A Double-Blind, Randomized Study of the Efficacy of OnabotulinumtoxinA (onaBoNT-A) versus Oral Tamsulosin in Men with BPH and LUTS (Protocol # 02-10-10-05)

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PRINCIPAL INVESTIGATOR: Christopher P. Smith, MD
Associate Professor
Scott Department of Urology
Baylor College of Medicine
Houston, TX 77030

CO-INVESTIGATORS: Gustavo Ayala, M.D.
Professor
Vice Chair for Outreach
Distinguished Chair in Pathology and Laboratory Medicine
University of Texas Health Science Center Medical School
Houston, Texas

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This clinical research protocol will be conducted in accordance with FDA, ICH and IRB regulations and guidelines. The Scott Department of Urology complies fully with the HIPAA guidelines.

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I. BACKGROUND

BPH and LUTS: Benign prostatic hyperplasia (BPH) with its related symptoms is a common condition that affects nearly half of men over age 50 and 90% of men over 80.[1] Lower urinary tract symptoms (LUTS) caused by BPH which include nocturia, frequency, urgency, hesitancy, intermittency and incomplete emptying, can be very bothersome, affect an individual's lifestyle significantly, and are costly.[2] The anatomical location of the human prostate and the urethra lend itself to the development of LUTS, including both obstructive and irritative symptoms. The initial evaluation of BPH/LUTS includes administration of the seven-item American Urological Association Symptom Index (AUASI) which evaluates the presence and severity of the main components of LUTS. The importance of symptoms in the pathophysiology of BPH is underscored by the fact that symptom measurements comprised 78% of all endpoints from the recent Medical Treatment of Prostate Symptoms (MTOPS) trial, compared to 22% of endpoints for objective measures such as urinary retention and urinary tract infection.[3] BPH, in large part, is a neural phenomenon given that sensory symptoms such as frequency, urgency, and nocturia are the key factors that push patients to seek treatment. Increasing basic and clinical evidence suggests that neural pathways within the prostate modulate bladder storage function and may play a significant role in LUTS.

Prostate Innervation**:** The prostate is dually innervated by the sympathetic and parasympathetic nervous systems.[4] Sympathetic fibers arise from the thoraco-lumbar cord (T10-L2) and course within the hypogastric nerve as either presynaptic neurons to terminate on short post-ganglionic adrenergic fibers near the prostate or as post-ganglionic neurons via the sympathetic chain to terminate directly on prostatic smooth muscle. The majority of norepinephrine containing nerves occurs in the thick stromal smooth muscle layer around the prostatic ducts and acini. Parasympathetic fibers arise from the lumbo-sacral cord (L5-S1) and course within the pelvic nerve to synapse on post-ganglionic fibers within peri-prostatic ganglion. Parasympathetic, cholinergic fibers localize to the prostatic epithelium, where their main function is thought to involve regulation of prostatic secretions. Recent data demonstrate a robust cholinergic innervation within the human prostate with muscarinic receptor densities exceeding those of alpha-adrenergic receptors.[5] In addition, the sensory nociceptor TRPV1 has been localized in abundance within prostatic urethral mucosa and prostatic acini. Taken into context, there should be no doubt that prostate innervation plays a key role in the development and pathophysiology of LUTS.

Alpha-adrenergic Receptor Antagonists for BPH**:** While surgery remains the gold standard for relieving obstruction and symptoms, more recent attention has been focused on pharmacotherapy as an alternative approach. The American Urological Association recommends alpha-adrenergic receptor blockers as first-line pharmacologic therapy for male patients suffering from LUTS/BPH. Alpha-adrenergic receptor blockers antagonize adrenergic receptors, blocking the effects of norepinephrine released from sympathetic nerve terminals. The end result is reduced smooth muscle tone. Of the three known α-adrenergic receptors ($\alpha1_A$, $\alpha1_B$, and $\alpha1_D$), $\alpha1_A$ is the most abundant within the prostate, the $\alpha1_B$ subtype is predominant within peripheral blood vessels, and the $\alpha1_D$ receptor subtype is localized to the bladder and spinal cord. Numerous studies have documented a beneficial effect of alpha-adrenergic receptor blockers for improving LUTS and urinary flowrates.[6] However, use of alpha-adrenergic receptor blockers can be associated with serious cardiovascular and sexual side effects that contribute to the fact that one-third of men discontinue drug use despite bothersome LUTS.[7] In addition, alpha-adrenergic receptor blockers directly target sympathetic

pathways and ignore the contributory effect of parasympathetic innervation on prostate growth and LUTS.

Effects of Surgical Denervation: McVary and colleagues demonstrated that surgical denervation of the rat prostate induces significant changes in the size of the rat ventral prostatic lobe.[8] Removal of sympathetic innervation caused a marked atrophy of the ipsilateral ventral lobe. In contrast, physical denervation of parasympathetic input had no effect on the ipsilateral lobe but promoted marked hyperplasia of the contralateral non-denervated lobe. The investigators hypothesized that growth factors secreted as a result of parasympathetic denervation may have contributed to enlargement of the contralateral ventral lobe. The effect of altered neural input on prostate growth was evaluated in a population of partial and complete spinal cord injured men.[9] The study found no difference in prostate volume with increased age, a finding that contradicts observations from longitudinal studies in neurally intact males demonstrating that prostate size positively correlates with age. Given that no abnormalities in pituitary-gonadal axis were observed, it appears likely that loss of neural input resulting from SCI stunts prostate growth. These basic and clinical studies support further research evaluating the effect of targeted prostate denervation on male LUTS associated with BPH.

OnabotulinumtoxinA (onaBoNT-A) Mechanism of Action: In the search for more effective non-invasive therapies to treat BPH, the role of somatic and visceral neuromuscular activity in the manifestation of LUTS has been studied.[10] OnaBoNT-A (BOTOX, Allergan, Inc., Irvine, CA) was first introduced as a therapeutic agent in the late 1970's for strabismus; thereafter, onabotulinumtoxinA has been safely used in the treatment of numerous other disorders characterized by excessive or inappropriate muscle contraction.[11] The drug is administered for a wide variety of medical conditions including muscular dystonia and axillary hyperhidrosis, and in a number of urological conditions including voiding dysfunction. OnaBoNT-A injections have already been successfully carried out to treat BPH[12, 13] and other urologic disorders such as detrusor-sphincter dyssynergia, neurogenic detrusor over activity, chronic retention, chronic pelvic pain and motor and sensory urinary urge incontinence.[14] These studies demonstrate that the use of onaBoNT-A in genitourinary disorders does not have significant side effects. OnaBoNT's primary function is to inhibit the exocytosis of acetylcholine from the presynaptic nerve terminal. After binding to the nerve cell membrane at a specific acceptor/receptor site and internalizing into the cell, onaBoNT cleaves one or more specific SNARE proteins that lead to reduced neurotransmitter release. At greater doses, it can also block the release of other neurotransmitters including norepinephrine, substance P, calcitonin-gene related peptide and glutamate.[15]

Chemical Denervation of Prostate with onaBoNT-A: When injected into rat prostates, onaBoNT-A produces: (1) a generalized atrophy of the prostatic gland with no evidence of inflammatory infiltrate; (2) an increase in apoptosis with DNA fragmentation and formation of apoptotic bodies on Tunnel stain and decrease in proliferation; (3) a reduction in the proliferation of epithelial cells and; (4) a reduction in $\alpha 1_A$ adrenergic receptor but not androgen receptor expression.[16, 17] Silva and associates found that onaBoNT-A induced prostate atrophy in rats could be prevented with daily injection of the adrenergic agonist phenylephrine but not from daily administration of the cholinergic agonist bethanecol.[18] Their investigations suggest that the predominant mechanism by which onaBoNT-A induces prostate atrophy and apoptosis in the rat is by inhibiting sympathetic neural transmission. Atrophy and apoptosis has also been observed in prostate tissue of dogs and humans following onaBoNT-A injection although the selective effect

of onaBoNT-A on parasympathetic or sympathetic pathways has not been evaluated in humans.[19]

OnaBoNT-A: Effective Treatment for Male LUTS Refractory to Medical Therapy*:* The effect of onaBoNT-A on LUTS associated with BPH has been evaluated in several studies in men that have failed standard therapy with an alpha-adrenergic antagonist + 5α-reductase inhibitor. The first off-label use of onaBoNT-A to treat BPH in humans was reported by Maria et al in 2003.[13] In a randomized, placebo-controlled study, 30 men with symptomatic BPH were randomized to receive either saline or 200 units of onaBoNT-A. One hundred units of onaBoNT-A in 2 cc of saline or saline alone were injected into each lobe of the prostate through the perineum via a 22-gauge spinal needle with transrectal ultrasound guidance. Clinical improvement was evident within 1 month of treatment in the onaBoNT-A group. By 2 months, 13 patients in the treatment group (87%) versus 3 patients in the control group (10%) reported subjective BPH symptom relief ($p = 0.00001$). At 12 months, the International Prostate Symptom Score (IPSS) decreased by 62%, max urinary flowrate (Qmax) increased by 85%, post void residual (PVR) urine decreased by 85%, and prostate size (determined ultrasonographically) decreased by 61%. The PSA values were also reduced by 38%. This degree of improvement was remarkable considering the fact that most patients had severe baseline symptoms (average IPSS = 23) and the mean prostate size was large (i.e. 52 cc) prior to injection. No urinary incontinence or systemic side effects were reported over the 19.6 month follow-up period.

