

Research Protocol

Initial Visit

Study population. Enrollment is planned to achieve a total of 68 patients, with each subject undergoing two injections. All patients will receive 2 injections over the study time period as part of this cross-over study. Participants must be adult patients (age 18 or older) with medication-refractory non-neurogenic OAB subject to the following inclusion and exclusion criteria (Table 1). Patients who elect to proceed with intravesical BTX-A injection as third line therapy, and are deemed appropriate for treatment by the clinician, will be considered for eligibility. Patients presenting for injection will be screened and consented prior to the first injection, and this consent will apply for the duration of the study (over two injections).

Demographic and clinical data: Participants will complete a standardized questionnaire assessing demographics and pertinent medical history including surgical history, current and past OAB therapy, history of urinary tract infections (UTIs) and urinary retention, and self-reported diagnoses of comorbid medical conditions. We will supplement this information with abstracted data from electronic medical records on past medical and surgical history, urinalysis, urine culture data, and post-void residual.

Note: Recurrent UTI will be defined as three or greater culture documented UTIs within a 12 month period.

Urinary tract infection: At enrollment, patients will complete a medical history questionnaire which will include information related to prior UTIs. Eligible patients will provide urine specimen for analysis along with physician assessment. Diagnosis of symptomatic UTI prior to injection is to be made at the discretion of the clinician with exception of nitrite positive urinalysis. Patients with nitrite positive urine on the day of injection, regardless of symptoms, will be excluded from enrollment. Nitrite positive urinalyses will be sent for urine culture, and patients may be enrolled in the trial at a later date following treatment. On the day of injection a voided urine specimen will be obtained from all patients and sent for urine culture. This allows for assessment of the bladder milieu at the time of injection, however will not be used for treatment purposes unless patient subsequently becomes symptomatic. Post-void residual will be obtained, per standard clinic protocol, for all patients receiving treatment. This will be done with bladder scanner in clinic and with catheterized specimen for patients receiving an injection in the operating room if PVR not previously recorded in the medical record within 8 weeks prior to injection.

Assessment of overactive bladder: Participants will complete *baseline* standardized, validated questionnaires documenting urinary symptoms and quality of life. Instruments will include the American Urologic Association Symptom Index (AUA-SI), Overactive Bladder Questionnaire short-form (OABq-SF), Patient Perception of Bladder Control questionnaire (PPBC) and the Patient's Global Impression of Improvement (PGII).

Inclusion/exclusion criteria. Study criteria are listed in the Table. A diagnosis of OAB and the decision to proceed to third line treatment will be determined by clinician according to AUA guidelines.

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none">• ≥ 18 years of age• Medication refractory OAB, identified per AUA guidelines	<ul style="list-style-type: none">• Post void residual urine >150ml on two occasions• Untreated, symptomatic UTI• Comorbid neurological conditions, including spinal cord injury, systemic neurologic illnesses (i.e. multiple sclerosis, Parkinson's disease) or central nervous system disease (i.e. brain tumor, stroke)• Prior pelvic irradiation• Current or prior bladder malignancy• Hematuria lacking a clinically appropriate evaluation• Chronic catheterization or SIC

Day of Injection

New Patients:

New patients will undergo complete history and focused physical exam. PVR will be obtained at initial clinic visit. If PVR has not performed or is not on file on the day of injection, PVR will be obtained prior to injection. If PVR >150cc, it will be repeated after double void. If again greater than >150cc, patient will be excluded from enrollment for that injection. Physician will be responsible for determining if patient has symptomatic UTI on the day of injection, and exclude the patient if appropriate (UA will be utilized in making this decision).

Established patients:

Established patients continuing injection will be screened and consented on the day of presentation for injection. PVR within 8 weeks must be documented in chart. If no PVR documented within 8 weeks, must repeat PVR prior to injection with either a bladder scanner or catheterized value. UA obtained per existing clinic policy. Eligibility determined by provider based on UA and PVR.

Demographic information including comorbidities will be abstracted from patients' electronic medical records, and a focused history and physical will be performed on day of injection.

Injection protocol: All patients will undergo injection per a standardized protocol of 1ml injection per site at a concentration of 10 units/ml. For patients undergoing injection in the clinic setting, an intravesical instillation of diluted local anesthetic will be used prior to injection, if no allergy to anesthetic is reported. For those performed in the operating room, anesthesia will be administered per the discretion of anesthesiology team. The number of units injected will be documented and accounted for during analysis by stratifying patients according to the amount of BTX-A injected.

Antibiotic protocol: On the day of injection patients will be randomized to either the experimental or control arm according to a computer generated randomization scheme (see explanation of randomization below). Patients randomized to the experimental arm will receive a single dose of trimethoprim/sulfamethoxazole (800/160mg) peri-procedurally. Patients randomized to the control arm will receive one dose of trimethoprim/sulfamethoxazole peri-procedurally and additionally be prescribed trimethoprim/sulfamethoxazole twice daily for three days. Patients who are allergic will receive amoxicillin/clavulanic acid (875/125mg) twice daily for three days. If allergic to both of the above antibiotics, patients will receive 100mg nitrofurantoin BID for three days. Our budget reflects published data regarding our local antibiogram and population estimates for antibiotic allergies.

