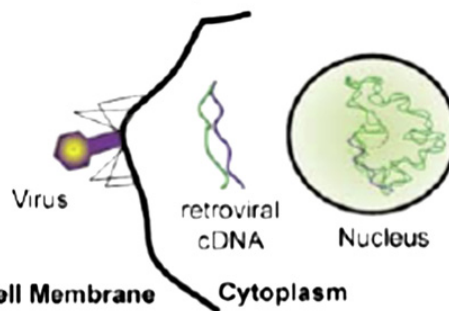


Fig. 3. Potential mechanism of Maxi-K gene transfer in old versus young animals. In young animals, most of the Maxi-K channel is expressed on the surface of the corporal smooth muscle cells. Upon activation, they hyperpolarize the membrane, an effect that inhibits the activity of the calcium channel. This results in a reduction in the intracellular free calcium levels and activity of the myosin light chain kinase (MLCK) through the action of the calcium-calmodulin complex. Less myosin light chain is in the phosphorylated (contracted) state, leaving the smooth muscle cell in a relaxed state. In older animals, increased expression of the dominant-negative Maxi-K channel (Sv1) reduces the amount of Maxi-K channel expressed on the cell surface. The reduction in the ability of the Maxi-K channels may cause hyperpolarization and, thereby, inhibit calcium channels, which leads to an increase in intracellular calcium that ultimately results in the smooth muscle cell being in a more contracted state. CaM, calmodulin; MLC20, myosin light chain 20; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; SMPP, smooth muscle myosin phosphatase.

Gene therapy using a retrovirus : nuclear integration



Gene therapy using naked DNA : NO nuclear integration

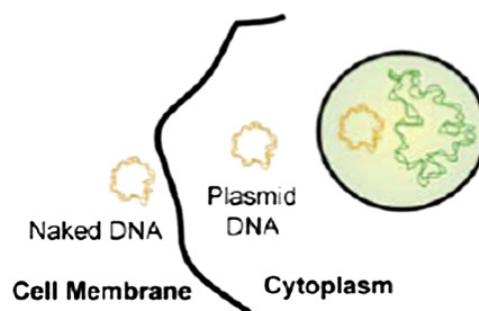


Fig. 4. Viral vector DNA. The difference between the use of viral and naked DNA to effect gene transfer. In the upper panel, in which a retrovirus is used as the vector, the DNA passes across the cell and nuclear membrane into the nucleoplasm, where it is integrated into the chromosomal apparatus. When naked DNA is used to effect the transfer, the DNA passes across both membranes but is not incorporated into the nuclear apparatus.

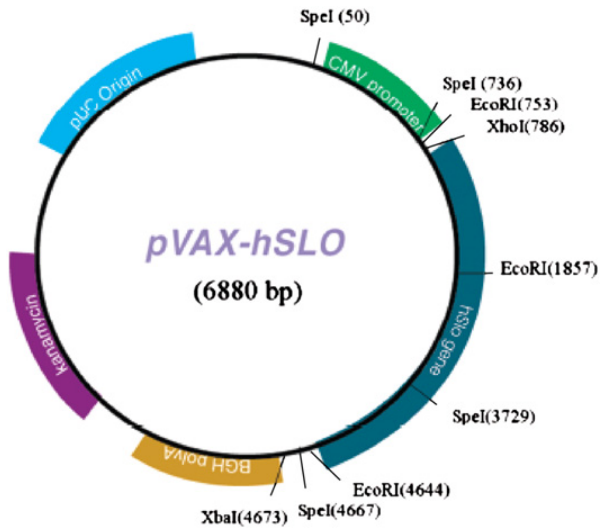


Fig. 5. *hMaxi-K*. Plasmid construct (*hMaxi-K*, 6880 base pairs): cytomegalovirus (CMV) promoter (positions 137–724), viral; *hSlo* gene (positions 888–4428), human; bovine growth hormone (BGH) polyadenylation [poly(A)] signal (positions 4710–4940), bovine; kanamycin gene (positions 5106–5901), bacterial; pUC origin of replication (positions 6200–6874), bacterial. Plasmid description: *hMaxi-K* is a double-stranded naked plasmid DNA molecule carrying the human *hSlo* gene, which encodes the α , or pore-forming, subunit of the human smooth muscle Maxi-K channel. *hSlo* is under the control of the CMV promoter positioned upstream of the transgene, and the construct also contains the BGH poly (A) site, kanamycin resistance gene, and pUC origin of replication. (From Melman A, Bar-Chama N, McCullough A, et al. The first human trial for gene transfer therapy for the treatment of erectile dysfunction: Preliminary results. *Eur Urol* 2005;48:314–8; with permission.)

