

## *h*Maxi-K Gene Transfer in Males with Erectile Dysfunction: Results of the First Human Trial

ARNOLD MELMAN,<sup>1</sup> NATAN BAR-CHAMA,<sup>2</sup> ANDREW McCULLOUGH,<sup>3</sup> KELVIN DAVIES,<sup>1</sup>  
and GEORGE CHRIST<sup>4</sup>

### ABSTRACT

Eleven patients with moderate to severe erectile dysfunction (ED) were given a single-dose corpus cavernosum injection of *h*Maxi-K, a “naked” DNA plasmid carrying the human cDNA encoding *hSlo* (for *human slowpoke*), the gene for the  $\alpha$ , or pore-forming, subunit of the human smooth muscle Maxi-K channel. Three patients each were given 500, 1000, and 5000  $\mu\text{g}$ , and two patients were given 7500  $\mu\text{g}$ , of *h*Maxi-K and monitored for 24 weeks. The primary objectives of this phase I study were safety and tolerability of escalating *h*Maxi-K doses as assessed by clinical evaluations and laboratory tests. Secondary efficacy objectives were measured primarily by use of the International Index of Erectile Function (IIEF) scale. Patient responses were validated by partner responses. There were no serious adverse events and no dose-related adverse events attributed to gene transfer for any patient at any dose or study visit. No clinically significant changes from baseline were seen in physical evaluations (general and genitourinary), hematology, chemistry, and hormone analyses, or in cardiac events evaluated by repeated electrocardiograms. Importantly, no plasmid was detected in the semen of patients at any time after the injections. Patients given the two highest doses of *h*Maxi-K had apparent sustained improvements in erectile function (EF) as indicated by improved IIEF-EF domain scores over the length of the study. One patient given 5000  $\mu\text{g}$  and one given 7500  $\mu\text{g}$  reported EF category improvements that were highly clinically significant and were also maintained through the 24 weeks of study. Efficacy conclusions cannot be drawn from results of a phase I trial with no control group. However, the promising primary safety outcomes of the study and preliminary indications of effectiveness provide evidence that *h*Maxi-K gene transfer is a viable approach to the treatment of ED and that further studies investigating the efficacy of *h*Maxi-K in patients with ED should be performed.

### OVERVIEW SUMMARY

The penis is an organ uniquely suitable for gene transfer because of its anatomic and ultrastructural features. Anatomically, the penis is a readily accessible external organ with internal features that promote distribution and long dwell-time for gene product in cavernous bodies with little potential for unwanted distribution to other tissues or organs. We report here promising results of the first human trial of gene therapy for the treatment of erectile dysfunction (ED). Injection of *h*Maxi-K into the corpus cavernosum of the penis was well tolerated with no adverse experiences or laboratory abnormalities attributed

to any dose of the gene product. Furthermore, no local physical events related to the injections were observed and sperm samples showed no detectable evidence of *h*Maxi-K in semen down to the 1-copy/ $\mu\text{g}$  level of the total DNA of any patient at any visit. This phase I safety study was not designed to provide efficacy answers; however, one patient in each of the higher dose groups (5000 and 7500  $\mu\text{g}$ ) reported clinically significant and sustained improvements in ED as indicated by erectile function (EF) domain scores of the International Index of Erectile Function (IIEF) scale. The study strongly supports continued investigation (phase II) of *h*Maxi-K gene transfer therapy for treatment of ED.

<sup>1</sup>Department of Urology, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY 10461.

<sup>2</sup>Mount Sinai School of Medicine, New York, NY 10029.

<sup>3</sup>New York University School of Medicine, New York, NY 10001.

<sup>4</sup>Wake Forest University School of Medicine, Winston-Salem, NC 27157.

## INTRODUCTION

**E**RECTILE DYSFUNCTION (ED) is a multifactorial neurovascular disease in which alterations in the tone and compliance of the corporal smooth muscle assume a major role in impotence, regardless of the exact etiology. Heightened contractility and/or impaired relaxation of the corporal smooth muscle are primary causes of ED in most men (Carrier *et al.*, 1993; Andersson and Wagner, 1995; Christ, 1995; Christ and Melman, 1997; Melman and Christ, 1997). Therefore most effective forms of therapy for ED exert their actions by inducing relaxation of corporal smooth muscle. The development and availability of oral phosphodiesterase type 5 (PDE-5) inhibitors, effective vasodilating agents that exploit this mode of action for the treatment of men with ED, have dramatically increased awareness of this common disorder (Melman *et al.*, 1999; Lewis, 2001; Seftel, 2003). More than 50% of men aged 40–70 years and 70% above age 70 were identified as having ED in the Massachusetts Male Aging Study (MMAS) (Feldman *et al.*, 2000). Worldwide it is estimated that 140 million men have some degree of ED and the prevalence is expected to increase to more than 300 million men by 2025 (Ayta *et al.*, 1999; Melman and Gingell, 1999).

A European study reported that diabetes mellitus, aging, and hypertension are the most important risk factors for the development of ED (Ponholzer *et al.*, 2005). Conversely, the second Princeton Consensus Conference (Princeton II) suggested that in otherwise asymptomatic men, ED is a warning sign of silent vascular disease, especially coronary artery disease (CAD) (Jackson *et al.*, 2006). This interrelationship between ED and CAD likely results from common risk factors and at least one pathophysiologic common denominator, that is, endothelial dysfunction (Kirby *et al.*, 2001; Jackson *et al.*, 2006). Princeton II concluded that a man with ED and no cardiac symptoms is a cardiac (or vascular) patient until proven otherwise (Jackson *et al.*, 2005).

Although the PDE-5 inhibitors have been successful and popular, there remain significant limitations in meeting patient needs. The short-lived duration of action of currently available members of this class, lasting from hours to a few days, results in the need for use on demand and limits spontaneity of the sexual act. In addition, 30–40% of patients are nonresponders. Nuisance side effects such as nasal stuffiness and headaches can be significant and the effectiveness of PDE-5 inhibitors in patients with diabetes is limited. PDE-5 inhibitors are absolutely contraindicated in all men taking nitrate compounds, an important issue with cardiovascular comorbidities. The report of nonarteritic anterior ischemic optic neuropathy in a small number of men with diabetes who were using PDEs has created a potential for reduced demand for the use of that family of drugs by the men who need them most (Pomeranz and Bhavsar, 2005). Because of limitations of current treatments, there is great potential for gene transfer therapy, assuming it can address the unmet needs of men with ED.

Gene therapy studies of erectile dysfunction to date have been limited primarily to preclinical, early-stage studies using a variety of vectors and genes (Melman, 2006). Both the cAMP and cGMP pathways have been investigated, but most commonly these approaches have targeted various points in the erectile pathway, which begins with nitric oxide (NO) production and involves cGMP-mediated activation of potassium channels

and other cellular and membrane proteins, ultimately resulting in hyperpolarization and relaxation of vascular and cavernosal smooth muscle cells. All of these have been short-term studies with responses measured within days to a few weeks after transfer.