Several case series have looked at specific BPH patient subpopulations to determine the effectiveness of onaBoNT-A treatment. Kuo et al. treated 10 patients who were either in frank urinary retention or carried a large PVR. These patients had already failed combination medical therapy (5α-reductase inhibitor and alpha-blocker) were not surgical candidates due to comorbid medical conditions.[20] Two hundred units of BoNT-A was diluted in 10 cc of saline and was injected via a cystoscope into 10 sites in the prostate (4 sites on each lateral lobe, 2 sites at the median bar, 20 units per site). After onaBoNT-A injection, 7 of 10 patients (70%) could void spontaneously while 3 required intermittent self-catheterization for 2 weeks. The therapeutic effects were experienced as early as one week after injection. By 3 and 6 months, significant improvements in Qmax, PVR, and prostate volumes were noted (53%, 85%, and 24%, respectively). Eight of ten patients continued to use combination medical therapy after BoNT-A injection to maintain good symptomatic relief.

Chuang et al. stratified patients who had failed medical treatment based on the prostate size: those with <30 cc were treated with 100 units onaBoNT-A while those with >30 cc were treated with 200 units (perineal injection).[12, 21] At 12 months, the percent improvements in IPSS, Qmax, and PVR were very similar to those of Maria et al, except that the percent shrinkage of prostate size was substantially smaller (13-19% versus 61%). In 12 of 41 men (29%) there was no change in prostate volume, yet 7 of these 12 men (58%) still had a > 30% improvement in IPSS, Qmax, and PVR, suggesting that onaBoNT-A may relieve BPH symptoms in ways other than reducing the prostate size alone (i.e. effect on sensory nerve pathways).

As the table below indicates, a handful of clinical studies have been conducted to date measuring the efficacy of onaBoNT-A prostate injections to treat BPH. However, only two studies provide level one evidence of clinical improvement from onaBoNT-A prostate injection, although they strongly disagree on whether onaBoNT-A has any effect on serum PSA level or prostate volume. The remaining open-label studies do not conclusively support the results of one randomized trial over the other. Our study will provide further level one evidence to either

support or refute the hypothesis that prostate denervation with onaBoNT-A reduces serum prostate levels and induces significant atrophy and prostate shrinkage.

Investigator	Study type, N	Symptom Score Decrease	PSA Decrease	Prostate Volume Decrease	Qmax Increase	Durability
Maria et al	Randomized N=30 200U	62%	51%	68%	90%	12 months
Brisinda et al.	Open Label N=77 200U	64%	58%	55%	92%	30 months
Kuo et al.	Open Label N=10 200U	NA	NA	30%	53%	6 months
Chuang et al.	Open Label N=16 100U	61%	NA	16%	73%	6 months
Chuang et al.	Open Label N=21 100U	62%	NA	19%	70%	12 months
Chuang et al.	Open Label N=20 200U	73%	NA	17%	70%	12 months
Silva et al.	Open Label N=21 200U	Indwelling Cath	24%	40%	Indwelling Cath	12-18 months
Crawford et al.	Randomized N=125 100U, 300U	38%, 46%	0%	0%	25%, 27%	12 months

No Effective Clinical Marker to Explain LUTS*:* The improvement in LUTS following onaBoNT-A injection appears unrelated to baseline prostate size or to change in prostate size following treatment. This observation is consistent with prior studies demonstrating a weak correlation between the symptoms of LUTS and prostate size, whether in men diagnosed with BPH or men in the general community over the age of 50 years.[22-24] While 5-α-reductase inhibitors (i.e. which reduce prostate volume) have virtually no benefit at relieving LUTS[25], drugs that do not impact prostate volume (i.e. alpha blockers, phosphodiesterase inhibitors, and anticholinergics) are often more effective in relieving LUTS.[26-28] Thus, prostate size does not equate with LUTS. Because LUTS includes a complex assortment of symptoms affecting urine storage, voiding and micturition, Bladder Outlet Obstruction (BOO) was linked with LUTS. Urological dogma reinforced the theory that BOO was linked with LUTS because of the observation that urinary flow decreases and LUTS increases with age.[26] Moreover, BOO can be present irrespective of prostate size. However, epidemiologic studies reveal a weak correlation between the severity of BOO and LUTS.[22-24] In addition, changes in urinary flow rate and changes in LUTS following alpha blocker therapy and TURP do not strongly correlate providing further evidence that the mechanism for symptom improvement following these treatments is not related to simply relieving BOO.[29, 30] Finally, phosphodiesterase inhibitors improve LUTS without having any

effect on flow rate giving further credence to the notion that neither the pathophysiology of BPH nor symptom improvement is dependent on relief of BOO.[27] The lack of correlation between clinical and subjective measures of LUTS/BPH demands the exploration of novel biological markers and molecular targets that not only are associated with moderate to severe LUTS but which respond appropriately to treatment and symptom resolution.

Gene Profile Changes with BPH**:** Gene expression patterns in BPH are distinct from those found in normal and cancerous prostate tissue.[31] Investigators have categorized BPH tissue based on clinical symptoms in an effort to identify genes that may be associated with a higher risk of symptomatic BPH. Changes in gene expression with symptomatic BPH have been segregated into 5 main categories: 1. Growth regulatory genes; 2. Immunologic/Inflammatory genes; 3. Transcription Factors/ Cell Signalling genes; 4. Stromal Component Genes; and 5. Hormone genes.

Bauman and colleagues reported on transcript profiling of proteins associated with androgen signaling in epithelial and stromal culture models from normal and BPH prostates.[32] While no significant differences in androgen signaling were noted between BPH and normal epithelial cells, dramatic differences suggesting an increased androgen response were noted in BPH compared to normal stroma cells.[32] The investigators noted an almost tripling of the AR:ERβ ratio in BPH stroma as well as significant increases in RL-HSD and a decrease in the ratio of AKR1C1:AKR1C2. These factors all point to the heightened production of DHT as well as the decreased metabolism of DHT to the proaptotic ligand 3-β diol.

Significant changes in genes that encode immunological regulation have also been described in men with BPH and LUTS including B-cell homing chemokine (i.e. 36 fold increase from normal), and MHC class II DP beta-1 (i.e. 5.5 fold increase compare to normal).[33] Increases in expression of cytokines such as IL-1-alpha, -2, -4, -8, -15, -17 as well as decreases in the level of the macrophage inhibitory cytokine-1 gene (MIC-1), pre-B-cell colony enhancing factor, and interferon-stimulated gene have been reported as well.[33-35] More recently, investigators have evaluated the molecular mechanisms of BPH in a rat model of phenylephrine induced histomorphologic BPH. The study demonstrated a significant involvement of inflammatory pathways, in particular those involving activation of the TGF-beta signaling cascade.[36]

Growth regulatory genes are thought to play an important role in the pathogenesis of BPH, particularly in epithelial cell growth and regulation. Investigators have found a number of these genes to be upregulated in BPH: 1. Neural epidermal growth factor-like 2 (nel-like 2) (i.e. 6-fold); 2. Early growth response 1 (i.e. 6-fold); 3. Insulin-like growth factor binding protein 10 (i.e. 5-fold); as well as significant increases in stromal expression of Insulin Growth Factors (IGF-2), Insulin Growth Factor Receptors (IGF-I-R), and Fibroblast Growth Factors (FGF-2).[33, 37] Finally, cell signaling genes noted to be more expressed in the prostates of symptomatic BPH patients include an increase in v-fos FBJ murine osteosarcoma viral oncogene homolog (i.e. 13 fold), an increase in mitogenic activated protein kinases (i.e. ERK, p-38), an increase in bcl-2 (anti-apoptotic factor), and an increase in the cell senescence marker SA-β-gal (epithelial).[33, 38, 39]

Although the relative significance of each of these changes in gene expression remains to be determined, we hypothesize that Gene expression profiling will allow us to assess the impact of Tamsulosin or onaBoNT-A on prostate cells. Systematic studies of gene expression in prostate tissues after treatment with onaBoNT-A or targeted blockade of adrenergic pathways with Tamsulosin should help us understand how these agents ameliorate BPH induced symptoms and identify better molecular targets to treat LUTS. Ultimately, the translational goal would be to

provide symptom relief beyond simplistically targeting a reduction in prostate size or relieving BOO since the mechanisms responsible for either treatment response remains to be determined.

II. PRELIMINARY STUDIES

Clinical: Our site was one of 7 centers involved in a 12-week Phase II trial of 100 and 300 units of onaBoNT-A for the management of BPH (Table 1).[40] A total of 134 men were enrolled and treated (68 at dose 100 U and 66 at dose 300 U). One hundred twenty five men provided complete primary outcome data (63 at dose 100 U and 62 at dose 300 U). A positive effect of onaBoNT-A was characterized as a ≥ 30% change from baseline in AUA Symptom Score (AUASS) and/or Qmax. Both arms passed the efficacy criteria: 73% passed at the 100 U dose and 81% passed at the 300 U dose (Table 1; yellow shading = pass efficacy criteria). Interestingly, no significant changes in Total Prostate Volume (TPV) or Transition Zone Volume (TZV) in the combined data of 100 U and 300 U (i.e. TPV increased slightly from 49.7 cc to 50.1 cc and TZV increased slightly from 24 cc to 24.4 cc) were observed. In addition, PSA values did not change significantly as a result of onaBoNT-A treatment (i.e. increased non-significantly from 2.4-2.7 at 12 weeks follow-up).