submitted protocol and has the option of requiring a public, Web cast presentation by the principal investigator or the sponsor, at which time a recommendation for approval or additional data is made.

Most clinical trials that use gene transfer are for the indications of cancer, end-stage vascular disease, or genetic disorders. The NIH's Genetic Modification Clinical Trial Research Information System (www.gemcris.od.nih.gov) lists only one ongoing gene transfer trial for the indication of ED, and that is the one described below.

The ethics of using gene transfer as a therapy for ED, a nonfatal disease, was addressed recently by Dr. Arthur Caplan [32] as a prelude to the published results of the clinical trial. The text was as follows:

The report by Melman and his team in this issue of *Human Gene Therapy* of a safety trial of gene therapy for men afflicted with erectile dysfunction is certain to raise more than a few ethical eyebrows. The history of clinical trials utilizing gene therapy is, to say the least, ethically contentious. Many would argue that the slow rate of progress and the established risks associated with various vectors should make it clear that gene therapy ought only be utilized in the pursuit of life and death disorders for which no current therapeutic modalities exist. Otherwise, many are likely to argue, the risks involved with gene therapy could not possibly be balanced by the potential for serious benefit.

This line of reasoning is however in my view deeply flawed. It presumes that conditions such as erectile dysfunction are not 'serious' enough to merit attention by those working on the cutting edge of gene therapy research. Dismissing erectile dysfunction out of hand as a condition for which risks ought not be permitted in research undervalues the importance of medical efforts aimed at maintaining or restoring the quality of life of patients. And, disparaging safety trials involving subjects with erectile dysfunction also fails to address the question of what sorts of conditions and what organ systems are likely to be the most

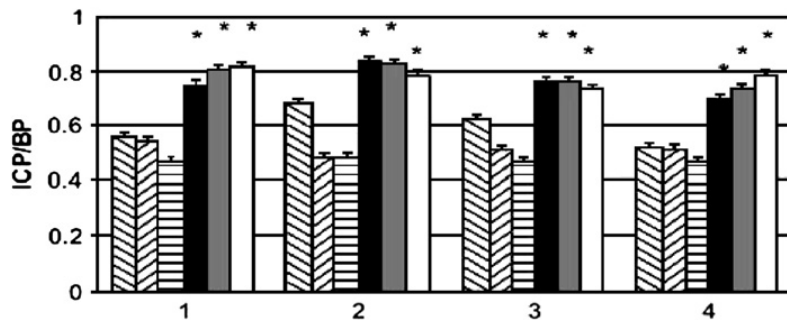


Fig. 6. Restoration of intracavernous pressure in impotent diabetic rats by *hSlo* gene therapy. Restoration of intracavernosal pressure (ICP) in impotent diabetic rats by *hSlo* gene therapy. Control treated and untreated groups demonstrate no improvement in ICPs compared with baseline pressure (BP), whereas all *hSlo*-treated rats demonstrated increases in ICPs that persisted up to 4 months after treatment: non-diabetic aged matched controls (hatched), plasmid cDNA (diagonal lines), untreated (horizontal lines), 100 μ g of *hSlo* (solid black), 300 μ g of *hSlo* (checkered), and 1000 μ g of *hSlo* (white) (* = significant difference from controls at the $p < 0.05$ level).

useful in understanding the utility and safety of various gene vectors and their potential for efficacy.