The penis is an organ uniquely suitable for gene transfer because of its anatomic and ultrastructural features, and the focus of the preclinical work leading to this paper was on the key role played by endogenous potassium channels (Christ, 2002; Melman, 2006). Anatomically, the penis is a readily accessible external organ with internal features that promote distribution and a long dwell time for gene product in the cavernous bodies. Gap junctions are a key ultrastructural feature of cell-to-cell communication, which allows the penile corpora to function as a syncytium and, thus, permits the use of inefficient transfer vectors such as naked DNA. Thus, despite an efficiency of cellular incorporation *in vivo* that is likely to be  $\leq 10\%$ , naked DNA can still be used for effective, physiologically relevant gene transfer in the smooth muscle of the genitourinary system (Christ *et al.*, 2006; Melman, 2006). Of specific relevance to this paper is the fact that potassium channels play an important role in modulating smooth muscle cell excitability, that is, the degree of smooth muscle cell contraction and relaxation, which translates into control of hollow organ function (Nelson and Quayle, 1995). At least four types of potassium channel are present in the plasma membranes of the human corpora (Christ *et al.*, 1993; Fan *et al.*, 1995; Karicheti and Christ, 2001; Melman and Christ, 2001). With respect to penile erection, these channels respond to physiologic intracellular events by opening and allowing  $K^+$  to flow out of the smooth muscle cell, resulting in hyperpolarization that limits calcium entry and relaxes the corporal and arterial smooth muscle cells. Thus a gene transfer approach that provides the ability to overexpress a potassium channel gene in corporal tissue could theoretically overcome diseases or aging that reduce neuronal input and lead to erectile dysfunction. Our laboratory focused on the ability of the cloned gene *hSlo* (for *human slowpoke*), which encodes the  $\alpha$ , or pore-forming, subunit of the  $Ca^{2+}$ -activated  $K^+$  potassium channel (Maxi-K), to improve erectile function. The *hSlo* gene was cloned into a commercially available expression vector, pVAX1 (Invitrogen, Carlsbad, CA), to construct *hMaxi-K*, the naked DNA construct used in this first human trial of gene transfer therapy for the treatment of erectile dysfunction. Preliminary safety results of this trial have been reported (Melman *et al.*, 2005; Melman, 2006). These reports were limited to adverse events reported and semen testing for *hMaxi-K* in patients given doses of 500, 1000, or 5000  $\mu\text{g}$  (results only to 3 months). No International Index of Erectile Function (IIEF) data were reported. The objective of this paper is to present comprehensive safety results of the completed trial (four *hMaxi-K* dose groups for 6 months) as well as secondary results on the potential effects of *hMaxi-K* on ED, using the erectile function domain of the IIEF.

## MATERIALS AND METHODS

### *Ethical and regulatory considerations*

This study was conducted in compliance with the Declaration of Helsinki, U.S. Title 21 Code of Federal Regulations, and the International Conference on Harmonization of Technical

Requirements for Registration of Pharmaceuticals for Human Use (E6, Guidelines for Good Clinical Practice; <http://www.fda.gov/cder/guidance/959fnl.pdf>). After a public presentation (June 21, 2002) to the Recombinant DNA Advisory Committee (RAC) of the Office of Biotechnology Activities of the National Institutes of Health, an Investigational New Drug (IND) application was submitted to and approved by the Center for Biologics Evaluation and Research (CBER) of the U.S. Food and Drug Administration (FDA) on August 1, 2003. The protocol and participant and partner informed consent documents were approved by the institutional review committee and institutional biohazard committee, Biomedical Research Alliance of New York (BRANY, Great Neck, NY). A data safety monitoring board (DSMB) approved each patient's entry. For each dose group the DSMB reviewed safety data on all patients approximately 4 weeks after administration of their hMaxi-K dose, before approving dose escalation to the next treatment arm.

### Study design and patient population

This was an open label, sequential four-arm, phase I study conducted at two study centers to evaluate the safety and tolerability of hMaxi-K in men with ED. The study was designed to evaluate a single administration of four escalating doses of hMaxi-K injected into the corpus cavernosum of the penis. Patients were monitored for 6 months after dosing and annual follow-ups are planned for 15 years as required by the FDA. Fifteen men were screened for the study and 11 qualified for entry.

Three men were treated at each of three dose levels, 500, 1000, and 5000  $\mu\text{g}$ , and two were treated with 7500  $\mu\text{g}$ . Dose levels for this safety study were selected on the basis of the lowest range of hMaxi-K used in preclinical studies in rodents. The 7500- $\mu\text{g}$  dose in an average 70-kg man was equivalent to 69  $\mu\text{g}$  in a 650-g retired breeder rat (approximately 106  $\mu\text{g}/\text{kg}$  in both species), but well below the maximum 1000- $\mu\text{g}$  dose used in rats (approximately 1538  $\mu\text{g}/\text{kg}$ , equivalent to 108,000  $\mu\text{g}$  in a 70-kg man). Even at the highest dose administered in the preclinical trials (i.e., 1000  $\mu\text{g}$ ), there were no immune responses or other adverse effects observed (Christ *et al.*, 2004).

The patient population consisted of men over 18 years of age with ED attributable to an underlying, stable medical condition but otherwise in good health. Men with ED secondary to aging as a solitary cause were excluded. Patients must have been in a stable monogamous relationship for at least 6 months and had no successful sexual intercourse for 6 months before study entry without specific ED therapy. They were eligible if they were unable to tolerate, did not wish to continue, or had unsuccessful results with prior therapy for ED. Patients agreed to use condom barrier contraception during the course of the study. Patients with diabetes were required to have  $\text{HbA}_{1c} \leq 7.0$  mg% at the time of enrollment and hypertensive patients had to have documented stable blood pressure. At screening patients were required to have a normal general and genitourinary physical examination and RigiScan results diagnostic for ED. The RigiScan (Timm Medical Technologies, Eden Prairie, MN) is a microcomputer that measures the number, duration, and hardness of erections during rapid eye movement (REM) sleep or visual

TABLE 1. SCHEDULE OF EVENTS

| Test or procedure                              | Visit 1<br>(week -2) | Visit 2<br>(week 0) | Visit 3<br>(week 1<br>$\pm 2$ days) | Visit 4<br>(week 2<br>$\pm 2$ days) | Visit 5<br>(week 4<br>$\pm 3$ days) | Visit 6<br>(week 8<br>$\pm 3$ days) | Visit 7<br>(week 12<br>$\pm 3$ days) | Visit 8<br>(week 24<br>$\pm 3$ days) |
|--|----------------------|---------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Informed consent process                       | ×                    |                     |                                     |                                     |                                     |                                     |                                      |                                      |
| Medical history                                | ×                    | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| Physical examination                           | ×                    | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| Physical examination of the penis <sup>a</sup> | ×                    | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| Blood chemistry                                | × <sup>b</sup>       | × <sup>c</sup>      | × <sup>b</sup>                      | × <sup>c</sup>                      | × <sup>b</sup>                      | × <sup>b</sup>                      | × <sup>b</sup>                       | × <sup>b</sup>                       |
| Urine analysis <sup>d</sup>                    | ×                    | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| Semen collection for <i>hSlo</i> cDNA          |                      | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | × <sup>e</sup>                       | × <sup>e</sup>                       |
| ECG  | ×                    | × <sup>f</sup>      | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| RigiScan                                       | ×                    |                     |                                     |                                     |                                     |                                     |                                      |                                      |
| IIEF <sup>g</sup>                              |                      | ×                   | ×                                   | ×                                   | ×                                   | ×                                   | ×                                    | ×                                    |
| Study drug administration                      |                      | ×                   |                                     |                                     |                                     |                                     |                                      |                                      |

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; CRP, C-reactive protein; ECG, electrocardiogram; *hSlo*, gene encoding the  $\alpha$ , or pore-forming, subunit of the  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  potassium channel (Maxi-K); IIEF, International Index of Erectile Function; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell; TSH, thyroid-stimulating hormone;  $\text{T}_4$ , thyroxine; WBC, white blood cell.