	100 Units		300 Units	
	Qmax fail	**Qmax pass**	**Qmax fail**	**Qmax pass**
AUA SS **fail**	17 (27%)	10 (16%)	12 (19%)	8 (13%)
AUA SS **pass**	20 (32%)	16 (25%)	27 (44%)	15 (24%)

Table 1: Results of 12-week Phase II clinical trial

Our results are consistent with other clinical studies of onaBoNT-A in BPH although the 3-month improvements in AUASS (39.1% for 100 U dose; 42.6% for 300 U dose) and Qmax (25.6% and 28.5%, respectively) demonstrated in our study were smaller than those described in prior studies (i.e. AUASS decrease 61-73% and Qmax increase 53-92%).[12, 13, 20, 21]

A decrease in prostate size that is known to occur following physical or chemical (i.e. onaBoNT-A) denervation in rats was not observed within this study.[8, 16] These results suggest that the mechanism of action of onaBoNT-A to improve LUTS in men with BPH is multi-factorial and not solely related to its effects on altering parasympathetic or sympathetic neural input. Thus, additional directed investigation into the mechanism of action for onaBoNT-A in prostate tissues is appropriate, particularly in view of clinical evidence in human bladders that onaBoNT-A injection can modulate both the expression of sensory nerve receptors and the release of sensory neurotransmitters and nerve growth factor.[41-43]

Basic: The following experiments were conducted in a rat model to examine the effects of chemical denervation (i.e. onaBoNT-A injection) or surgical denervation (i.e. Major Pelvic Ganglion (MPG) dissection) on gene expression profiles of different compartments of the prostate in a prostate cancer model. These results are proof of principle that validate our ability to measure changes in gene expression of prostatic epithelial and stromal cells following chemical denervation with onaBoNT-A. We will use the same methods in human prostate tissue to identify genetic changes occurring with BPH/LUTS development and after successful treatment of LUTS with either Tamsulosin or onaBoNT-A.

Reduction of tumor size by prostatic denervation: To determine the functional significance of innervation within the prostate and in PCa, we used an orthotopic rat animal model. Transgenic mice do not survive the complications caused by prostatic denervation (i.e. neurogenic bladder and rectum). The experiments have been performed as a component of the Tumor Microenvironment Network. Athymic NIH-RNU rats were denervated mechanically through removal of the male pelvic ganglion (MPG). This technique has been widely used in rats [49]. Rat prostates were also denervated chemically through the use of BOTOX®. VCaP cells were injected orthotopically into the prostates one week after the denervation procedures. Control animals were injected at the same time. Animals were followed for 7 weeks. Of the 8 animals per group, we only lost one rat from the pelvic ganglion denervation arm. Animals were sacrificed and full autopsies performed. The prostates were halved and one section was formalin-fixed and paraffin-embedded, while the other was frozen. Hematoxylin and eosin-stained sections were obtained from both halves. These were imaged and the tumors mapped and quantified using J Image. The results suggest that nerves are functionally required for tumor growth in this orthotopic rat model. While large tumors were present in 50% of the control arm, only 1/8 were identified in the BOTOX® group and 2 in the pelvic ganglion denervation group. The tumors were much smaller in the denervated group (Fig. 1). To evaluate gene expression changes in normal epithelial, stromal, and PCa tissues after denervation, we laser-captured each cell type and performed RNA expression array analysis. Cluster analysis showed that BOTOX® chemical and physical denervation had similar gene profile effects on all compartments studied (Fig. 2). Our results demonstrate that denervation of the prostate has profound effects on both the stromal and epithelial compartments of the prostate.

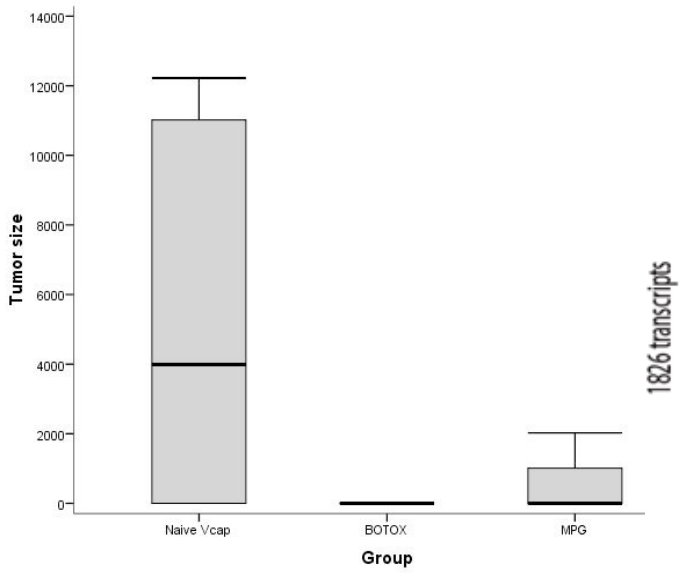

Figure 1: The size of tumors in rats with mechanical MPG and Botox denervation is smaller than sham operation controls.

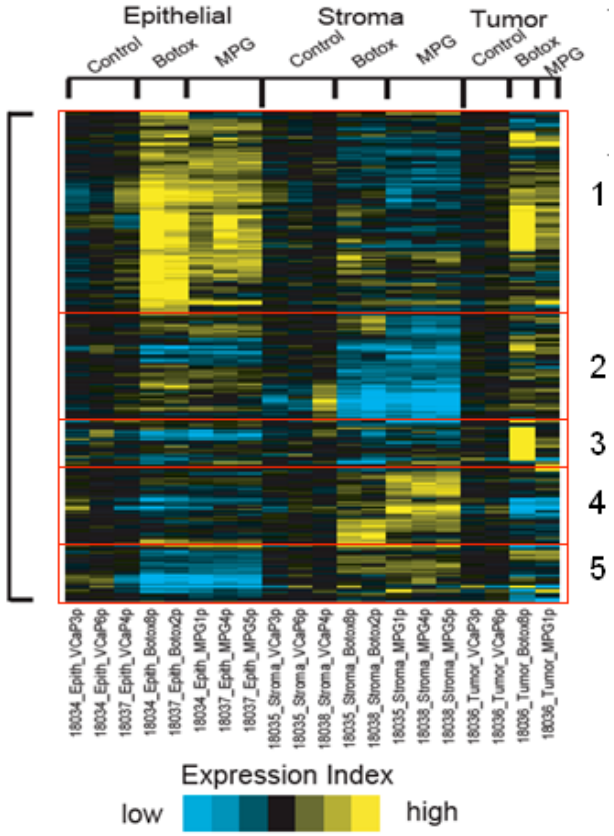

Figure 2: Unsupervised clustering of gene expression of normal epithelial and stromal, and PCa tissue in denervated and control mice.

Denervation Effects on Non-Neoplastic [Rat Prostate] Epithelium: To determine the effects of denervation on the non-neoplastic epithelium we laser captured the viable non-cancer epithelial cells away from the tumors in both the treated and control animals. We extracted RNA and performed gene array studies. Initial gene ontology studies of genes downregulated in the denervated animals showed that major cellular processes were affected. These included translation and translation initiation factor activity (signal transducer and activator of transcription 3, eukaryotic translation initiation factor 4A1, eukaryotic translation elongation factor 1 gamma*, eukaryotic translation initiation factor 3, subunit B, C and F); ribosomes and structural constituents of ribosome's (ribosomal protein L22*, L5, L6, S17 and S6, mitochondrial ribosomal protein S14), and metabolic pathways (ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase). Other genes included proteasome (prosome, macropain) subunit, alpha type 5, protein tyrosine phosphatase domain containing 1, tyrosine kinase with immunoglobulin-like and EGF-like domains*, heat shock protein 1-like, cAMP responsive element binding protein 1, Forkhead box K2 and RING1 and YY1 binding protein*.

We validated successfully 12 out of 16 genes, marked previously with a "*". Validation was done on a second laser capture microdissection, as material from the first was utilized for array studies. The histologic translation of these findings is generalized atrophy, as seen in figure 3. There is significant reduction in cell height and secretions into the lumens, as previously demonstrated.[16] However, the phenotype was variable, and other patterns were noted. The second most seen pattern was apoptotic epithelial cells and collagenous nodules attempting to close down damaged acini (Figure 4). The latter phenotype can be explained by the upregulated gene profile pattern of these cells.

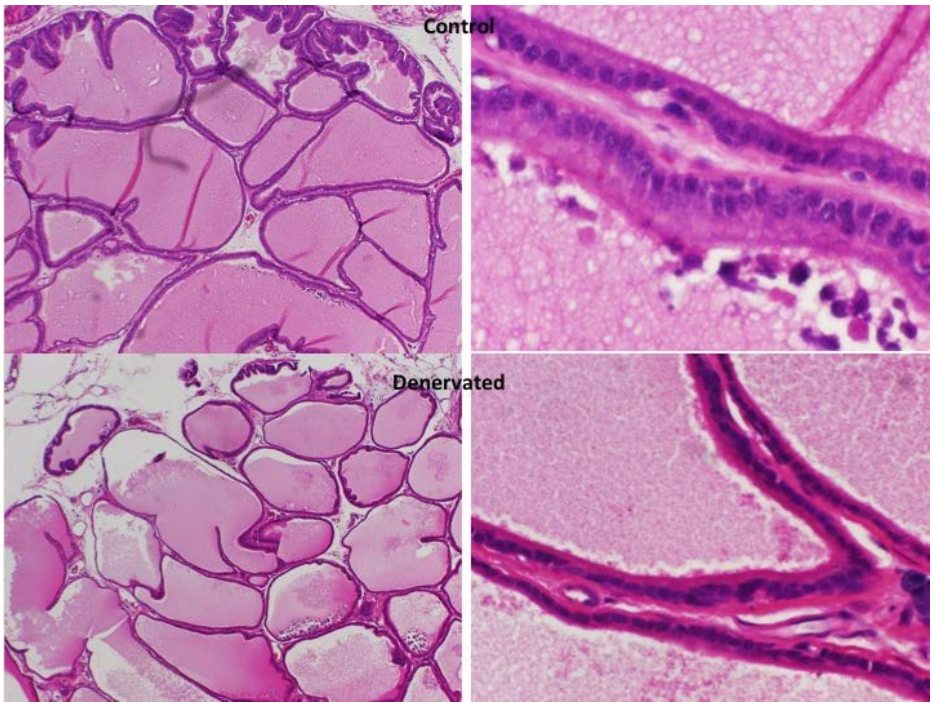

Figure 3: Effects of Saline or onaBoNT-A injection on rat prostate morphology. Note flattening of epithelium in onaBoNT-A treated tissues (denervated).