Erectile dysfunction is a serious medical problem. There are many men who decline life-saving surgery for prostate cancer when told that such surgery may leave them impotent. The degree to which this condition matters in relationships and to the individual self-esteem of persons is reflected in the surgical measures that men were willing to endure before the advent of pharmacological treatments for impotency. Sexual behavior is a key aspect of the quality of life that we all enjoy. If existing treatments cannot help all those in need then there is absolutely no reason not to pursue new and pioneering strategies to resolve dysfunction and disorder in this area of life.

As Melman and his co-authors note there are sound physiological reasons to think that gene transfer into smooth muscle in the penis might provide a safer and more useful model for understanding gene therapy than is afforded by targeting other tissues and organs. The generation of key safety data from subjects suffering from a serious and sometimes devastating medical condition is an ethical course for gene therapy researchers to follow as long as their science is sound, their consent of subjects thorough and their reports of results modest, balanced and fair.

In August of 2003, the FDA approved an IND application for Ion Channel Innovations, LLC to conduct the first phase 1 human trial to study the safety of gene transfer for the treatment of ED. Detailed methodology and results were published recently [6].

Following institutional review committee and biohazards committee approvals, 11 patients who had moderate to severe ED were given single-dose corpus cavernosum injection of *hMaxi-K* naked DNA plasmid carrying the human cDNA encoding hSlo, the gene for the α , or pore-forming, subunit of the human smooth muscle Maxi-K channel. Men 18 years or older participated in the study and had moderate to severe ED according to their International Index of Erectile Function (IIEF) scores. The ED was attributable to underlying, stable medical conditions. The men were otherwise in good health, with normal blood pressure and general and genitourinary physical examination at screening. Patients and their sexual partners signed the respective informed consents, and approved patients returned for the baseline visit 2 weeks later when gene transfer was given.

Eleven of 15 men screened qualified for entry. Three men each were treated with 500-, 1000-, or 5000- μ g doses and two were given 7500 μ g. The dose level selection was based on the lowest range of *hMaxi-K* used in preclinical studies in rodents [4]. *hMaxi-K* was injected into the corpus cavernosum of patients after placing a tourniquet (Actis venous flow controller; Vivus, Inc., Menlo Park, California) at the base of the penis. The tourniquet remained in place for 30 minutes to ensure that the vector was largely limited to the penis. Patients were monitored for 6 months after dosing, and annual follow-ups are planned.

The primary objective of this study was the safety and tolerability of a single injection of *hMaxi-K* at four escalating dose levels. This was measured by assessment of changes in clinical evaluations and laboratory tests that included general and genitourinary physical examinations, blood pressures and heart rates, ECG, general blood electrolyte and liver chemistries, hematologic parameters, endocrine tests, thyroid profiles, and urine and semen analysis. Adverse events were assessed and recorded at each visit. The DNA of semen was tested for the presence of pVAX-*hSlo* plasmid using reverse transcriptase polymerase chain reaction with primers specific to the plasmid [27].

Although the primary objective of this phase 1 study was safety, the key secondary study objective was assessment of the effect of *hMaxi-K* on ED using the erectile function (EF) domain category of the IIEF scale [33,34]. The EF domain, questions 1 through 5 and 15 of the IIEF, has been validated to assess erectile changes only [34]. Additional IIEF subdomain scores were recorded to confirm the IIEF-ED, including the mean intercourse satisfaction score—questions 6, 7, and 8—as an indicator of overall sexual satisfaction.

The study was conducted from May, 2004 to May, 2006. The mean age of the study population was 59.0 ± 10.6 years (range, 42–80 years), six subjects were white, four subjects were African American, and one subject was Hispanic. The duration of ED ranged from 1 to 20 years, the mean baseline IIEF-EF score was 6.8 ± 4.05 , and 9 subjects were categorized with severe ED and 2 subjects with moderate ED according to standard classifications [34].

