

# Gene Therapy for Erectile Dysfunction: What Is the Future?

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**Abstract** Gene transfer for the treatment of erectile dysfunction has completed phase I safety testing and has shown the necessary safety to proceed to the next level of clinical trial. This review focuses on the background of the components of that have led to US Food and Drug Administration acceptance of human gene transfer trials for nonlethal disease.

**Keywords** Gene transfer · Erectile dysfunction · Plasmid · Naked DNA, Overactive bladder · Phase I trial · Smooth muscle · Corpora cavernous

## Introduction

Gene therapy to treat inherited and acquired diseases represents a potential large step forward in medicine. The concept of gene transfer is to introduce into specific somatic cells new genetic material that will produce a beneficial effect by replacing or correcting a defective or deficient gene in the nucleus of the cell [1]. This approach has the advantage that the introduced gene will produce a specific protein product with a specific action in the cell. This is in contrast to drug-based therapies that may produce diverse effects in nonspecific cell and organ groups [2]. The application of gene transfer in human clinical therapy for urological diseases places our specialty in the forefront of medical advancement. To date, gene transfer has been directed toward the treatment of erectile dysfunction (ED)

and the syndrome of urinary urgency and frequency, also known as overactive bladder (OAB). Each of these diseases is caused by, and can be ameliorated or prevented by directing the treatment to, the organ's smooth muscle cells. A primary hurdle to the use of gene transfer as therapy to treat nonfatal disease was passed when the US Food and Drug Administration (FDA) first approved a gene transfer trial to treat ED in 2003 and one to treat OAB in 2007.

The gene chosen for both trials was *hSlo*. The *hSlo* gene encodes the expression of the  $\alpha$ , or pore-forming, unit of the large-conductance calcium (Ca) and voltage-activated potassium (K)-ion channel known as the BK<sub>Ca</sub>, or Maxi-K, channel. The BK<sub>Ca</sub> channel is composed of a tetramer of six membrane-spanning proteins that, when in the open state, selectively conduct K<sup>+</sup> ions down the electrochemical gradient out of the cell at a rate of 10<sup>6</sup> to 10<sup>8</sup> ions/sec in all cells in the body except cardiomyocytes [3]. The consequence of the transient rapid efflux of K<sup>+</sup> ion is the hyperpolarization of the cell and closure of voltage-dependent calcium channels that decrease Ca<sup>2+</sup> entry into the cell. The inhibition of Ca<sup>2+</sup> influx into vascular smooth muscle cells causes the smooth muscle cells to relax with consequent vasodilation. As a result, the K<sup>+</sup> channels are required for maintenance of vascular tone as they counteract and balance vasoconstrictor events within the smooth muscle cells [4]. The BK<sub>Ca</sub> channels can be thought of as a natural feedback mechanism to oppose smooth muscle contractility. Alterations in K<sup>+</sup> channel physiology and function increasingly are being recognized as major contributing factors to the development of the vascular pathological conditions associated with diabetes and aging [5, 6]. In mouse models in which the *BKCa* gene has been knocked out, the animals exhibit both ED and bladder overactivity [7, 8]. Therefore, the possibility of correcting

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channelopathies with long-lasting and safe gene transfer methodology is an exciting concept.

The mechanism of each of the extant successful medical therapies for ED is the initiation or prolongation of corporal smooth muscle relaxation. Nonsurgical drug-delivered treatments for ED include oral drugs such as sildenafil (eg, Viagra; Pfizer, New York, NY), tadalafil (Cialis; Eli Lilly and Co., Indianapolis, IN), and vardenafil (Levitra; Bayer HealthCare Pharmaceuticals, Wayne, NJ), which act as phosphodiesterase type 5 (PDE5) inhibitors; intraurethral alprostadil; and intracavernous injection of prostaglandin E<sub>1</sub> or a combination of prostaglandin E<sub>1</sub>, papaverine, and phentolamine. Despite the availability of these therapies, lack of efficacy, their potential side effects, and loss of spontaneity have limited therapy for ED to about less than 20% of the overall ED patient population.