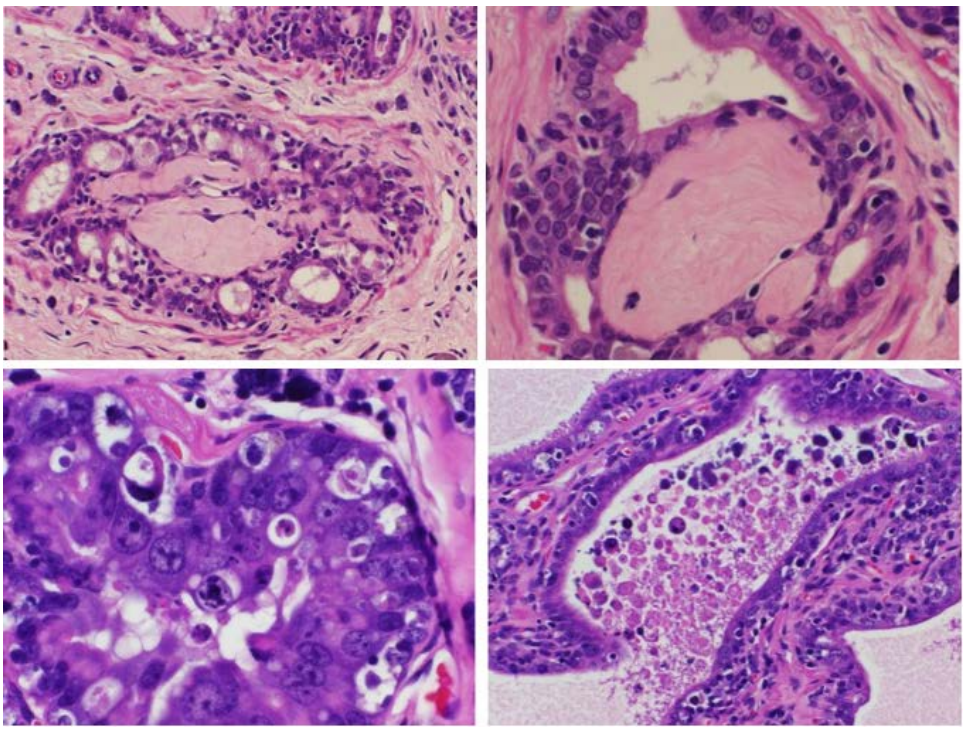

Figure 4: In the second most common histologic pattern observed, ona-BoNT-A treated prostate tissues demonstrated apoptotic epithelial cells and excessive collagenous deposits within acini.

We identified that the epithelial cells gene profile indicated an expected acute phase response, driven most likely by serum response factor, as this seems to be the most common motif that regulates this set of genes (SRF:Serum response factor (c-fos serum response element-binding transcription factor)). Endothelin* and endothelin receptors types A and B were among the most upregulated genes. Insulin I* and II* and insulin-like growth factor 1 and insulin-like growth factor binding protein 4 were also significantly upregulated. Numerous other growth factors were also upregulated including c-fos induced growth factor, connective tissue growth factor as well as epidermal growth factor receptor. Fas ligand (TNF superfamily, member 6) and tumor necrosis factor receptor superfamily, member 1b were also upregulated.

The epithelial cells acquired a more mesenchymal phenotype with production of multiple collagens (collagen, type XVI, alpha 1, collagen, type XIV, alpha 1, collagen, type III, alpha 1, collagen, type I, alpha 1), probably responsible for the collagenous nodules. Genes usually identified with stemness properties were also upregulated (noggin, secreted frizzled-related protein 4, wingless-related MMTV integration site 11, dickkopf homolog 4).

Denervation effects on Non-Neoplastic Human Prostate Epithelium: As an initial clinical translation and proof-of-concept study, we next evaluated tissues from three patients with prostate cancer enrolled in a Phase I clinical trial examining the effects of onaBoNT-A injections on prostate epithelial biology. Patients received unilateral injection with onaBoNT-A (100 units in 1.0 ml) into one transition lobe and a vehicle control injection (i.e. saline) into the contralateral lobe. Examination of tissue from radical prostatectomies performed 4 weeks after injection

revealed a significant or trend increase in the apoptotic ratio by TUNEL studies in two out of three patients (Figure 5). No significant changes were observed in the proliferation index, measured by Ki-67.

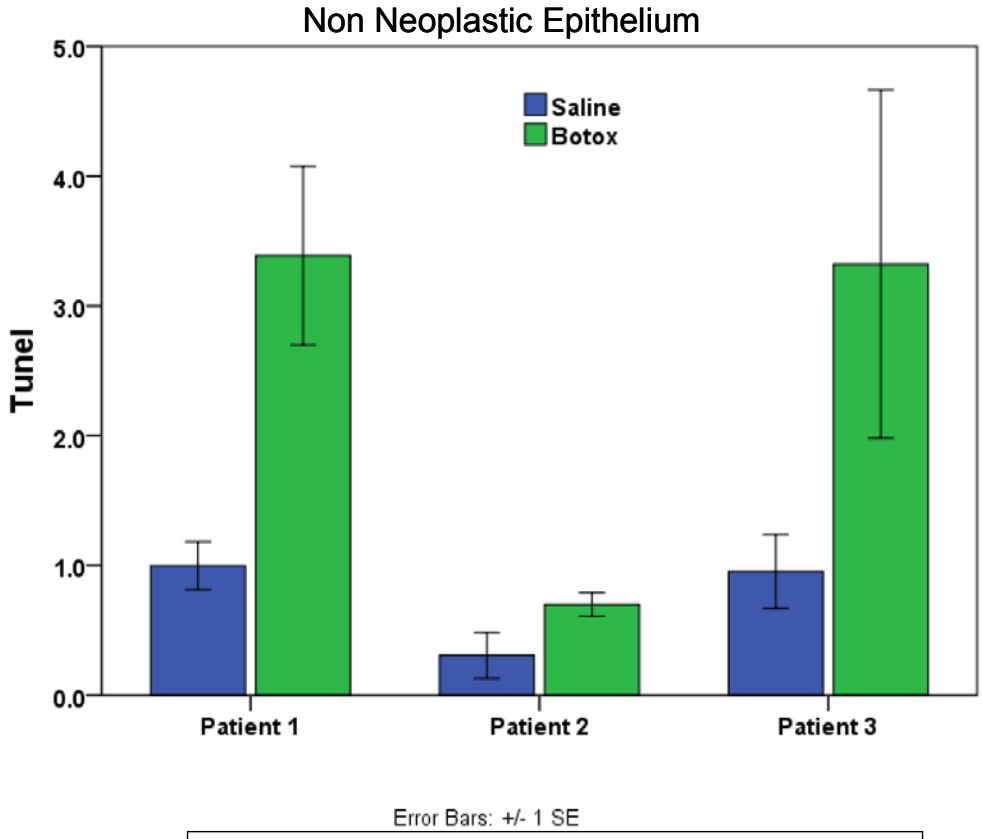

Figure 5. Effect of onaBoNT-A on human prostate epithelial tissue by TUNEL studies.

In summary, these studies suggest that nerves exert trophic effects on normal epithelial cells through the regulation of basic cellular processes such as gene expression, protein translation, and metabolism. The loss of nerve function induces a pattern of gene expression associated survival and wound repair as well as a general atrophic phenotype in all experimental denervation conditions. These studies provide proof of principle that nerves have the ability to affect cell growth directly.

Our ability to measure meaningful changes in gene expression profiles in an orthotopic rat animal model and in a small group of human patients following chemical denervation with onaBoNT-A substantiate the need to apply these same methods to a greater extent in human prostate tissue following injection with onaBoNT-A. Such studies would allow for the identification of potential molecular targets highlighted by the onaBoNT-A effect for future investigation.

III. SIGNIFICANCE AND RATIONALE

Our proposed intervention is the first randomized clinical trial comparing the effects of onaBoNT-A prostate injection versus alpha adrenergic antagonist medication for LUTS

associated with BPH. Up to this point, clinical studies using onaBoNT-A in the prostate has been limited to patient's refractory to α-1 adrenoceptor blocker therapy. Our study will directly compare onaBoNT-A against α-1 adrenoceptor blockers as frontline therapy in a male veteran cohort suffering from moderate to severe LUTS. Besides its obvious efficacy in patients' refractory to α-1 adrenoceptor blocker therapy, onaBoNT-A injection has several potential advantages over oral agents. Focal prostate injection has been shown to be safe and obviates the systemic side effects observed with α-1 adrenoceptor blockers (i.e. orthostatic hypotension, sexual dysfunction). In addition, most clinical studies demonstrate a durable response to onaBoNT-A treatment exceeding 12 months. Although this study is of modest length (i.e. total 4 years), significant results could drive paradigm shifts in how LUTS associated with BPH is treated, even with regards to frontline therapy.