<sup>a</sup>Physical examination of the penis included inspection and palpitation.

<sup>b</sup>Blood tests included hematology CBC with differential, platelet count, PTT, PT, sedimentation rate, CRP), chemistry (BUN, Cr,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{CO}_2$ , Cl, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein), and endocrine (testosterone, random cortisol, TSH,  $\text{T}_4$ ).

<sup>c</sup>Blood tests included hematology (CBC with differential, CRP) and chemistry (BUN, Cr,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{CO}_2$ , Cl, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein).

<sup>d</sup>Urine analysis included microscopic RBC and WBC, protein, glucose, and specific gravity.

<sup>e</sup>Semen specimen was to be collected only if *hSlo* DNA was detectable in either of two preceding semen specimens.

<sup>f</sup>ECG was done before study drug administration and 1 and 3 hr after study drug administration.

<sup>g</sup>IIEF was completed by both the patient and his partner at all time points. Week 0 (baseline) tests were completed before administration of study drug.

sexual stimulation (Timm, 1994; Benet *et al.*, 1996). Tip rigidity less than 55% for less than 5 min on each of two nights tested is diagnostic for ED. Patients were excluded if they had any major cardiovascular events within the 6 months before enrollment, gonadal failure not treated with hormone replacement, a prior penile implant, current urinary tract infection, Peyronie's disease, abnormal electrocardiogram (ECG), or abnormal baseline laboratory values.

Patients and their sexual partners signed the respective informed consent forms and patients underwent the screening procedures indicated in Table 1. The screening data were reviewed by the DSMB. Approved patients returned for the baseline visit in 2 weeks, when gene transfer was given. They were seen after 1 week and then at monthly intervals for 6 months (Table 1).

### Assessments and analysis

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 the clinical evaluations and laboratory tests shown in Table 1. The procedures and tests 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 also assessed and recorded at each visit.

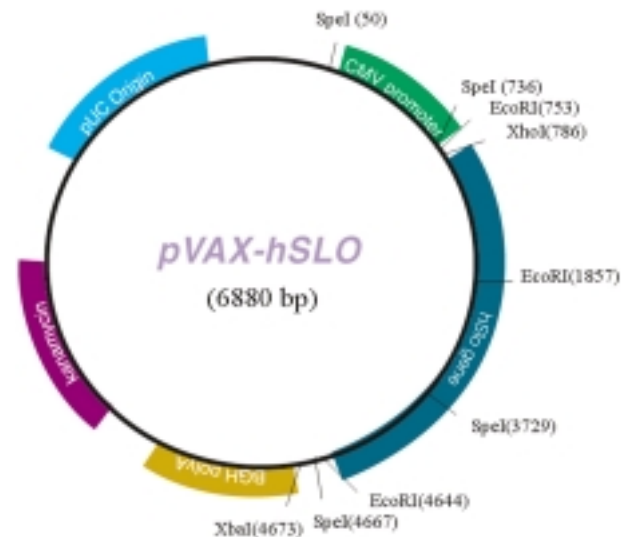
The DNA of semen was tested for the presence of pVAX1-*hSlo* plasmid, using reverse transcriptase-polymerase chain reaction (RT-PCR) with primers specific to the plasmid. The sensitivity of the assay was determined to be in the range of 1 copy/ $\mu$ g of total DNA as previously described (Christ *et al.*, 1998). Specimens were routinely collected at visits 2–5 (Table 1); however, specimens were to be collected at visits 6 and 7 only if *hSlo* DNA was detectable in either of two preceding semen specimens. If semen was still positive at week 24, the participant was to return monthly until two successive semen specimens were negative for *hSlo* DNA.

The key secondary study objective was assessment of the effect of *hMaxi-K* on ED, using the erectile function domain category of the International Index of Erectile Function (IIEF) scale (Rosen *et al.*; 1997; Cappelleri *et al.*, 1999). The IIEF is a validated, self-administered questionnaire shown to be a cross-culturally and psychometrically valid measure of male ED (Rosen *et al.*, 1997). The erectile function (EF) domain, questions 1–5 and 15 of the IIEF, has been validated to assess erectile changes only (Cappelleri *et al.*, 1999). Sexual partner responses to questions appropriate for the partner's assessment before and after gene transfer were used to validate patient responses.

The primary study outcome variables were adverse events during each study period, clinical laboratory tests, electrocardiogram, blood pressure, heart rates, and physical examination results. The secondary study outcome variables reported here were the EF and sub-EF (items 3 and 4) domain scores of the IIEF and change from baseline in the IIEF domain scores. Both the safety data and data to assess activity were analyzed using summary descriptive statistics for each dose group and the total population.

### Gene transfer product: *hMaxi-K*

**Plasmid derivation.** *hMaxi-K* was constructed by inserting the human Maxi-K<sup>+</sup> channel cDNA *hSlo* (~3900 nucleotides, i.e., 3.9 kb) (McCobb *et al.*, 1995) into the *XhoI-XbaI* cloning site of the pcDNA3 vector (Fig. 1). The original *hSlo* cDNA was obtained in the pMXT-*hSlo* plasmid vector from L. Salkoff (Washington University School of Medicine, St. Louis, MO). The plasmid pMXT is a derivative of pBlueScript (Stratagene, La Jolla, CA), modified to incorporate 5' and 3' untranslated sequences from the *Xenopus*  $\beta$ -globin gene. The pBlueScript vector contains an f1(–) origin,  $\beta$ -galactosidase  $\alpha$  fragment, a pUC origin of replication, and an ampicillin resistance gene. The procedure for isolation and cloning of the *hSlo* gene into pMXT by L. Salkoff was previously described (McCobb *et al.*, 1995). Briefly, the murine *Slo* gene was used as a low-stringency probe to screen human arterial smooth muscle and genomic libraries, and the human *Slo* homolog was cloned into pMXT to generate the plasmid pMXT-*hSlo*. For early preclinical studies the *hSlo* gene was removed from this plasmid, by digestion with *XhoI* and *XbaI*, and subcloned into pcDNA3 (Invitrogen) as described in Christ *et al.* (1998). This procedure generated the plasmid pcDNA3-*hSlo*. The pcDNA3 vector backbone drives expression of the *hSlo* gene from a cytomegalovirus (CMV) promoter and expresses ampicillin and neomycin resistance genes. Additional elements include T7,



**FIG. 1.** Plasmid construct (*hMaxi-K*, 6880 bp): CMV promoter (positions 137–724), viral; *hSlo* gene (positions 888–4428), human; BGH polyadenylation 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  $\alpha$ , 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 bovine growth hormone poly(A) site, kanamycin resistance gene, and pUC origin of replication. Reprinted from Melman *et al.* (2005) with permission from the European Association of Urology.