The intracellular mechanism of action of the popular PDE5 inhibitors is the prolongation of the intracellular concentration of cyclic guanosine monophosphate (cGMP), an agent that has both a direct and an indirect effect on ion channels and pumps. Therefore, control of potassium channels is downstream from the intracellular events initiated by the release of nitric oxide and its product, cGMP. The prostaglandins stimulate the second messenger cyclic adenosine monophosphate, and their actions produce a similar downstream effect on ion channels and pumps that cause smooth muscle relaxation. Papaverine is a nonspecific phosphodiesterase inhibitor. Phentolamine blocks the binding of adrenergic agonists to their receptors, thereby reducing the intracellular release of calcium and promoting relaxation of the smooth muscle cell.

### Previous Animal Studies

One of the observations of ED research in lower mammalian species is that there is a wealth of published studies where the introduction of a large and diverse variety of genes has been positive [9•]. One of the common biochemical characteristics of these treatments is that they result in increased relaxation of corporal smooth muscle. The implication of the positive results for a diverse range of materials, models, and diseases is that erection is such an important event for continuing of the species that a great deal of redundancy and plasticity are present through evolutionary events to maintain the continued ability to procreate.

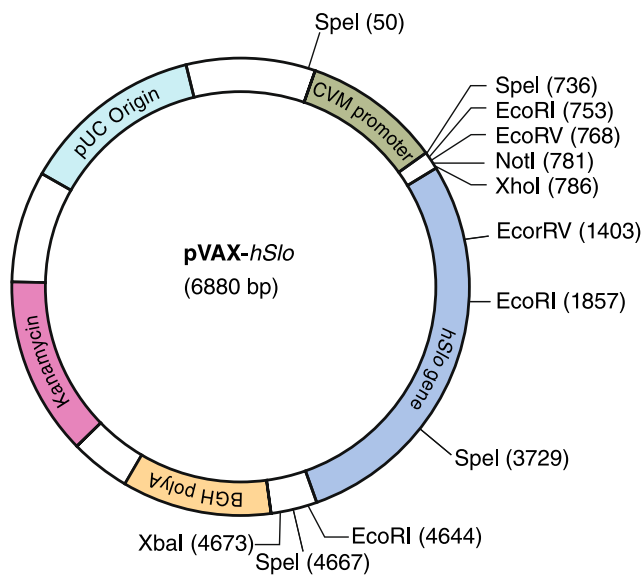
One of the major interests of our group has been the clinical translation of animal studies in which the gene encoding the Maxi-K channel has been used to treat ED or OAB. Clinical phase 1a and 1b safety trials for the use of gene transfer of plasmids expressing Maxi-K to treat ED now are complete. As noted in previous publications, the trial was conducted on men with moderate to severe ED

who previously failed, or did not wish to use, other forms of medical therapy [10]. The men had to be willing to use condoms (until it was proven that the gene did not integrate into sperm), be willing to sign to have their bodies examined at autopsy should an adverse event occur, and their long-term sexual partner had to sign a similar informed consent. The informed consents were explained to the potential participants by an unbiased clinical coordinator.

The specific hurdle for gene transfer in men for nonfatal, nonmalignant disease hinged upon acceptance of the vector chosen to effect transfer of the gene into the smooth muscle cells of the penis. The untoward events that led to interruption in gene therapy trials occurred after the death of an 18-year-old patient who was treated with a gene to correct a genetic ornithine decarboxylase deficiency with the use of a viral vector [11]. As a result, we chose to use a plasmid to express our gene of interest because it is a nonviral vector widely recognized as having a far greater safety profile than viral vectors. Plasmids are not associated with immune-related toxicity, possible mutagenesis and oncogene activation, cytotoxicity, and other possible problems associated with the use of viral vectors. However, the reputation of the plasmids is that there is only short duration of expression, and only limited numbers of cells take up the plasmid. However, our animal studies demonstrated that, in a rodent model of ED associated with aging, gene transfers of plasmids expressing hMaxi-K not only were efficacious, but also had a beneficial effect lasting for several months. Such a long duration of action would represent a distinct advantage over the repeated need for on-demand oral therapy.