Secondly, although sophisticated molecular techniques (i.e. LCM with Microarray Analysis) have been used by other investigators to characterize gene profile changes with BPH and LUTS, this will be the first study examining gene profile changes in BPH patients following treatment with the α-1 adrenoceptor blocker Tamsulosin or onaBoNT-A. This study is important because scant knowledge exists on the true mechanisms by which α-1 adrenoceptor blockers like Tamsulosin or onaBoNT-A improve patient urinary tract symptoms and quality of life. It is clear, however, that nerves not only regulate prostate growth and function but also account for LUTS that drive patients to seek therapy. This investigation will utilize onaBoNT-A as a biological tool to identify potential novel mechanistic pathways for future investigation that will push the development of targeted therapy to benefit those patients refractory to all pharmacologic treatment. Potential inflammatory pathways or neural sensory signaling alterations induced by BPH, which are modified by onaBoNT-A or Tamsulosin to improve symptoms via gene profile changes, can be explored by expert laboratories in the Texas Medical Center. This is a highly collaborative project utilizing expertise across departments that will foster translational work from the laboratory to the patient. Although not the primary goal of this study, we will also search for possible biological markers with prognostic value that could be confirmed in a future multi-center trial.

IV. OBJECTIVES

A Primary objective:

The primary objective of this Phase 2 clinical research study is to compare the efficacy of 200 U onaBoNT-A injected into the prostate versus oral tamsulosin for the treatment of lower urinary tract symptoms caused by BPH in male veteran volunteers at the Michael E. DeBakey Veterans Affairs Medical Center, Houston (MEDVAMC).

B Secondary objective:

The secondary objective is to determine the impact of tamsulosin and onaBoNT-A on the pathologic parameters and RNA profiles of epithelium and stroma in BPH tissues.

V. DRUG INFORMATON

A Botulinum toxin A is marketed in two pharmaceutical distinct formulations that are not equipotent in terms of mouse units. Differences in species sensitivities to different botulinum neurotoxin serotypes preclude extrapolation of animal-dose activity relationships to human dose estimates. For the purposes of this study, patients are

treated with the formulation onaBoNT-A which is marketed in the U.S. as BOTOX® by Allergan. OnaBoNT-A is a purified neurotoxin complex supplied as a sterile, vacuum dried purified botulinum toxin type A, produced from fermentation of Clostridium botulinum type A.

One unit of onaBoNT-A corresponds to the calculated median intraperitoneal lethal dose (LD 50) in mice. The specific activity of onaBoNT-A is approximately 20 units/nanogram of neurotoxin protein complex.

BOTOX® is the brand name for onaBoNT-A. For this study's treatment injections, we will be using a total of 200 units of BOTOX® in 4 cc of preservative free normal saline, or 4 cc of preservative free normal saline for placebo.

BOTOX® blocks neuromuscular transmission by binding to acceptor sites on motor or sympathetic nerve terminals, entering the nerve terminals, and inhibiting the release of acetylcholine. This inhibition occurs as the neurotoxin cleaves SNAP-25, a protein integral to the successful docking and release of acetylcholine from vesicles situated within nerve endings. When injected intramuscularly at therapeutic doses, BOTOX® produces partial chemical denervation of the muscle resulting in a localized reduction in muscle activity. In addition, the muscle may atrophy, axonal sprouting may occur, and extra-junctional acetylcholine receptors may develop. There is evidence that reinnervation of the muscle may occur, thus slowly reversing muscle denervation produced by BOTOX®.

BOTOX® is not expected to be present in the peripheral blood at measurable levels following IM or intradermal injection at the recommended doses. The recommended doses of neurotoxin administered at each treatment session are not expected to result in systemic, overt distant clinical effects, i.e., muscle weakness, in patients without other neuromuscular dysfunction. However, some clinical systemic effects have been shown by a single fiber electromyography after IM doses of botulinum toxins appropriate to produce clinically observable local muscle weakness.

BOTOX® is contraindicated in the presence of infection at the proposed injection site(s), and in individuals with known hypersensitivity to any ingredient in the formulation.

BOTOX® is supplied in a single-use vial. Each vial contains 100 units of vacuum-dried Clostridium botulinum type A neurotoxin components.

Unopened vials of BOTOX® should be stored in a refrigerator (2° to 8° C) for up to 24 months (or the expiration date on the product label). Administer BOTOX® within four hours of reconstitution and within 30 minutes of drawing into syringe; during this period the reconstituted BOTOX® should be stored in a refrigerator (2° to 8° C).

Additional BOTOX® information is available in the BOTOX® Prescribing Information package insert.

B Tamsulosin, marketed as Flomax®, is an alpha adrenoreceptor-blocker that is used to improve symptoms associated with an enlarged prostate (BPH). It works by relaxing muscles in the bladder and prostate. This may improve urine flow rates and decrease urinary symptoms such as hesitancy and urgency.

VI PHASE OF STUDY Phase 2

VII ELIGIBILITY CRITERIA

A Inclusion

1 Males at least 50 years of age
2 AUA Symptom Score greater than 8
3 Voided volume greater than125 ml
4 Maximum urinary flowrate less than 15 ml/sec.
5 Must agree to all procedures and willfully consented

B Exclusion

1 Any prior surgical intervention or use of 5-alpha reductase medical treatment for BPH
2 Current diagnosis of acute or chronic prostatitis (which may cause LUTS that mimic BPH)
3 Previous exposure to onabotulinumtoxinA
4 Overactive bladder without obstructive symptoms (i.e. decrease in force of stream, hesitancy, intermittency, post-void dribbling);
5 Active urinary tract disease or biopsy of the prostate within the past 6 weeks;
6 Two documented urinary tract infections of any type in the past year (UTI defined as greater than 100,000 colonies per ml urine from midstream clean catch or catheterized specimen)
7 Uncontrolled diabetes
8 History of bladder calculi (stones)
9 Penile prosthesis or artificial urinary sphincter [placement]
10 Documented bacterial or acute prostatitis within the past year
11 Episode of unstable angina pectoris, myocardial infarction, transient ischemic attack, or cerebrovascular accident (stroke) within the past 6 months
12 Known primary neurologic conditions such as multiple sclerosis, myasthenia gravis or Parkinson's disease, or other neurological diseases known to affect bladder function
13 History or current evidence of carcinoma of the prostate or bladder, pelvic radiation or surgery, urethral stricture, or bladder neck obstruction
14 Cancer that is not considered cured, except basal cell or squamous cell carcinoma of the skin (cured defined as no evidence of cancer within the past 5 years)
15 Any serious medical condition that is likely to impede successful completion of the study, such as certain mental disorders, hypersensitivity to onabotulinumtoxinA or anesthetics used in the study, syncope
16 Daily use of a pad or device for incontinence required
17 Interested in future fertility
18 PVR greater than 350 ml
19 Serum prostate specific antigen level greater than 8 ng/ml (Hybritech). For those with a PSA between 4-8 ng/ml, the PSA elevation must be considered to

be from a benign cause in the opinion of the PI. This decision can be based on PSA velocity, previous TRUS biopsy, percent free PSA, or other clinical estimations in keeping with sound urologic care

20 No alpha-blockers in the past 7 days before biopsy.

21 Has taken phenylephrine, pseudoephedrine, imipramine, an anticholinergic, or cholinergic medication within the past 2 weeks

22 Has taken estrogen, androgen, any drug producing androgen suppression, or anabolic steroids within the past 4 months

23 Is taking aminoglycosides or any drug that interfere with neuromuscular transmission. Eaton-Lambert syndrome, hemophilia, hereditary clotting factors deficiency, or bleeding diathesis

24 Must be off aspirin, NSAIDS, and Coumadin for 7 or more days prior to onabotulinumtoxinA injection

25 Enrolled in another treatment trial for any disease within the past 30 days

VIII DESIGN

This Phase 2 clinical research trial is a double-blind, randomized, placebo-controlled, parallel-group study to compare the treatment effects of onaBoNT-A 200 U versus 0.4 mg per day of oral tamsulosin in male veterans diagnosed with moderate to severe LUTS (AUASS equal to or greater than 8) associated with BPH. A total of 74 volunteers will be recruited to participate in this clinical trial. Volunteers will include only males who are greater than 50 years of age and diagnosed with LUTS associated with BPH. They are veterans who visit the MEDVAMC. There are no eligibility restrictions as to race or ethnicity.

Transition zone biopsies will be performed at Visit 2, 30 days before treatment, and again at 3 months post-treatment. A total of 6 biopsy cores (i.e. 3 cores from the left and right transition zone) will be obtained from each volunteer. Prostate biopsy cores will be rapidly snap frozen in liquid nitrogen in coded and labeled containers and sent to Dr. Gustavo E. Ayala's laboratory located at University of Texas Health Science Center - Houston Medical School for further processing.