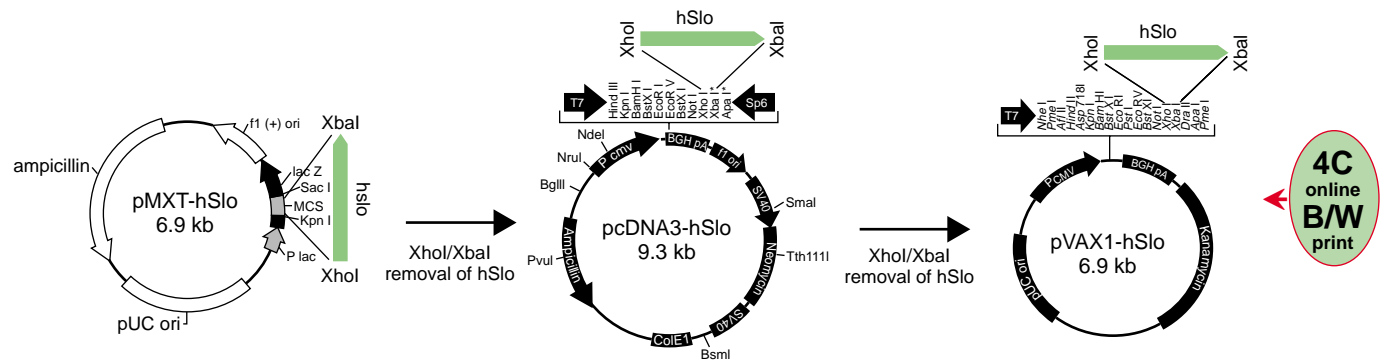


FIG. 2. Derivation of pVAX1-*hSlo* from pMXT-*hSlo*.

Sp6, and simian virus 40 (SV40) promoters, bovine growth hormone (BGH) and SV40 polyadenylation sites, a ColE1 origin, and SV40 origin of replication.

The plasmid pVAX1 (Invitrogen) contains the kanamycin resistance gene and no extraneous expression cassettes and is suitable for clinical testing. Therefore, in the present clinical trial, the *hSlo* gene was subcloned from pcDNA3-*hSlo* into pVAX1 (Invitrogen), by digestion with *Xho*I and *Xba*I, to generate pVAX1-*hSlo* (Fig. 2). pVAX1 uses a CMV promoter to drive expression of *hSlo*. In addition to the kanamycin resistance gene the pVAX1 backbone contains a T7 promoter, a BGH polyadenylation site, and a pUC origin of replication. This pVAX1-*hSlo* construct has efficacy indistinguishable from that of the pcDNA3-*hSlo* construct as shown in bioactivity testing (data not shown). This construct was sequenced (Althea Technologies, San Diego, CA) and the *hSlo* gene was confirmed to be identical to the original *hSlo*. The clinically used construct is called *hMaxi-K*.

*Plasmid testing and qualification.* Final plasmid testing before release for clinical use included a battery of purity and

identity evaluations. Identity testing was performed by additional restriction enzyme analysis and gel electrophoresis. Bioactivity of the final plasmid product was determined using the *in vivo* bioassay system in the retired breeder Sprague-Dawley rat model previously described in detail (Christ *et al.*, 1998; Melman *et al.*, 2005). The bioactivity assay was performed to assess the stability of *hMaxi-K* plasmid DNA that had been stored at  $-80^{\circ}\text{C}$  for approximately 11 months. Briefly, 100  $\mu\text{g}$  of the final *hMaxi-K* plasmid DNA product was injected intracorporally into a total of five rats; five control animals were injected with PBS vehicle. Animals were anesthetized 1 week after injection and subjected to a series of surgical and experimental procedures (Christ *et al.*, 1998; Melman *et al.*, 2005). For plasmid testing, cavernous nerve stimulation was performed as described in Christ *et al.* (1998) and Melman *et al.* (2003). This protocol accurately discriminates between gene therapy-treated and untreated age-matched control animals. In the untreated age-matched control animals, extensive experience with hundreds of animals indicated that during stimulation the intracavernous pressure (ICP)-to-blood pressure (BP) (i.e.,

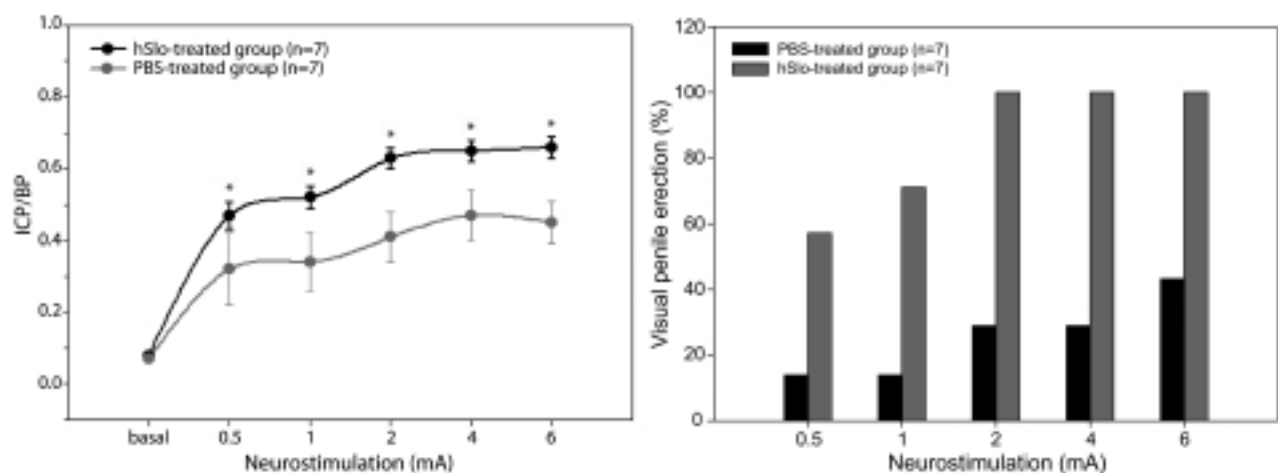


FIG. 3. Illustration of *in vivo* *hMaxi-K* bioassay system. Shown on the left is the mean ICP:BP ratio for a subset of *hSlo*-treated and age-matched control rats 1 week after injection of either *hSlo* or PBS (vehicle). Note that at all levels of current stimulation the gene transfer rats have significantly greater ICP:BP ratios and, moreover, shown on the right a much greater percentage of the *hSlo*-treated rats had visible penile erections; note especially that 100% of the *hSlo*-transfected rats achieved erections when their ICP:BP ratio was above 0.6.

ICP:BP) ratio is routinely between 0.3 and 0.5, and not associated with visible erections. *hMaxi-K*-treated animals have routinely produced ICP:BP ratios of 0.6–0.8, and these were associated with visible erectile responses. As per the IND application, the specification for *hMaxi-K* bioactivity required that animals treated with the *hMaxi-K* plasmid attain an average ICP:BP ratio of 0.6 to 0.8 and that control animals have an ICP:BP ratio of <0.6 when stimulated at the 4-mA level. Data illustrated in Fig. 3 demonstrate the ability of this assay to determine the potency and activity of the *hMaxi-K* plasmid DNA. The results of this study 1 week after transfer were consistent with results of studies involving hundreds of rats (ICP:BP ratio  $\geq$ 0.6), in which the study duration was 1 to 6 months.

**Final *hMaxi-K* gene transfer product.** Subcloning of *hSlo* into a commercially available, nonviral, closed-loop (circular), double-stranded piece of DNA (pVAX1; Invitrogen) creates the “naked” DNA plasmid (Fig. 1). The *hMaxi-K* plasmid construct is the double-stranded naked plasmid DNA molecule carrying the human cDNA encoding *hSlo*, the gene for the  $\alpha$ , or pore-forming, subunit of the human smooth muscle Maxi-K channel. *hMaxi-K* in phosphate-buffered saline (PBS)–20% sucrose was produced in an FDA-approved facility (Althea Technologies) under good manufacturing practices (GMP) conditions.