The plasmid used for the trial is called hMaxi-K (Althea Technologies, San Diego, CA), a double-stranded naked plasmid DNA molecule carrying the human cDNA encoding the  $\alpha$ , or pore-forming, subunit of the human smooth muscle Maxi-K channel *hSlo*. It consists of a 3-kb backbone, pVAX carrying a 3.9-kb insert, *hSlo* cDNA. Expression of the *hSlo* gene in pVAX is from the upstream cytomegalovirus (CMV) promoter. The plasmid backbone also contains a T7 priming site, a multiple cloning site polylinker, the kanamycin resistance gene, the bovine growth hormone polyadenylation site, and pUC origin of replication. The CMV promoter and bovine growth hormone poly(A) sites promote expression of the transgene. The plasmid hMaxi-K (pVAX/*hSlo*) was manufactured from an *Escherichia coli* DH10B master cell bank under cGMP conditions. The 6880 base pair map of the pVAX/*hSlo* construct is shown in Fig. 1.

The authors recognize that plasmid maps are not part of the typical purview of clinical urology. However, a brief description of the map is useful to provide background information to understand the permutations of gene transfer in



**Fig. 1** Plasmid map of pVAX/*hSlo* construct *bp* base pair; *CMV* cytomegalovirus

general and the site of manipulation of the plasmid that can drastically alter the product to obtain the desired physiological (clinical) effect. The parent plasmid is a commercially available product from Invitrogen (Carlsbad, CA). The plasmid is designed to be flexible, in that a particular gene of interest can be inserted into the plasmid at the multiple cloning site using appropriate restriction enzymes. After the plasmid is constructed, it is transfected into *E. coli* and grown in a broth containing kanamycin, a selectable marker present on the plasmid backbone. The *E. coli* containing the plasmid then is allowed to grow, vastly expanding the plasmid copy numbers. Subsequently, the plasmid is purified from the bacteria under good manufacturing conditions, as required by the FDA, and then prepared for human use in an appropriate format depending upon the route of delivery. The CMV promoter of the pVAX/*hSlo* plasmid will drive expression of the gene of interest in any cell type in which the plasmid gains entry into the nucleus. We subsequently have developed and manufactured a plasmid in which the Maxi-K gene is driven by the  $\alpha$ -actin smooth muscle promoter, which restricts expression of the *hSlo* gene to smooth muscle cells only. This construct also improves erectile function in animal models of ED [12••] and has the clinical advantage of improved safety profile because of restricted tissue expression. The ability to refine the effect of the therapy to target a specific cell is one of the exquisite refinements of gene therapy.

#### Preclinical or Animal Supportive Studies

Preclinical animal research to prove that the treatment was both efficacious and safe was performed in the following

manner. The effect of *hSlo* gene transfer on erectile response in vivo was evaluated using two models of ED: streptozotocin (STZ)-induced Fisher diabetic rats and retired breeder Sprague–Dawley rats. The effect of single intracorporal/intracavernous doses of 10, 30, 100, 300, and 1,000  $\mu$ g *hSlo*/pcDNA plasmid on the intracorporal pressure/blood pressure (ICP/BP) ratio was compared with that in control patients. The *hSlo*/pcDNA plasmid was the prototype for the pVAX/*hSlo* plasmid in which *hSlo* still is expressed from the CMV promoter, but has a different backbone and selectable marker. In these experiments, the ICP/BP ratio was measured after external electrostimulation of the cavernous nerve.

As in humans, to achieve an erectile response, the arterial and cavernous smooth muscle cells of the penis must be relaxed sufficiently to permit transmission of systemic pressure into the corpus cavernosum. As long as the corporal venoocclusive mechanism functions properly, blood engorges the corporal spaces under systemic pressure and the penis becomes rigid. The ICP/BP ratio is a physiological measurement that correlates with engorgement of the corpora with blood. After an extensive series of experiments, a visually observable erectile response was seen in most rats when the ICP/BP ratio was 0.5 or higher, whereas a visually observable erectile response was seen in all animals regardless of age when the ICP/BP ratio was 0.6 or higher.

The effect on the ICP/BP ratio of a single intracorporal/intracavernous injection of *hSlo*/pcDNA followed by external electrostimulation of the cavernous nerve was evaluated in the STZ-induced diabetic rat and retired breeder Sprague–Dawley rat models. In the STZ-induced diabetic rat model, rats confirmed to be diabetic 2 months after STZ-induced diabetes were injected with *hSlo*/pcDNA plasmid. Systemic blood pressure and nerve-stimulated ICPs were measured at various stimulus intensities ranging from 0.5 to 10.0 mA at monthly intervals ranging from 1 to 4 months after injection of plasmid DNA. Age-matched nondiabetic rats, untreated STZ-diabetic rats, and STZ-diabetic rats injected with 1,000  $\mu$ g of the pcDNA plasmid backbone alone served as controls.