A Randomization

Volunteers will be randomized using a blocked randomization approach by the MEDVAMC Research Pharmacy to either: ARM 1: onaBoNT-A 200 U prostate injection and placebo oral capsule daily or ARM 2: Placebo prostate injection (saline) and tamsulosin 0.4 mg capsule daily. Subjects will be randomized into one of the two treatment arms, using a block size of 4. The order in which the treatments are assigned in each block is randomized and this process is repeated for consecutive blocks of subjects until all subjects are randomized. This process ensures that after every fourth randomized subject, the number of subjects in each treatment group is equal. The MEDVAMC will prepare both pills (i.e. tamsulosin and placebo pill) and injections (i.e. onaBoNT-A and placebo saline injection) so that the study subject, investigator and all personnel treating or evaluating the subject will be blinded to treatment allocation.

B Schedule Of Events During Treatment Protocol

Tests/Time	Visit 1: Screening	Visit 2: Biopsy	Visit 3: Injection (4 weeks Post-biopsy ± 3 days)	Visit 4: Telephone Visit (Day 3 Post Prostate Injection)	Visit 5: Telephone Visit (1 Week Post-Injection ± 3 days)	Visit 6: (4 Weeks Post-Injection ± 3 days)	Visit 7: End of Study (3 months Post Injection ± 3 days)
Physical with DRE	X						X
Medical History	X						
Vital signs	X	X	X			X	X
Flowrate	X					X	X
PVR	X					X	X
PSA	X						X
CBC /platelet count	X						
Urinalysis (dipstick)	X	X	X			X	X
Biopsy(Transrectal Ultrasound Guided - TRUS)		X					X
Prostate Volume		X					X
Antibiotic		X	X				X
Fleets Enema		X	X				
Anesthesia (periprostate block)		X	X				
onaBoNT-A or saline injection via TRUS			X				
Informed Consent	X						
Antibiotic dispensing	X	X				X	
Instructions to stop alpha blocker meds 7 days prior to biopsy	X						
Drug/Placebo dispensing			X			X	
Drug/Placebo accountability						X	X
AUA Symptom score	X					X	X
BPH Impact Scale	X					X	X
Bladder Function	X					X	X
Erectile Function	X					X	X
Ejaculatory Function	X					X	X
Adverse Events			X	X	X	X	X
Concomitant Meds	X	X	X	X	X	X	X

C Procedures

1 Visit 1: Screening

- Informed consent will be obtained prior to any research-related procedures.
- A physical including a digital rectal exam (DRE) will be performed.
- The subject's medical history and concomitant medications will be discussed and documented.
- The subject's vital signs will be taken and documented.
- The subject will complete the following questionnaires:
- The AUA symptom score
- BPH Impact Scale

- Bladder Function
- Erectile Function
- Ejaculatory Function
- The subject will undergo uroflow testing and will provide a post-void residual urine measurement.
- Blood (about 10 ccs or 2 teaspoons) will be drawn for CBC with platelets and PSA testing.
- A urinalysis (dipstick) will be performed.
- Dispense a Bactrim DS (antibiotic) 160/800mg tablet orally twice a day [Cipro (generic is permissible) 500 mg twice a day] with instructions to take for 1 day prior to biopsy, day of biopsy, and for 1 day following biopsy (total of 3 days).
- Subjects will be instructed to stop taking alpha blocker medications 7 days prior to biopsy.

2 Visit 2: Biopsy

- The subject's concomitant medications will be discussed and documented.
- The subject's vital signs will be taken and documented.
- A urinalysis (dipstick) will be performed to ensure that no infection is noted or suspected.
- Dispense an antibiotic
- Prostate size is measured via transrectal ultrasound on patients after receiving a Fleet's enema the morning of the examination.
- Transition zone biopsies will be performed.

3 Visit 3: Injection (4 weeks post-biopsy, ± 3 days)

- The subject's concomitant medications will be discussed and documented.
- The subject's vital signs will be taken and documented.
- Any adverse events experienced by the subject will be documented.
- Injection Procedures: onaBoNT-A solution does not contain a preservative, it should be administered within 4 hours after reconstitution and within 30 minutes of drawing into the syringe. The drug should be stored in a refrigerator when not in use during this period.
  - Patient Preparation
    a. Patient is examined and submits urine for urinalysis which is examined by standard dipstick analysis or microscopic examination prior to randomization and preparing onaBoNT-A solution to ensure that no infection is noted or suspected.
    b. Prostate size is measured via transrectal ultrasound on patients after receiving a Fleet's enema the morning of the examination. The patient is placed in the lateral decubitus position with knees tucked against the chest and the transrectal probe introduced. A multi-planar probe that gives a 112° sector image is used to allow viewing in both transverse and longitudinal planes without moving the probe. The prostate size is measured initially using the height (H), width (W), and length (L) functions available on the ultrasound machine. Scanning of the prostate is carried out in two planes at

right angles to each other. The height and width are identified on a transverse image and recorded. The length is identified and recorded in the perpendicular longitudinal plane. The volume is calculated as a machine function using the formula H x W x L x π/6 and recorded on hard copy.

- OnabotulinumtoxinA or Saline placebo Injection
  a. Anesthesia is delivered according to the clinical practice of the center PI. Transrectal periprostatic block may be used with a 5 ml bolus of 1% lidocaine given just superiolateral to each seminal vesicle as visualized using the transrectal sound probe in the sagittal plane. The bolus is given via an appropriately long 22-gauge needle.
  b. The onabotulinumtoxinA toxin type A solution is given via the same approach using a second needle.
     i. For men randomized to the 200 unit onaBoNT-A arm, 50 units per 1.0 ml volume is given at the following points of the transitional zone: left and right inferior and superior transitional zone.
     ii. For men randomized to the placebo arm, 1.0ml of saline is given at the following points of the transitional zone: left and right inferior and superior transitional zone.
  c. The needle is passed from posterior to anterior through the peripheral zone (PZ) into the base of the prostate at the transition zone/central zone junction on the left side. The needle is advanced to superior aspect of the transition zone.
  d. Prior to injection of onabotulinumtoxinA the needle is aspirated to insure that there is no blood return. This is repeated prior to each injection.
  e. The initial 1.0 ml of solution is first deposited at the superior (bladder base) transition zone (TZ). The needle is withdrawn to the inferior (apex) transition zone/central zone junction and the second 1.0 ml of solution is deposited on the same side as the needle is withdrawn.
  f. The needle is passed from posterior to anterior through the peripheral zone (PZ) on the right side into the base of the prostate at the transition zone/central zone junction on the left side. The needle is advanced to superior (bladder base) aspect of the transition zone.
  g. The 1.0 ml of solution is first deposited at the inferior (apex) transition zone (TZ). The needle is withdrawn to the inferior (apex) transition zone/central zone junction and the second 1.0 ml of solution is deposited on the same side as the needle is withdrawn.
  h. The needle, syringe, and vial are disposed of in a biohazard container according to institutional policy, and a witness signs a form.
  i. After the injection, the patient remains in the clinic until a spontaneous void has occurred.

- Subjects will receive an adequate supply of tamsulosin/placebo with instructions on dosing.

- Because of the theoretical possibility of diffusion of onabotulinumtoxinA into seminal fluid, and no evidence to the contrary, those patients who engage in sexual intercourse are required to use condoms for 48 hours after the injection.

4 Visits 4 (Day 3 post-injection)

The subjects will be contacted by telephone 3 days after injection to monitor for symptoms of infection or bleeding.

5 Visits 5 (1 Week ± 3 days post-injection)

The subjects will be contacted by telephone 1 week after injection to collect medical events, concomitant medication update, and status.

6 Visit 6: (4 Weeks post-injection ± 3 days)

- The subject's concomitant medications will be discussed and documented.
- The subject's vital signs will be taken and documented.
- Any adverse events experienced by the subject will be documented.
- The subject will undergo uroflow testing and will provide a post-void residual urine measurement.
- A urinalysis (dipstick) will be performed.
- The subjects returned drug product packaging will be reviewed and discussed with him. A new supply of tamsulosin will be dispensed.
- The subject will complete the following questionnaires:
- The AUA symptom score
- BPH Impact Scale
- Bladder Function
- Erectile Function
- Ejaculatory Function
- Dispense an antibiotic

7 Visit 7: End of Study (3 months post-injections ± 3 days)

- A physical including a digital rectal exam (DRE) will be performed.
- The subject's concomitant medications will be discussed and documented.
- Any adverse events experienced by the subject will be documented.
- The subject's vital signs will be taken and documented.
- The subjects returned drug product packaging will be reviewed and discussed with him.
- The subject will complete the following questionnaires:
- The AUA symptom score
- BPH Impact Scale
- Bladder Function
- Erectile Function
- Ejaculatory Function
- The subject will undergo uroflow testing and will provide a post-void residual urine measurement.

- Blood (about 5 ccs or 1 teaspoon) will be drawn for PSA testing.
- A urinalysis (dipstick) will be performed.
- Transition zone biopsies will be performed and the prostate volume will be measured.

IX BIOLOGICAL SPECIMENS

A Prostate tissue will be collected at the time biopsy to conduct gene array studies to determine the effects of onaBoNT-A and tamsulosin on the genetic profile of human normal and BPH tissues. The specimens will be coded and will not include any subject identifying information. Testing on the tissue specimens will be conducted in Dr. Ayala's laboratory. The specimens will be stored until the end of the study at which time any unused specimens will be destroyed. These specimens will not be released to anyone not listed as an investigator on the protocol, nor will they be sold or transferred to any third party. If at any time the subject withdraws from this study, he will not be able to get the specimens back. Upon written request to Dr. Smith, the stored samples will be destroyed but the data collected up until that time will not be deleted.