#### Dosing and administration

*hMaxi-K* was diluted in PBS–20% sucrose buffer and stored at 1000  $\mu$ g/0.4 ml in 5-ml sterile borosilicate glass vials at  $-20^{\circ}\text{C}$  in a locked freezer with limited access. Immediately before patient administration, *hMaxi-K* was diluted with PBS–20% sucrose to achieve the desired doses in volumes of 2–3 ml.

Appropriate doses were injected at a single site into the corpus cavernosum of the penis. In this first-in-human phase I

study, maximum precautions were taken to prevent unwanted biodistribution of the gene product. Rapid breakdown of the product in blood ( $t_{0.5}$  of  $\sim$ 30 min; unpublished data) limits the opportunity for unwanted biodistribution. As an additional precaution, a tourniquet (ACTIS venous flow controller; Vivus, Mountain View, CA) was placed at the base of the penis before injection of the gene product and remained in place for 30 min to ensure that the vector was limited largely to the penis.

## RESULTS

#### Disposition, demographics, and baseline characteristics

This study was conducted from May 2004 to May 2006. Fifteen men were screened for the study and 11 men fulfilled the criteria for enrollment and were given injections of *hMaxi-K*. Three men each were given the 500-, 1000-, and 5000- $\mu$ g doses, respectively, and two men were given the 7500- $\mu$ g dose. The mean age of the study population was  $59.0 \pm 10.6$  years (range, 42–80 years), six subjects were white, four subjects were black, and one subject was Hispanic (Table 2). The primary cause of ED in six of the subjects was diabetes and/or was cardiovascular related, two subjects had unspecified organic causes, one subject had fracture of the penis, one subject had a radical prostatectomy, and one subject was diagnosed as psychogenic (i.e., erectile domain score on IIEF of 12, but no discernible physical cause of ED). Subjects were taking concomitant medications appropriate to their diagnoses in addition to ED, for example, hypertension, diabetes, and hypercholesterolemia medications. The duration of ED ranged from 1 to 20 years. Five subjects had previously taken tadalafil (Cialis) or sildenafil (Viagra), seven subjects had undergone prior intracavernous therapy, and one subject had used alprostadil (Muse).

TABLE 2. PATIENT BASELINE CHARACTERISTICS

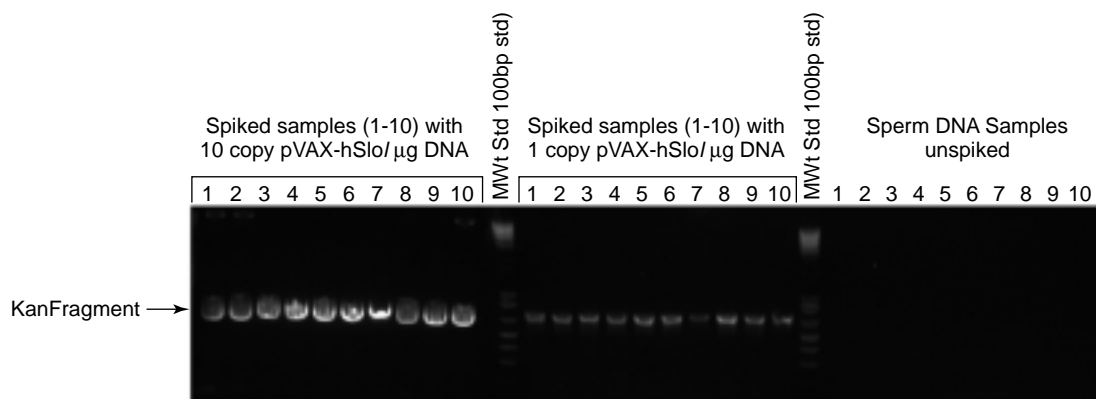
| Patient number | <i>hMaxi-K</i> dose ( $\mu$ g) | Age <sup>a</sup> (years) | Race     | Primary cause of ED                     | Duration of ED (months) | Severity <sup>b</sup> of ED (IIEF-EF <sup>c</sup> ) |
|----------------|--------------------------------|--------------------------|----------|---|-------------------------|---|
| 003            | 500                            | 56                       | White    | Organic                                 | >60                     | Severe (1)  |
| 005            | 500                            | 80                       | White    | Hypertension                            | 37–60                   | Severe (9)  |
| 006            | 500                            | 60                       | White    | Diabetes                                | 37–60                   | Moderate (11)                                       |
| 009            | 1000                           | 46                       | Black    | Organic                                 | 37–60                   | Severe (1)  |
| 251            | 1000                           | 65                       | Hispanic | Atherosclerosis                         | 13–36                   | Severe (8)  |
| 252            | 1000                           | 65                       | White    | Fracture of penis                       | >60                     | Severe (8)  |
| 011            | 5000                           | 64                       | Black    | Diabetes and hypercholesterolemia       | 37–60                   | Severe (9)  |
| 012            | 5000                           | 49                       | Black    | Diabetes and hypertension               | >60                     | Severe (1)  |
| 254            | 5000                           | 64                       | White    | Hypertension                            | 37–60                   | Severe (6)  |
| 013            | 7500                           | 58                       | White    | Pelvic surgery and radial prostatectomy | 13–36                   | Severe (9)  |
| 016            | 7500                           | 42                       | Black    | Psychogenic                             | 13–36                   | Moderate (12)                                       |

Abbreviations: ED, erectile dysfunction; EF, erectile function.

<sup>a</sup>Mean age,  $59 \pm 10.6$  years.

<sup>b</sup>Severe (EF 6–10), moderate (EF 11–16), mild to moderate (EF 17–21), mild (22–25), no ED (EF 26–30) (Cappelleri *et al.*, 1999).

<sup>c</sup>Mean IIEF,  $6.8 \pm 4.05$ .



**FIG. 4.** Analysis of total DNA extracted from sperm for the presence of pVAX1-*hSlo*. Plasmid DNA was extracted from sperm, using a Qiagen (Valencia, CA) total DNA extraction kit according to the manufacturer’s instructions. Five milligrams of total DNA was subjected to PCR 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 (KD); lanes 2–4, treated patient (RDS/252), samples taken on 02/02/05 (repeat), 4/19/05, and 7/14/05; lanes 5–7, treated patient (PH/011), samples taken on 5/24/05, 5/31/05, and 7/12/05; lanes 8 and 9, patient (6-C/251), samples taken on 3/9/05 and 5/4/05; lane 10, water (negative control).

The mean baseline IIEF-EF score was  $6.8 \pm 4.05$ ; nine subjects were categorized as having severe ED and two subjects as having moderate ED according to standard classifications (Cappelleri *et al.*, 1999).

*Semen testing by PCR*

Figure 4 represents typical results of analysis of total DNA extracted from sperm samples for the presence of the 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 *h*Maxi-K in semen down to a

level of 1 copy/μg of total DNA in any participant at any of the visits.

*Primary study end point: safety*

Table 3 is a summary of adverse events reported by patients during the study. 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

TABLE 3. ADVERSE EVENT SUMMARY BY DOSE

|  | Dose (μg)      |                 |                 |                 | Total     |
|--|----------------|-----------------|-----------------|-----------------|-----------|
|  | 500<br>(n = 3) | 1000<br>(n = 3) | 5000<br>(n = 3) | 7500<br>(n = 2) |           |
| Patients reported at least one 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 events.