The initial safety question to be answered was the possible presence of detectable *hMaxi-K* in the semen of subjects during the 6 months after transfer. Fig. 7 shows a representative analysis of total

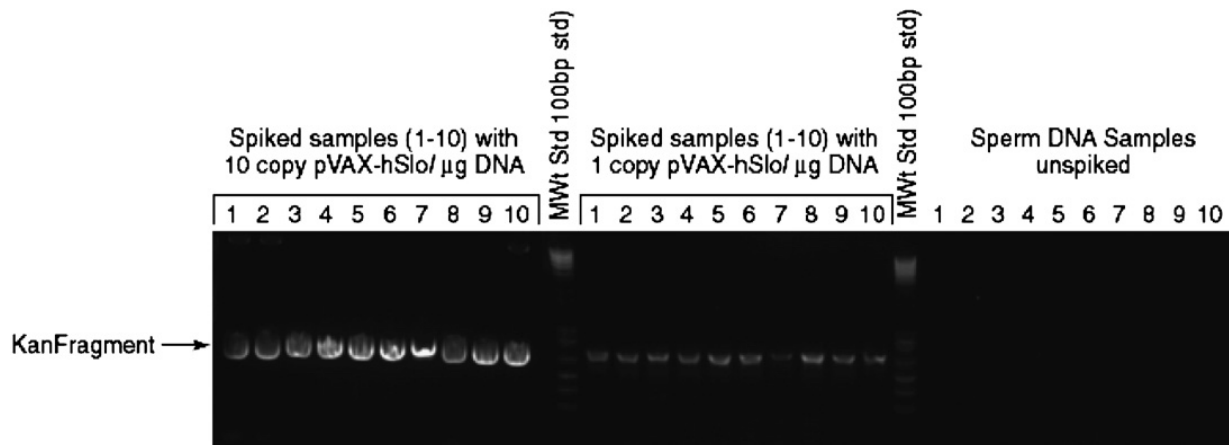


Fig. 7. Analysis of total DNA extracted from sperm for the presence of pVAX1-hSlo. Plasmid DNA was extracted from sperm, using a Qiagen (Valencia, California) total DNA extraction kit according to the manufacturer's instructions. Five milligrams of total DNA was subjected to polymerase chain reaction with primers amplifying the kanamycin (Kan) gene present in pVAX1-hSlo. In addition to unspiked samples, samples were also spiked with 1 or 10 copies of pVAX1-hSlo per milligram of total DNA as labeled above the gel. Unspiked samples gave no signal, whereas spiked samples gave a signal of the expected size for the kanamycin gene in pVAX1-hSlo. The amount of plasmid present in the sperm samples was less than the limit of detection (1 copy/ μg of total DNA). Sperm DNA samples were as follows: lane 1, untreated; lanes 2–4, treated patient, samples taken on February 2, 2005 (repeat), April 19, 2005, and July 14, 2005; lanes 5–7, treated patient, samples taken on May 24, 2005, May 31, 2005, and July 12, 2005; lanes 8 and 9, patient, samples taken on March 9, 2005 and May 4, 2005; lane 10, water (negative control).

DNA extracted from sperm samples for the presence of pVAX1-hSlo plasmid. Samples spiked with 1 or 10 copies of pVAX1-hSlo per microgram provide reference comparisons for semen samples from three patients, taken at several time points. There was no detectable evidence of hMaxi-K in semen down to the 1 copy/ μg of the total DNA level in any participant at any of the visits.

Table 1 is a summary of adverse events reported by patients during the study. All three patients given 500 μg , one of three patients given 1000 μg , and one of three patients given 5000 μg had adverse experiences. All the reported events occurred at least 30 days after gene transfer, and none of the events were considered related to the gene product

transfer by the investigators. All three patients in the 500- μg dose group had adverse experiences; one had knee arthroscopy, one had atrial flutter with ablation reported as severe, and one had kidney stone removal by lithotripsy, also reported as severe. The atrial flutter and lithotripsy also were classified as serious adverse events. One patient given 1000 μg reported acid reflux, sciatic pain, and an upper respiratory infection, and one patient had a parasitic intestinal infection and foot edema. One patient given 5000 μg had bladder stone removal; neither patient given 7500 μg reported an adverse experience. No patients reported any discomfort from the injection, and no local physical events related to the injections were observed.