The gene expression profiles in epithelial and stromal BPH cells of the prostates injected with onaBoNT-A and saline (i.e. Tamsulosin group), respectively, will be compared. Pre and post treatment biopsies will be frozen for laser capture microdissection and subsequent RNA extraction. All 6 biopsies will be frozen to guarantee adequate representation of both epithelia and stromal compartments of BPH. These sections will be used to orient laser capture microscopy in immediate frozen serial sections. Epithelial and stromal cells of the BPH nodules will be laser captured. Laser captured microdissection (LCM) tissues will be processed for gene expression profiling using Agilent Oligo Microarrays. Samples will be hybridized on a 4x44K slide formatted with the Whole Human Genome Oligo Microarray Kit that contains 41,000 unique genes and transcripts. Gene expression profiles will be evaluated using BioConductor software that will be used to process and normalize data. Significant differences in mean mRNA levels between groups will be determined with two-sided t-tests. Visualization of data (color maps) will be performed using Cluster and Java TreeView software. We will search for GO annotation terms in gene sets and will establish protein interaction networks analysis using the Human Protein Reference Database as we have reported previously and visualization will use Cytoscape software. Our next step is always to confirm the array analyses using qRT-PCR using pre-designed and validated primers when available. Otherwise primers will be designed and validated as described previously in numerous publications by Dr. Ittmann and our prostate research group. As a result of this study we will identify differential gene expression profiles in epithelial and stromal cells in the onaBoNT-A versus Tamsulosin treated prostates. In addition, we will be able to assess changes in gene expression profile before and after treatment with either agent. This analysis will permit us to assess whether Tamsulosin and onaBoNT-A have similar effects on human tissues as we have identified in rats. These results will serve as substantiation for further human clinical trials.

In addition to determining the effects of onaBoNT-A and tamsulosin on the genetic profile of human normal and BPH tissues, another goal is to determine the effects of

Tamsulosin and onaBoNT-A on the human BPH prostate tissue proliferation, apoptosis, innervation, stromal response, and angiogenesis.

1 Apoptosis: The apoptotic rates of normal and BPH epithelial cells will be measured, as onaBoNT-A denervation is known to induce apoptosis. For the detection of apoptosis in situ will use the TUNEL technique. Apoptotic bodies will be counted under a light microscope (x400). The area with highest positive stain within areas of BPH will be selected for counting. The number of apoptotic bodies will be determined in a total of approximately 1000 cells, normalized to 100 cells and defined as apoptotic index (AI). We expect to see an increase in apoptosis with onaBoNT-A compared to tamsulosin treatment.

2 Proliferation: The proliferation rate of BPH cells in human tissues will be determined using the immunoperoxidase method with antibodies against Ki-67. The proliferation index (PI) will be defined as the ratio of KI-67 positive BPH cells to total prostate BPH epithelial cells in the highest positive stain fields (at least 2000 cells), using a microscopic grid at 400x magnification. We expect to see a decrease in proliferation.

3 Nerves: To measure nerve density in these specimens we will immunostained with PGP9.5 antibodies using IHC Detection System as described previously**.** Total area of nerves will be quantified visually and with image analysis. We expect to identify a decrease in the density of nerves following onaBoNT-A treatment. It is possible that we will identify a time dependent compensatory effect.

4 Reactive stroma formation: Previous studies suggest that paracrine stromal interactions may be important factors in inducing and maintaining BPH. Therefore, we will determine if the stroma shows changes induced by denervation with onaBoNT-A. Tissue staining will be evaluated following procedures we have reported previously for quantitative determination of expression indexes for reactive stroma markers. A decrease in the stromal reactive response to BPH is expected.

5 Angiogenesis: Angiogenesis and neurogenesis are concomitant processes in embryologic development and wound response. Therefore we will look at the effect of denervation with onaBoNT-A on vessel density using standard vessel count methodology.

B Blood samples collected during the screening visit are routine for patients with BPH and LUTS and will be handled according to standard of practice. The test results will be included in the subject's research and medical charts.

C Urine specimens collected for dipstick analysis during the screening visit are routine for patients with BPH and LUTS and will be handled according to standard of practice. The test results will be included in the subject's research and medical charts.

X. STATISTICAL ANALYSIS

The primary endpoint, change in AUASS score from baseline to 3 months, between the onaBoNT-A and tamsulosin groups will be analyzed using the 2-sided independent t-test.

Baseline measures of the data such as age and ethnicity as well as secondary endpoints will be examined using summary statistics to determine if any differences exist between the 2 study groups.

Graphical methods will be used to access distributional properties of the secondary endpoints (urinary flow rate, serum PSA, prostate volume symptom relief, post-void residual, BPH impact score, Bladder Function, Erectile Function and Ejaculatory Function questionnaires). If extreme departures from normality are found, use of non-parametric analytical methods such as the Wilcoxon rank-sum test or transformation of the data will be considered. Questionnaire scores will be examined for reliability using Cronbach's alpha. The distributions of the scores will be examined to see if they are evenly distributed around the mid-point of the scales or are clustered at the top or bottom of the scales (ceiling or floor effect). If strong ceiling or floor effects are found, this could limit the usefulness of these measures. Changes in these secondary endpoints will be assessed using either the independent t-test or its non-parametric equivalent, the Wilcoxon rank sum-test. Statistical analysis will be done using SAS, version 9.2 (SAS Institute, Cary NC).

The endpoints apoptotic index, proliferation index, nerve density, stromal reactive indexes, and vessel density, will be checked for their distributional properties using the methods described for the data collected in Aim 1. Change from baseline to 3 months will be analyzed using either the 2 sided t-test or the Wilcoxon rank sum test.

Statistical analysis of expression data will be performed similar to that of previous studies [44], using public datasets of pathway-associated genes to identify general pathways involved. Our data indicates that epithelial cells are directly targeted by nerves. Denervation induces generalized atrophy of the epithelium and a significant decrease in protein production that cannot be reversed through supraphysiologic levels of testosterone. Mechanistically, denervation induces a translational and metabolic shutdown of the epithelial cells, which undergo a non-proliferative and more stem like phenotype with epithelial mesenchymal transformation. Particular emphasis will be given to specific markers within categories of genes we hypothesize onaBoNT-A treatment will have a greater impact given evidence within the BPH literature and our own experience with onaBoNT-A in rat and human prostate tissues: 1. Immune/Inflammation: IL-8, B cell homing chemokine, SRF:Serum response factor (c-fos serum response element-binding transcription factor), and MIC-1; 2. translation and translation initiation factor activity (signal transducer and activator of transcription 3, eukaryotic translation initiation factor 4A1, eukaryotic translation elongation factor 1 gamma*, eukaryotic translation initiation factor 3, subunit B, C and F); 3. Metabolic pathways (ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase);] 4. Growth Factors: IGF-2, FGF-2, IGF-I-R; and 5. Androgen Regulation: AR, ERβ, RLS-HD, AKR1C1, AKR1C2, AR:ERβ ratio, AKR1C1:AKR1C2 ratio. Corroborative studies will be performed with qPCR, immunofluorescence, and immunohistochemistry experiments.

XI. SAMPLE SIZE

Mean AUA score reduction in the alpha blocker group is expected to be at most 9.6 with a standard deviation of 6.3 based on published results of a randomized trial comparing tamsulosin versus placebo.[29] However, that same study showed a strong pill placebo effect with a mean reduction of 5.5, resulting in a treated reduction of 4.1 above the placebo group. Another study[13], comparing botulinum toxin to placebo, showed a mean score reduction in the botulinum group of 15.2 and no placebo change. For our study, we will assume a mean reduction of 15.2 in the onaBoNT-A group which is 9.7 more than the pill placebo effect, a mean reduction of 9.6 in the alpha blocker group and a standard deviation of 6.3 for both groups. Based on these assumptions, a sample size of 29 subjects per group will achieve 90% power to detect a difference of 5.6 with an alpha level of 0.05 using the 2-sided independent t-test. Sample size calculations were done using PASS 2008 software (Hintze, J. (2008). NCSS, LLC. Kaysville, Utah. www.ncss.com).

The drop-out rate in the alpha-blocker study was as large as 20% and our own experience with onabotulinumtoxinA (onaBoNT-A) in the MIST-2 trial has demonstrated a dropout rate of 14%. So adjusting our sample size for an expected dropout rate of 20%, we will enroll 37 patients into the onaBoNT-A and tamsulosin groups for a total of 74 patients. To address some of the problems with possible attrition bias, we will use intent to treat analysis and patients will be kept in the study as long as possible even if they are no longer on treatment. We will also compare results between the intent to treat groups and the subjects who completed treatment to determine if differences exist.

XII. RETENTION AND DROPOUT/WITHDRAWAL

Minimal dropout or loss to follow-up is expected in this cohort, especially given the short follow-up. However, if a patient drops out before 4 weeks, he will be replaced so that the required sample size is realized. For purposes of tracking the feasibility of conducting a trial of treatment with onaBoNT-A, reasons for dropout or withdrawal will be documented.

XIII. CRITERIA FOR DISCONTINUING THE STUDY

A Subjects unable to tolerate [tamsulosin or] onaBoNT-A due to side effects.
B Subjects can request removal from the study for any reason at any time.
C Subjects may be withdrawn at any time at the discretion of the investigator for safety, compliance, or other reasons.

A discontinuation occurs when an enrolled subject ceases participation in the study, regardless of the circumstances, prior to completing the protocol.

The reasons for a subject discontinuing from the study will be recorded in the Case Report Form.