TABLE 4. CHANGE FROM BASELINE IN KEY BLOOD CHEMISTRY AND ENDOCRINE TESTS

|                                 | <i>n</i> | <i>Baseline (mean)</i> | <i>Week 24 (mean)</i> | <i>Mean change from baseline (±SD)</i> |
|---------------------------------|----------|------------------------|-----------------------|--|
| ALT/SGPT (IU/liter)             | 9        | 28.9                   | 30.8                  | 2.78 ± 18.21                           |
| AST/SGOT (IU/liter)             | 10       | 23.8                   | 27.2                  | 3.70 ± 8.38                            |
| Alkaline phosphatase (IU/liter) | 10       | 72.0                   | 68.9                  | -1.70 ± 25.55                          |
| Glucose (mg/dl)                 | 6        | 116.5                  | 105.1                 | -18.3 ± 68.42                          |
| Cortisol (μg/100 ml)            | 9        | 10.9                   | 11.65                 | 0.72 ± 3.11                            |
| T <sub>4</sub> (μg/100 ml)      | 10       | 8.04                   | 8.21                  | 0.03 ± 1.65                            |
| TSH (mg/dl)                     | 11       | 1.47                   | 1.65                  | 0.17 ± 0.75                            |
| Testosterone (μg/100 ml)        | 10       | 484.0                  | 433.0                 | -41.1 ± 124.20                         |

Abbreviations: See Table 1.

lithotripsy were also classified as serious adverse events. One patient given 1000 μg reported acid reflux, sciatic pain, and an upper respiratory infection (URI) and one patient had a parasitic intestinal infection and foot edema. One patient given 5000 μg had bladder stone removal and 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.

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 electrocardiograms as determined by shift analysis (no normal to abnormal occurrences) were observed with the exception of the atrial flutter in one patient, considered unrelated to treatment.

Table 4 presents a summary of the change from baseline to the last study visit for key blood chemistry and endocrine tests. No clinically significant changes were seen in mean 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 as well as heart rates did not show notable changes over time in any

dose group. However, individual subject values varied from visit to visit and no clinically significant pattern of changes was evident. Therefore, no adjunctive therapies or changes in therapy were required.

#### Secondary study end point: efficacy

**IIEF results.** The mean IIEF-EF domain scores by visit are shown in Table 5. A decrease in mean scores at each dose was observed after 1 week. Mean scores for the two lower dose groups (500 and 1000 μg) fluctuated around the baseline score throughout the 24 weeks of the study. However, improvements in mean IIEF-EF scores were observed for the two higher dose groups beginning 2 weeks after transfer. Improvements were maintained in both groups through the 24 weeks of study. Figure 5 displays the IIEF-EF for each patient at each visit. The positive changes from baseline for most patients were small and did not indicate improvement by IIEF scoring. However, one patient given 5000 μg and one patient given 7500 μg showed notable improvement in IIEF-EF beginning 2 weeks after transfer. The IIEF-EF scores continued to improve (from severe to mild or to no ED) at 4 weeks, and the improvement was maintained through the 24-week study.

Table 6 displays the mean scores for the EF subdomain of the IIEF (items 3 and 4, penetration ability and maintenance

TABLE 5. MEAN ERECTILE FUNCTION DOMAIN<sup>a</sup> Score over Time by Dose Level

| <i>Dose level (μg)</i> | <i>Week 0 (visit 2)</i> | <i>Week 1 (visit 3)</i> | <i>Week 2 (visit 4)</i> | <i>Week 4 (visit 5)</i>  | <i>Week 8 (visit 6)</i>  | <i>Week 12 (visit 7)</i> | <i>Week 24 (visit 8)</i> |
|------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 500 ( <i>n</i> = 3)    | 7.0 ± 5.29              | 4.0 ± 3.00              | 5.7 ± 4.04              | 4.7 ± 2.52               | 4.3 ± 3.06               | 4.3 ± 3.51               | 2.3 ± 1.53               |
| 1000 ( <i>n</i> = 3)   | 5.7 ± 4.04              | 3.7 ± 3.79              | 5.0 ± 1.00              | 4.7 ± 1.53               | 6.0 ± 1.73               | 5.7 ± 4.16               | 6.7 ± 6.03               |
| 5000 ( <i>n</i> = 3)   | 5.3 ± 4.04              | 5.0 ± 2.00              | 9.0 ± 8.49 <sup>b</sup> | 17.5 ± 16.3 <sup>b</sup> | 16.0 ± 12.7 <sup>b</sup> | 10.7 ± 10.0              | 11.7 ± 11.9              |
| 7500 ( <i>n</i> = 2)   | 10.5 ± 2.12             | 7.0 ± 8.49              | 18.0 ± 17.0             | 18.0 ± 17.0              | 18.0 ± 17.0              | 15.5 ± 20.5              | 15.5 ± 20.5              |
| Combined total         | 6.8 ± 4.05              | 4.7 ± 3.77              | 8.6 ± 8.41              | 9.9 ± 10.4               | 9.9 ± 9.54               | 8.5 ± 9.32               | 8.5 ± 10.2               |

<sup>a</sup>EF = items 1, 2, 3, 4, 5, and 15 of the IIEF.

<sup>b</sup>*n* = 2.

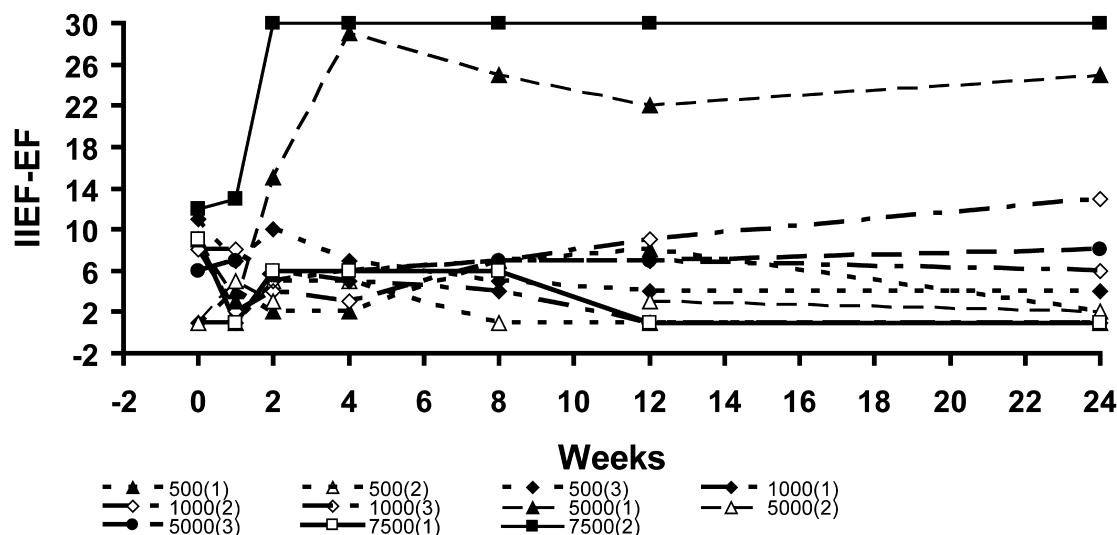


FIG. 5. hMaxi-K: Change in patient IIEF-EF (items 1, 2, 3, 4, 5, and 15): scores over time by dose for each patient.

frequency). Patterns similar to the EF domain results are seen for each dose group. Little change from baseline was noted in the two lower dose groups whereas mean scores for two higher dose groups improved and were maintained after 2 weeks. Figure 6 displays the subdomain scores for each patient at each visit. Again the patterns are similar to those for the IIEF-EF domain. One patient given the 5000- $\mu$ g dose and one given the 7500- $\mu$ g dose had clinically significant improvements in EF subdomain scores that were maintained for 24 weeks.