Table 1
Phase I trial of hMaxi-K in men who have erectile dysfunction: adverse event summary by dose

Events	Dose (μg)				Total (n = 11)
	500 (n = 3)	1000 (n = 3)	5000 (n = 3)	7500 (n = 2)	
Patients reported ≥ 1 AE	3 (100%)	2 (67%)	1 (33%)	0 (0%)	6 (54.5%)
Patients with AEs related to study treatment	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Patients with serious AEs	2 (67%)	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)
Patients with AE leading to early withdrawal	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Abbreviation: AE, adverse event.

No clinically significant changes were seen in the general or genitourinary physical examinations during the study. No emergent transfer-related cardiac events were noted or reported during the study, and no significant changes in ECG, as determined by shift analysis (no normal to abnormal occurrences), were observed with the exception of atrial flutter considered unrelated to treatment in one patient.

No clinically significant changes were seen in the mean blood chemistry or endocrine test values at the end of the study or at any of the interim study visits. In addition, no clinically significant changes from normal to abnormal in any blood chemistry, endocrine, hematology, or urinalysis values were seen at any visit for any patient. Mean systolic and diastolic blood pressures and heart rates did not show notable changes over time in each dose group; however, individual subject values varied from visit to visit, but no clinically significant pattern of changes was evident. No adjunctive therapies or changes in therapy were required.

The three patients given the 500- μ g dose and one patient given 1000- μ g of *hMaxi-K* have now completed 2-year safety follow-up safety examinations with no reported complications or adverse experiences. One patient given 1000 μ g was lost to follow-up. The other patient given 1000 μ g, three patients given 5000 μ g, and two patients given 7500 μ g doses have completed 1-year follow-up examinations with no reported complications or adverse experiences.

Examination of secondary efficacy end points indicated some suggestion of improvement in ED symptoms. Decreased IIEF-EF domain scores at each dose were observed 1 week after injection. Mean scores for the two lower dose groups (500 and 1000 μ g) fluctuated around the baseline values throughout the study; however, improvements in the mean IIEF-EF scores were observed for the two higher dose groups (5000 and 7500 μ g) beginning 2 weeks after transfer. Improvements were maintained in both groups through the 24 weeks of study. The positive changes from baseline for most patients were small and did not indicate improvement by IIEF scoring; however, two patients, one given 5000 μ g and one given 7500 μ g, showed notable improvement in IIEF-EF beginning 2 weeks after transfer and continuing improvement (from severe to mild or to no ED) at 4 weeks. The improvement was maintained through the 24-week study.

Fig. 8 displays the IIEF-mean intercourse satisfaction score for each patient at each visit. The results showed that for those men who responded to the transfers at the two higher doses there was a clinically significant increase in sexual satisfaction.

The most important finding of the study was that single injections of *hMaxi-K* at doses of 500, 1000, 5000, and 7500 μ g were well-tolerated and safe, and furthermore, that no safety issues emerged during the 6 months of follow-up. No significant drug-related changes from baseline were seen in physical evaluations (general and