XIV. DATA ANALYSIS

Analysis will begin with descriptive statistics. Implausible and missing values will be identified and double-checked for accuracy. Parametric methodology will be used unless measurements have tested positive for violation of normality assumption. The differences between the study subjects groups will be evaluated using the paired-samples t-tests. The clinical outcomes will be compared using Fisher's test. The criterion for significance (alpha) has been set at 0.05. All tests are 2-tailed, which means that an effect in either direction will be interpreted. Analysis will be conducted using SPSS 17.0. ("SPSS Statistics 17.0", SPSS Inc., Chicago, IL, 2008).

XV. RISKS AND DISCOMFORTS

A BOTOX®: It is expected that some participants may have some or all of the following side effects when given BOTOX®. Other side effects may occur which were not seen before. Side effects are usually temporary and manageable. However, it is possible they could cause serious disease or death. The study may include risks that are unknown at this time.

There have been rare reports of serious and/or immediate or even deadly abnormally sensitive reactions after treatment with BOTOX. These reactions include allergic reaction, skin rash, itching, swelling, and difficulty in breathing.

It is a rare possibility that the injection of BOTOX® could lead to botulism. The classic symptoms of botulism include double vision, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. The doctor's examination may reveal that the gag reflex and the deep tendon reflexes like the knee jerk are decreased or absent.

There have been rare reports of sudden death, sometimes associated with difficulty in swallowing or pneumonia. There have also been rare reports of heart problems (including irregular heart beats and heart attack, some resulting in death). Some of these patients were already at risk for heart disease. It is not known if BOTOX actually caused these problems.

It should not be used when infection is present at the injection site or in people known to be abnormally sensitive to BOTOX.

The following events have been observed since it has been marketed: skin rash, itching, and allergic reaction. In general, these side effects occur within the first week following injection and, while usually temporary, they may last several months. Pain, tenderness, or bruising around the injection site may also occur. Local weakness of the injected muscle(s) is expected. Weakness of nearby muscles may also occur due to spread of BOTOX.

There are also possible risks of urinary retention, fecal incontinence and infection as a result of intraprostatic onabotulinumtoxinA injection.

BOTOX contains albumin, which comes from human blood. Although the blood is rigorously tested, there is an extremely remote risk for the transmission of viruses and similar infectious agents.

B Flomax®: Possible side effects of FLOMAX may include:

Flomax capsules may cause a sudden drop in blood pressure upon standing, especially after the first dose or when changing doses. Symptoms may include fainting, dizziness, and lightheadedness.

Allergic reactions may include rash, itching, and hives. Rare and more serious allergic reactions may also include swelling of face, tongue, or throat, and difficulty breathing.

Priapism (a painful erection that will not go away) may occur.

During cataract surgery, a condition called intra-operative floppy iris syndrome (IFIS) can happen if FLOMAX is being taken or has been taken in the past.

Common side effects of FLOMAX capsules may include runny nose, dizziness, and decreased semen.

Adverse experiences with Lidocaine® are, in general, dose-related and may result form high plasma levels caused by excessive dosage, rapid absorption or inadvertent intravascular injection, or may result from a hypersensitivity, idiosyncrasy or diminished tolerance on the part of the patient.

Central nervous system symptoms that may be experienced are lightheadedness, nervernousness, apprehension, euphoria, confusion, dizziness, drowsiness, tinnitus, blurred or double vision, vomiting, sensations of heat, cold or numbness, twitching, tremors, convulsions, unconsciousness, respiratory depression and arrest.

Cardiovascular manifestations are usually depressant and are characterized by bradycardia, hypotension, and cardiovascular collapse, which may lead to cardiac arrest.

Allergic reactions are characterized by cutaneous lesions, urticaria, edema, or anaphylactoid reactions. These reactions to Lidocaine are extremely rare.

C Lidocaine®

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Allergic reactions are characterized by cutaneous lesions, urticaria, edema, or anaphylactoid reactions. These reactions to Lidocaine are extremely rare.

D Antibiotic

1 Bactrim DS may cause allergic reactions: hives, difficult breathing, and swelling of face, lips, tongue, or throat. It may also cause diarrhea, pale skin, rapid heart rate, trouble concentrating, sudden weakness, fever, chills, new or worsening cough, vomiting, headache, jaundice, skin rash.

2 Cipro may cause hypoglycemia and mental health issues, such as, disturbances in attention, disorientation, agitation, nervousness, memory impairment, and delirium. Other side effects may be pounding heart or very fast pulse, dizziness, pale skin, feeling shaky, sweating, unusual hunger, trembling, headaches, irritability, unusual anxiety, tendinitis, and changes in sensation and nerve damage.

E Transrectal Ultrasound (TRUS): The ultrasound rarely results in physical discomfort but some anxiety before and during the procedure may be experienced.

F Blood Draw: Inserting needles into veins for collecting blood may be uncomfortable. Risks include slight bruising at the injection site, fainting, the formation of a small blood clot or swelling of the vein and surrounding tissue, bleeding from the site, and the remote possibility of infection at the site of the needle puncture. Fainting is usually harmless, of short duration, and typically produces feelings of weakness, sweating, slowing of the heart rate and an abnormal decrease in blood pressure. Care will be taken to avoid these complications.

G Questionnaires: Completing the questionnaires may cause the subject to have or to experience some level of emotional discomfort due to the personal nature of the questions. The study doctor and staff will maintain a professional and caring attitude while administering the questionnaires.

H Loss of Confidentiality: The loss of confidentiality regarding research information is a possibility; although, the risk is very small.

I Birth Control: Because of the theoretical possibility of diffusion of onabotulinumtoxinA into seminal fluid, and no evidence to the contrary, those patients who engage in sexual intercourse are required to use condoms for 48 hours after the injection.

XVI. BENEFITS

A Subject: Patients with LUTS should see a benefit to onaBoNT-A injection within one week after injection. This may be manifested by a decrease in AUA symptom scores and an increase in urinary flowrate.

B Society: A secondary benefit of this study is to society because we will gain insight into the molecular changes in BPH and bladder tissues induced by onaBoNT-A

injection. The results of this study could potentially lead to novel treatments for BPH and LUTS.

C Risk to Benefit Ratio: The risks of the injection are minimal (i.e. bleeding, infection) and are mitigated in this protocol (i.e. no blood thinner medications, peri-injection antibiotics). The benefit of offering novel, minimally invasive treatments for BPH and LUTS far exceed the risk of prostate injection.

XVII. ALTERNATIVES

The alternative to participating in this research study is watchful waiting, medical therapy with approved drugs, or surgical treatment.

XVIII. COSTS TO SUBJECTS

Other than the study investigative products and anesthesia, all events and procedures are considered standard of care for this patient population.

XIX. PAYMENT TO SUBJECTS

Subjects will not receive payment for participating in this study.

XX. ADVERSE EVENT REPORTING

Adverse Events will be reported in accordance to all applicable FDA, ICH, and IRB rules, regulations, and guidelines.

XXI. DATA HANDLING AND RECORD KEEPING

A Case Report Forms

B Protocol-specific data will be collected on Case Report Forms (CRFs). The completed dataset should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from BCM.

C Record Retention

D To enable evaluations and/or audits from Health Authorities/BCM, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all CRF's, and detailed records of drug disposition. To comply with international regulations, the records should be retained by the investigator in compliance with regulations.

XXII. ETHICS AND GOOD CLINICAL PRACTICE

This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice (GCP) in accordance with International Conference on Harmonisation (ICH) Tripartite Guidelines for Good Clinical Practice and the US 21 Code of Federal Regulations dealing with clinical studies.

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of GCP that it conforms to.

A Institutional Review Board (IRB) and Other Institutional Review Committees

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted and other institutional review committees, as required. A signed and dated statement that the protocol and informed consent document(s) have been approved by the IRB and committees will be provided to the FDA in a general correspondence submission.

B Informed Consent Process

Subjects will be identified from those visiting in the Urology clinic at MEDVAMC. The investigator or his designated personnel must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician. Informed consent will be obtained in every patient prior to performing a biopsy or any protocol-related procedures or tests.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his informed consent has been obtained. The original signed informed consent form will be kept with the research study documents and a signed copy will be given to the subject.

C Confidentiality

Each subject will be assigned a unique, consecutive three-digit identification number (i.e., 001, 002, …). The study number will be utilized for identification of the subject throughout the study. The clinical study coordinator of the trial will maintain the code that links the name of the participant to their study identification number. The study coordinator will maintain the code in a secured cabinet and the code will be kept confidential. Only the clinical study coordinator and principal investigator can access and view the codes.

Data for all participant enrolled at MEDVAMC will be managed under the privacy guidelines as determined by MEDVAMC.

D Regulatory Documents

All regulatory documents will be managed by Research Administration of the Scott Department of Urology located at Main Baylor in rooms 502 and 506D in the Jewish Wing. The offices have keyed entries. All computers are password protected.

The Scott Department of Urology complies fully with the HIPAA Privacy Rule.

E Protocol Changes

If it is necessary for the study protocol to be amended, the amendment will be submitted to the BCM IRB for approval. Amended procedures will not be in effect until after IRB approval has been given. The principal investigator is responsible for the distribution of the approved documents to the staff.

Revisions to questionnaires or CRFs will not require IRB; therefore, these revisions will not be reported.

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XXIV. APPENDICES

A. Questionnaires

1 AUA Symptom Score
2 BPH Impact Scale
3 Bladder Function Questionnaire
4 Erectile Function Questionnaire
5 Ejaculation Function Questionnaire

B. SDU QA/QC protocol