Overall, cavernous nerve stimulation in STZ-diabetic rats 2 months after intracavernous injection of *hSlo* resulted in ICP/BP of 0.6 or higher in the gene therapy groups, whereas the STZ-diabetic controls had ICP/BP ratios less than 0.6. Analysis of the tested doses of *hSlo*/pcDNA on the ICP/BP ratio demonstrated the following: 1) the 10- $\mu$ g dose is near the threshold for physiological effect; 2) the 30- to 1,000- $\mu$ g treatment groups consistently had ICP/BP ratios significantly greater than those observed in the untreated groups, and in a large majority of cases, these values were greater than 0.6; and, 3) response was seen to last at least to 6 months after study drug administration, but

the magnitude of the response elicited by all gene therapy doses declined with elapsed time after injection.

Retired breeder Sprague–Dawley rats typically display a significant age-related decline in erectile capacity. In this second animal model, the ICP/BP ratio was measured in male Sprague–Dawley rats larger than 500 g and older than 9 months that received a single intracorporal injection of 10, 30, 100, 300, and 1,000  $\mu\text{g}$  *hSlo*/pcDNA plasmid or 1,000  $\mu\text{g}$  pcDNA plasmid as control. Cavernous nerve stimulation ranged from 0.5 to 6 mA. Similar to the STZ-induced diabetic rat model, a dose-related effect on ICP/BP ratio was seen in the retired Sprague–Dawley rat model. The 10- $\mu\text{g}$  *hSlo*/pcDNA dose was less effective than the higher doses. With time, the overall effect of all doses decreased and greater levels of neuronal stimulation (ie, 6 mA current stimulation) were needed to elicit a response. In all cases, more *hSlo*-treated rats had visible erectile responses in contrast to the untreated rats that also had ICP/BP ratios less than 0.5 to 0.6.

In these two distinct animal models of ED, increased expression of Maxi-K channels after *hSlo* gene expression resulted in an increased ICP/BP ratio. For most doses evaluated, the increase in ICP/BP ratio was to a level that has been shown to correlate physiologically with penile erection. Importantly, the ICP (erectile) response seen with cavernous nerve stimulation in both models returned to basal (resting) levels immediately on termination of the external peripheral neural stimulus. This return to baseline intracorporal pressure suggests that potentially undesirable effects (eg, priapism) resulting from expression of *hSlo* in the corporal smooth muscle cells of the penis are unlikely to be seen in the clinic.

### Effect of Repeat Intracavernous Doses

Because the effect of gene transfer of hMaxi-k is finite and seems to have duration of expression of about 6 months, investigation of the effect of repeat exposure to *hSlo* has been initiated. In a study of retired breeder rats, we evaluated the effects of the intracavernous injection of 100  $\mu\text{g}$  *hSlo*/pcDNA plasmid or pcDNA/*rSlo* (the rat Maxi-K gene) once every 4 weeks for 16 weeks on the ICP/BP ratio and cardiovascular parameters. Rats treated with *hSlo*/pcDNA had significantly increased ICP response after cavernous nerve stimulation compared with rats receiving phosphate-buffered saline (PBS) containing 20% sucrose or plasmid backbone alone. However, no cumulative effect of repeat dosing was observed. Mean ICP values after the fourth injection of *hSlo*/pcDNA were similar to those seen in the earlier single-dose experiments.

Importantly, cardiovascular parameters, measured weekly, demonstrated no adverse effects after repeat administration of

*hSlo*/pcDNA. All measurements of systolic and diastolic blood pressure and heart rate in the treated animals were within normal range and were similar to measurements in the controls. There were no differences in any of the outcome measures (ICP/BP ratio).

The results from the preclinical in vivo studies suggested that after administration of pVAX/*hSlo*, increased expression of the  $\alpha$ , or pore-forming, subunit of the human large conductance, calcium-sensitive K channel subtype (*hSlo* or Maxi-K) can ameliorate age- and diabetes-related ED in rat models, and these seem to be long-term effects.

Those results, along with other safety and biodistribution data, were presented to the FDA, with resultant approval to initiate human trials.