3-week Post-Procedure Visit:

All patients at 3-week post-procedure visit will receive:

- a. AUA-SI, PPBC, OABq-SF, PPBC (per standard clinical practice)
- b. PVR (per standard clinical practice)
- c. UA (per standard clinical practice)
- d. Urine culture if determined necessary by physician (per standard clinical practice)
- e. Post-Botox 3-Week Post Procedure Survey

Symptoms Assessment: Participants will complete standardized, validated questionnaires documenting urinary symptoms and quality of life for comparison. Instruments will include the American Urologic Association Symptom Index (AUA-SI), Overactive Bladder Questionnaire short-form (OABq-SF), Patient Perception of Bladder Control questionnaire (PPBC) and the Patient's Global Impression of Improvement (PGII).

Urinary tract infection: Patients will be assessed at a 3-week follow up visit with urinalysis. If a patient reports symptoms concerning for UTI, urine culture and antibiotic treatment will be ordered at the discretion of the clinician. All patients will be provided with instructions for reporting adverse events including clinic nurse contact information should symptoms of UTI develop. Given that our institution is a tertiary referral center and patients may report to local providers, a system will be in place to obtain all outside urine culture and antibiotic records for analysis.

Note: For the purposes of this study, UTI will be defined as $\geq 10^5$ bacterial colonies in the presence of symptoms.

Treatment success: Patients will be assessed at post-procedure appointments at 3 weeks and a phone call at 3 months. Follow up visit will consist of urinalysis, ultrasound PVR, patient-reported questionnaires, and recording of adverse events. Efficacy of injection will be assessed via inter-injection times for patients receiving multiple injections and will be followed for the duration of the study period.

3-month Post-Procedure Phone Call:

All patients at 3-month post-procedure visit will receive:

- a. AUA-SI, PPBC, OABq-SF, PPBC (per standard clinical practice)
- b. Post-Botox 3-Month Post Procedure Survey

Symptoms assessment: Participants will be asked, by phone, standardized, validated questionnaires documenting urinary symptoms and quality of life for comparison. Instruments will include the American Urologic Association Symptom Index (AUA-SI), Overactive Bladder Questionnaire short-form (OABq-SF), Patient Perception of Bladder Control questionnaire (PPBC) and the Patient's Global Impression of Improvement (PGII).

Urinary tract infection: Patients will be asked about presence and frequency of UTI including treatment and duration of treatment.

Treatment success: Patients will be assessed at post-procedure phone call at 3 months. Efficacy of injection will be assessed via inter-injection times for patients receiving multiple injections and will be followed for the duration of the study period.

2nd Injection:

Patients will have been consented at the beginning of the study to include both 1st and second injections and therefore do not need to be re-consented. The exact protocol is followed as stated above, however, patients will receive the treatment from the arm NOT received after the first injection. For example, patients who received extended 3 day course of antibiotics after the first injection, will receive only peri-procedural antibiotics after the 2nd injection.

The timing of the second injection will be determined by the clinician and patient based on symptoms and will not be standardized.

Randomization

Every patient will receive Extended Dose Antibiotics (EDA) and Peri-Procedural Antibiotics (PPA). The patients will be randomized to receive EDA or PPA at the first visit or second visit. A block randomization with block sizes of 2 and 4 will be used. The exact numbers of size 2- and size 4- blocks will be randomly chosen so that approximately 70% of blocks will be allocated to 'block of size 4'.

Statistical analysis plan

The primary endpoint is the presence (Yes/No) of urinary tract infection 3 weeks post procedure. We will estimate the probability of urinary tract infection for both treatment groups. Baseline patient-characteristics data will be collected and tabulated. The trial design (crossover) will guarantee that the baseline covariates will be distributed similarly for both treatment groups. Carryover effect is minimized since the time between the first and second injections is sufficiently long for any residual treatment effect from the first period to wear off. For each treatment, the probability of UTI will be estimated and presented with 95% Wilson confidence intervals. We aim to establish non-inferiority of EDA to PPA with the non-inferiority margin of 10 percentage points. We will estimate the proportion of patients who will have infection with EDA but not with PPA.

Sample size and power were computed using the method described in Chow et al ¹. To compute the sample size, we need to specify, in addition to the usual parameters (type I error rate of 5%, type II error rate of 10%, non-inferiority margin of 0.10, and difference under the alternative of 0), the variance of difference, which takes value of 1 if only EDA is successful, -1 if only PPA is successful, and 0 if EDA and PPA yield the same outcome. This variance, in turn, depends on the true probability of infection with EDA and PPA and also concordance of the result (success/failure) within a given patient. Under a pessimistic scenario where P[infection] is 25% and 35%, and the concordance is weak (Knowing the result of other treatment only changes the probability by 5 percentage points for the patient), the variance is 0.40, and the corresponding sample size is 68. Even if the true P[infection] is 35% and 45%, we will maintain the excellent power as shown below in the Table.

Power with n=68

P[infection] with EDA	0.25	0.25	0.35	0.35
P[infection] with PPA	0.35	0.35	0.45	0.45
Concordance	+0.05	+0.10	+0.05	+0.10
Power	90%	92%	83%	86%

¹ Chow SS, Shao J, Wang H. Sample size calculation in clinical research. New York: Marcel Dekker, 2003.