*Partner IIEF results.* Partner responses to items 3 and 4 of the IIEF were consistent and validated the responses of each patient at each visit (results not shown).

DISCUSSION

Gene transfer therapy is extremely promising for prevention of the effects of aging and provision of long-term treatment for genetic and acquired diseases. Gene transfer focused on ion channel therapy in the smooth muscle of organs such as the penis and bladder offer a promising new treatment strategy. This novel therapeutic approach may address limitations of current

therapies for diseases such as ED and overactive bladder disease. This first human trial of gene transfer therapy for ED evolved from a series of promising preclinical studies. These studies initially showed that transfer of the gene encoding the large-conductance, calcium-sensitive K channel subtype (*hSlo*) restored the age-related decline in erectile capacity of rats *in vivo* (Christ *et al.*, 1998; Melman *et al.*, 2003). A single intracavernous injection of “naked” pcDNA-*hSlo* (100  $\mu$ g), which encodes the  $\alpha$  subunit of the human Maxi-K channel, was associated with physiologically significant increases in magnitude of the cavernous nerve (CN)-stimulated intracavernous pressure (ICP) response that lasted for up to 6 months (Christ *et al.*, 1998; Melman *et al.*, 2003). Similar results were seen in a rat model of diabetes, where the effect was maintained for at least 4 months (Christ *et al.*, 2004). These studies provided evidence of the effectiveness and long-term duration of gene transfer, with no indications of safety issues.

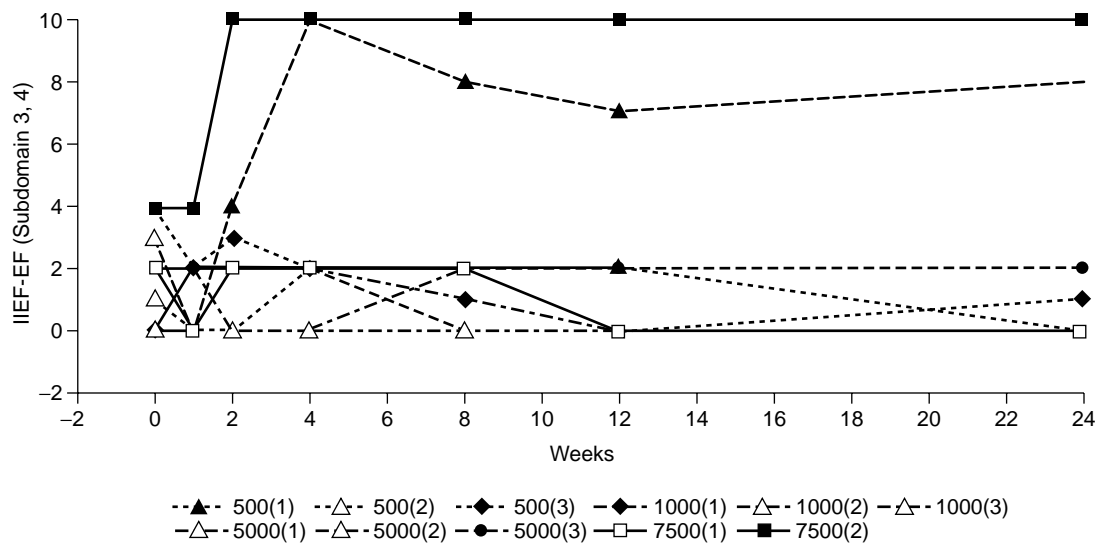
Unique anatomic and ultrastructural features of the penis may account for the positive *in vivo* gene transfer results. Gap junctions located in the membranes of apposing smooth muscle cells of the penis provide the ultrastructural feature that allows rapid cell-to-cell propagation of second messengers after cellular activation by a variety of vasoactive compounds (Christ *et al.*,

TABLE 6. MEAN ERECTILE FUNCTION SUBDOMAIN<sup>a</sup> Score over Time by Dose Level

| Dose level ( $\mu$ g) | Week 0 (visit 2) | Week 1 (visit 3) | Week 2 (visit 4)            | Week 4 (visit 5)            | Week 8 (visit 6)            | Week 12 (visit 7) | Week 24 (visit 8) |
|-----------------------|------------------|------------------|-----------------------------|-----------------------------|-----------------------------|-------------------|-------------------|
| 500 (n = 3)           | 1.7 $\pm$ 2.08   | 0.7 $\pm$ 1.15   | 1.0 $\pm$ 1.73              | 1.3 $\pm$ 1.15              | 0.7 $\pm$ 1.15              | 0.7 $\pm$ 1.15    | 0.3 $\pm$ 0.58    |
| 1000 (n = 3)          | 1.0 $\pm$ 1.73   | 0.7 $\pm$ 1.15   | 0.7 $\pm$ 1.15              | 0.7 $\pm$ 1.15              | 1.0 $\pm$ 1.00              | 0.0 $\pm$ 0.00    | 0.0 $\pm$ 0.00    |
| 5000 (n = 3)          | 1.3 $\pm$ 1.15   | 1.3 $\pm$ 1.15   | 2.0 $\pm$ 2.83 <sup>b</sup> | 6.0 $\pm$ 5.66 <sup>b</sup> | 5.0 $\pm$ 4.24 <sup>b</sup> | 3.0 $\pm$ 3.61    | 3.3 $\pm$ 4.16    |
| 7500 (n = 2)          | 3.0 $\pm$ 1.41   | 2.0 $\pm$ 2.83   | 6.0 $\pm$ 5.66              | 6.0 $\pm$ 5.66              | 6.0 $\pm$ 5.66              | 5.0 $\pm$ 7.07    | 5.0 $\pm$ 7.07    |
| Combined total        | 1.6 $\pm$ 1.57   | 1.1 $\pm$ 1.38   | 2.1 $\pm$ 3.14              | 3.0 $\pm$ 3.80              | 2.7 $\pm$ 3.47              | 1.9 $\pm$ 3.42    | 1.9 $\pm$ 3.59    |

<sup>a</sup>EF subdomain = IIEF items 3 and 4.

<sup>b</sup>n = 2.



**FIG. 6.** *hMaxi-K*: Change in patient IIEF subdomain (items 3 and 4): scores over time by dose Q3. When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner? Q4. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?