### The Completed Phase 1 Erectile Dysfunction Human Trials

The completed phase 1a and 1b studies evaluated the safety of hMaxi-K in a target population of men between 18 and 80 years of age having moderate to severe ED attributable to an underlying medical condition of longer than 6-months duration. The trial was conducted in seven cohorts at doses of 500, 1,000, 5,000, 7,500, 10,000 and 16,000  $\mu\text{g}$  of plasmid. The plasmid solution in a total of up to 4 mL of PBS containing 20% sucrose was injected as a solution into the corporal sinusoids with the use of a 5-mL syringe and a 25-gauge needle. To minimize even the potential for unintended biodistribution an Actis tourniquet (VIVUS, Inc., Mountain View, CA) was placed at the base of the penis before the injection and left for 30 min to prevent any possible biodistribution. If the plasmids are not taken up by the cells within 30 min, the plasmids are degraded by nucleases present in the blood.

The men were studied at two clinical sites: Mt. Sinai and NYU, by Drs. Natan Bar-Chama and Andy McCullough, respectively. The study procedures after screening and drug administration were conducted at weeks 0, 1, 2, 4, 8, 12, and 24.

The primary goal of the study was to assess safety by analysis of adverse experiences, clinical laboratory tests, and physical examinations. The men were followed for 18 months after completion of the study. Safety data were analyzed using summary descriptive statistics.

The results of the trial showed no serious adverse event related to gene transfer in any of the men up to 2 years after the transfer. One participant noted a transient slight tingling sensation in the glans penis immediately after transfer. Most importantly from a regulatory viewpoint, there were no gene transfer-related electrocardiogram changes, nor was there evidence of expression of the gene in the semen in any trial participant. Physical examination and blood chemistries showed no changes after gene transfer.

As indicated in the preclinical trials, there was no evidence of prolonged erection. Even though priapism was not expected because of the channel's mechanism of action, the trial proved that it did not occur during sexual excitement at any dose in any participant.

The phase 1 trial was designed to prove safety. They did so at seven escalating doses.

Does the gene transfer increase erection in man? Preclinical trials in rats and hypertensive crab-eating monkeys demonstrated normalized and prolonged erectile duration after gene transfer. As noted earlier, preclinical experiments are not designed to prove efficacy in man. However, there was some indication of efficacy, as two of the men had normalized erections (by the International Index of Erectile Function criteria) for 6 months after treatment, the expected duration of action shown in the preclinical studies, in the first phase of the trial [10]. In the second phase, two of the men and their partners reported significant improvement in their erection as measured by the by the Treatment Satisfaction Scale [13].

### Will Gene Transfer Be Accepted by Men and the Urological Community?

There is no question that the public will accept gene transfer if approved as safe and effective by the FDA. Proof of that statement is derived from the hundreds of emails received from men who have requested to be added to the next trial despite absence of advertising or marketing. We previously have reported the results of a web-based survey done by Navigant Consulting, Inc. under the direction of the sponsor, Ion Channel Innovations, LLC. That study demonstrated that, if approved by FDA, the product would be used for both a primary and secondary treatment modality by most urologists with an interest in ED [14•].

### Conclusions

What Is the Future of Gene Transfer for Erectile Dysfunction?

The next step in the development of clinically approved gene transfer treatment of ED is a placebo-controlled phase 2 trial, powered to have a sufficient number of participants to prove or disprove the efficacy of gene transfer of hMaxi-K [15•]. Unfortunately, there has been a delay in initiating the phase 2 trials. The issue causing the delay in initiating these trials is not the expectations for the product, patient demand, availability of clinical sites, or product availability, but funding. The transfer sponsor, Ion Channel Innovations, LLC, is a private, academically

based company that has begun the trials on private capital. The period between phase 1 and phase 2 trials is typically time the most difficult for small biotech companies to raise capital from the pharmaceutical or venture capital world. At this moment, the ongoing challenges in raising capital hopefully will abate, providing the full potential of gene transfer to treat ED, OAB, and other medical issues to be fully realized.

**Disclosures** Dr. Arnold Melman is a cofounder of Ion Channel Innovations, LLC, and has been issued patents, owned by Einstein Industries and licensed to Ion Channel Innovations, LLC. Dr. Kelvin Davies has received a grant from the National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases.

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