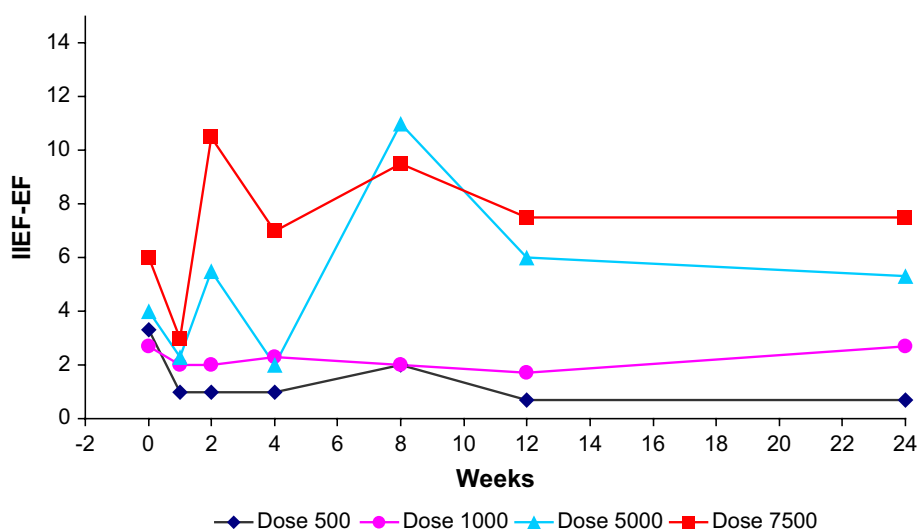


Fig. 8. *hMaxi-K*: Change in patient IIEF-MIS scores over time by dose for each Patient. MIS, mean intercourse satisfaction. (From Melman A, Bar-Chama N, McCullough A, et al. Plasmid-based gene transfer for treatment of erectile dysfunction and overactive bladder: results of a phase I trial. *Isr Med Assoc J* 2007;9:143–6; with permission.)

genitourinary), hematology, chemistry and hormone analyses, or in cardiac events evaluated by repeated ECGs (one patient who had preexisting atrial arrhythmia had a recurrence at ~1 month after dosing). No plasmid was detected in the semen of patients at any time after the injections. FDA requires that the participants in gene transfer trials, in which naked DNA is used as the vector, be followed for at least 2 years after transfer. The lack of complications or adverse experiences has now been confirmed for up to 2 years following gene transfer.

We cannot draw conclusions about efficacy from the results of phase 1 trials without randomized controlled groups. Nonetheless, efficacy measurements, made at each study visit, may provide insight into potential clinical activity. The IIEF is the standard instrument accepted as the best measure of efficacy in ED clinical trials, and patients given the two highest *hMaxi-K* doses had apparent sustained improvements in erectile function indicated by improved scores of the IIEF-EF domain over the length of the study. Specifically, one patient in the 5000- μg group and one in the 7500- μg group reported mostly equivalent EF improvements that approached the no ED IIEF-EF score, and they maintained the improvements for 24 weeks. Sexual satisfaction scores confirmed patient improvements.

Because the participants in this trial were not blinded to their treatments the improvement in IIEF score may have been a consequence of their belief in the effectiveness associated with the treatment; however, the preliminary results indicate that gene transfer with *hMax-K* has significant potential as a therapy for patients who have ED. Overall, the results suggest that further studies in a larger group of patients, with the addition of a placebo control and multiple doses, should be conducted to confirm the safety and efficacy of *hMaxi-K* in patients who have ED.

Summary and future directions

It remains to be confirmed in controlled clinical trials that *hMaxi-K* is efficacious as treatment for men who have ED. Following the promising safety outcomes at the doses of *hMaxi-K* in the initial phase 1 trial, cohorts of patients are being recruited to extend the observations to a series of higher doses preliminary to design of a controlled clinical trials program. The results of the first human trial of gene transfer therapy for ED suggest that gene

transfer focused on ion channel therapy in the smooth muscle of organs, such as the penis and bladder, offers a promising new treatment strategy. This novel therapeutic approach may address limitations of current therapies for ED.

Preclinical studies recently reviewed [3,35] documented the important role of Maxi-K channel-mediated hyperpolarizing currents to the modulation of bladder myocyte function. Results of this work suggested that *hMaxi-K* gene transfer therapy also may be effective for the treatment of urinary incontinence related to bladder overactivity. In December, 2006, the FDA approved an IND for Ion Channel Innovations, LLC to conduct the first phase 1 human trial to study the safety of *hMaxi-K* gene transfer for the treatment of detrusor overactivity. The phase 1 trial was initiated in April, 2007.

The potential clinical advantages of a gene transfer therapy-based approach to treatment of genitourinary smooth muscle-based disorders are several: potential single therapy for restoration of normal bladder or erectile function; elimination of the need for daily medication; use in combination with other therapies to reduce dose requirements and side effects; and the development of mechanism-based, patient-specific treatment approaches. With the safe administration of *hMax-K* to men who have ED in the first human phase 1 trial and the initiation of the phase 1 trial of *hMaxi-K* for patients who have detrusor overactivity, we have entered an exciting new era in the development of safe enduring therapies for genitourinary disorders.

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