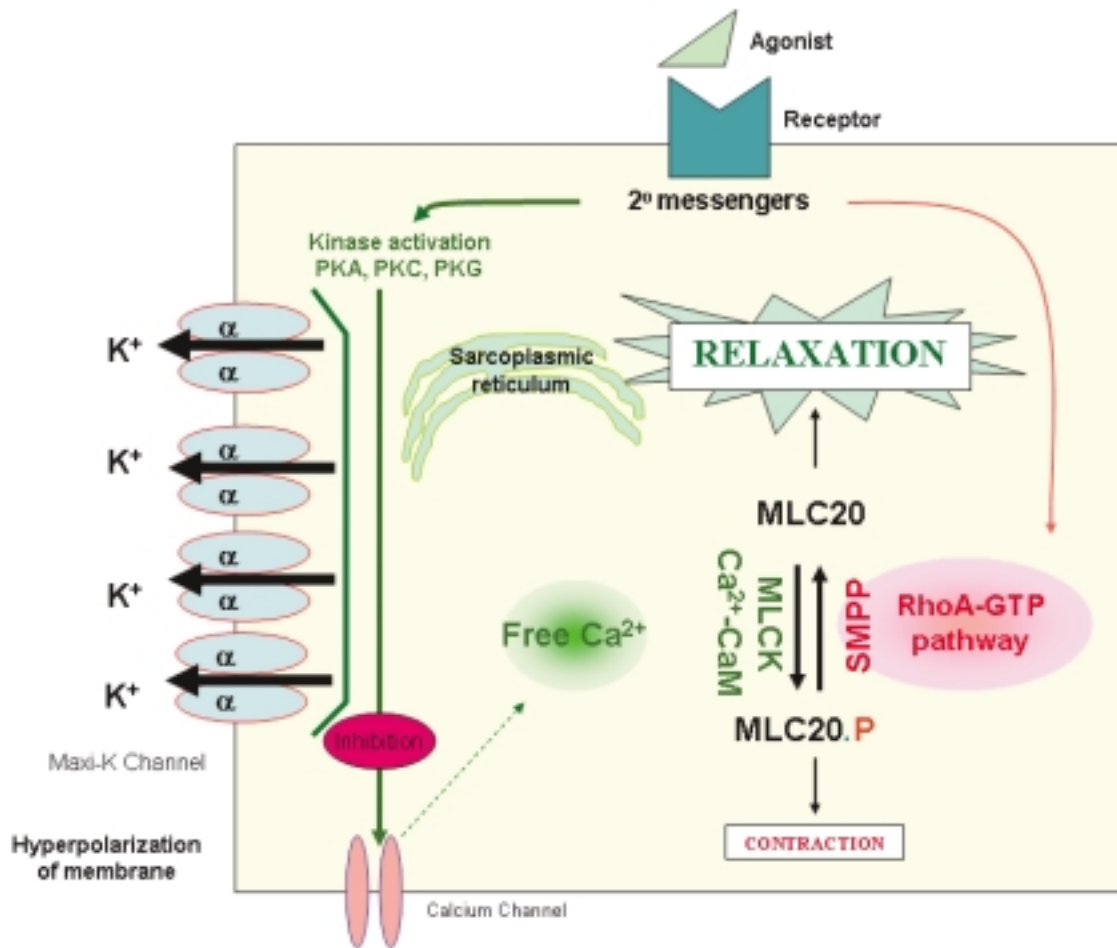
1993, 2006; Melman and Christ, 2001; Schultheiss, 2004; Melman *et al.*, 2005). The function of gap junctions in the penile corpora has been reviewed (Melman, 2006). In the presence of gap junctions, nonviral gene therapy offers an attractive opportunity to achieve appropriate therapeutic response while minimizing adverse effects. Potassium channels are critical for contraction and relaxation of the smooth muscle of the penis as well as other hollow organs such as the bladder, gut, and blood vessels. They alter membrane potential and excitability of smooth muscle cells by modulating  $Ca^{2+}$  influx through L-type voltage-dependent  $Ca^{2+}$  channels (Nelson and Quayle, 1995). 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 cells, which in turn determines the degree of smooth muscle cell contraction. The Maxi-K channel, that is, the large-conductance, calcium-sensitive K channel, is prominent among the K channel subtypes in corporal smooth muscle cells. Increased Maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. The need for rapid, robust, and syncytial corporal smooth muscle cell relaxation to obtain and maintain an erection is the basis for the use of K channel therapy in the treatment of ED (Andersson, 2000, 2001; Karicheti and Christ, 2001). Intracorporal administration of *hMaxi-K* presumably increases expression of the Maxi-K channel in smooth muscle cells, which, with appropriate stimuli, will generate increased efflux of  $K^+$  across the cell membrane, resulting in enhanced cellular hyperpolarization and, thus, decreased entry of  $Ca^{2+}$  ions. Expression of *hMaxi-K* in the membranes of smooth muscle cells after gene transfer is illustrated in Fig. 7. The enhanced effect on the outward  $K^+$  currents across the smooth muscle cells allows the smooth muscle of the corpora to relax and, in turn, the corporal sinusoids to become engorged with blood, and the penis to become rigid. When the sexual/neural stimulation is terminated the  $K^+$  efflux also stops and accounts for

the lack of priapism observed in animal studies (Melman *et al.*, 2003).

The highly significant preclinical evidence showing the safety, effectiveness, and long duration of action of *hMaxi-K* led to the design and approval of this study as the first human trial of gene transfer therapy for ED.

The primary objective of this single dose escalation study was to determine whether single escalating doses of *hMaxi-K* given to men with ED would be tolerated and safe. Thus the most important finding of the study is that single injections of *hMaxi-K* at doses of 500, 1000, 5000, and 7500  $\mu\text{g}$  were well tolerated and safe on injection and 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 genitourinary), hematology, chemistry, and hormone analyses, or in cardiac events evaluated by repeated ECGs (one patient with preexisting atrial arrhythmia had a recurrence approximately 1 month after dosing). No plasmid was detected in the semen of patients at any time after the injections. This is an extremely important finding because germ line transmission is a major concern in gene transfer therapies. Localization of the plasmid to the penis conserves the advantage of using the naked DNA vector, that is noted for a lack of potential immunogenicity.

Conclusions about efficacy cannot be drawn from the results of phase I trials without randomized controlled groups. However, efficacy measurements were made at each study visit and may provide insight into potential clinical activity. The IIEF is the standard instrument accepted as the best measure of efficacy in ED clinical trials. Patients given the two highest *hMaxi-K* doses had apparent sustained improvements in erectile function indicated by improved scores in the IIEF-ED domain over the length of the study. One patient in the 5000- $\mu\text{g}$  group and one in the 7500- $\mu\text{g}$  group reported relatively equivalent EF improvements at the two doses that approached the no-ED IIEF-



**FIG. 7.** Illustration of a representative cell into which the  $\alpha$  subunit of the Maxi-K channel has been transferred. Additional Maxi-K channels are shown inserted in the cell membrane. The  $\beta$  subunit is not shown as at present we have not determined whether there is sufficient  $\beta$  subunit to associate with the plasmid-derived Maxi-K channel. In the presence of an appropriate neural signal, the potassium channels open, hyperpolarize the cell, and inhibit the influx of calcium ion, thus causing the cell to relax (Melman, 2006). CaM, calmodulin; MLC20, myosin subunit, 20-kDa regulatory light chain; MLC20.P, phosphorylated MLC20; MLCK, myosin light chain kinase; PKA, PKC, PKG, protein kinase A, C, and G, respectively; SMPP, smooth muscle myosin phosphatase.

EF score and they maintained these improvements for 24 weeks. IIEF-EF subdomain scores (penetration and maintenance) were consistent with the primary EF domain results and partner self-reports confirmed patient improvements. In this trial the participants were not blinded as to their treatments, and the improvement in IIEF score may have been a consequence of their belief in the effectiveness associated with the treatment. Thus the preliminary results indicate that gene transfer with *hMaxi-K* has significant potential as a therapy for patients with ED. The intriguing results of preclinical studies also suggest that the therapy may be effective in ameliorating the ED associated with diabetes and aging. Overall, the results indicate 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 with ED. In addition, these positive results of the first *hMaxi-K* gene transfer therapy in patients provide promise for the possibility of a platform technology application of this novel approach to

other smooth muscle-related diseases such as overactive bladder (i.e., detrusor overactivity), irritable bowel syndrome, and asthma.

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Address reprint requests to:  
 Dr. Arnold Melman  
 Department of Urology  
 Albert Einstein College of Medicine/Montefiore Medical Center  
 Room 744, Forchheimer Building  
 1300 Morris Park Avenue  
 Bronx, NY 10461

E-mail: amelman@montefiore.org

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