EFFECTIVENESS OF ONABOTULINUM A TOXIN ON REDUCTION OF REST TREMOR IN PARKINSON'S DISEASE: A PILOT STUDY

NCT number NCT03301272
Document Date 12/03/2018

EFFECTIVENESS OF ONABOTULINUM A TOXIN ON REDUCTION OF REST TREMOR IN PARKINSON'S DISEASE: A PILOT STUDY

Daniel A. Roque, M.D.
Nina Browner, M.D., Miriam Sklerov, M.D., Melissa Collins, Diana Drazheva, Manisha Chopra, William Powers, M.D., Kevin Robertson, PhD

Version 8
December 3, 2018

Table of Contents

I. Background

II. Objectives and Hypotheses

III. Subjects

i. Number, Type, Gender, Ethnicity, Race, Age

ii. Eligibility Criteria

IV. Research Plan

i. Recruitment & Consent Process
ii. Data Collection
iii. Study Design

V. Benefits and Risks/Minimization of Risk

VI. Data Monitoring and Statistical Analysis

i. Power Calculation
ii. Data Analysis
iii. Interpretation of Results
iv. Data Storage & Security/Confidentiality/Sharing

VII. Safety Monitoring Plan

VIII. Potential Problems and Limitations

IX. References

I. Background

Among the most prevalent signs and symptoms of idiopathic Parkinson's disease is that of rest tremor: a rhythmic oscillation of a limb or component of a limb which occurs primarily at rest but can reemerge during the maintenance of a posture or while attempting to approach a target. Although rigidity in Parkinson's can be disabling, rest tremor is frequently reported as a symptom which leads to the inability to perform fine motor tasks especially when attempting to approximate targets. This can lead to the rapid inability for patients to maintain their current employment and even perform instrumental activities of daily living.

Although a number of treatment options have been developed to provide symptomatic relief over the course of the last 50 years, rest tremor remains one of the most medically-refractory symptoms of Parkinson's disease. Among antiparkinsonian medications, levodopa replacement therapy has been reported to be perhaps least effective in reducing tremor, with dopamine agonists having a reasonably higher chance of reducing rest tremor per earlier studies. Alternative therapies for rest tremor do include amantadine and anticholinergic medications, ultimately still with suboptimal outcomes on tremor reduction while leaving a large portion of patients unable to tolerate the side effects. As such, patients with medically-refractory rest tremor are left with one remaining option: to consider implantation of deep brain stimulation hardware via elective brain surgery. This rather invasive procedure has been shown to have success rates of 80-85% on reducing rest tremor and in many cases eliminating rest tremor even for at least 15 years after implantation. Understandably, many patients are wary of elective brain surgery and would prefer to lose their functional independence as opposed to undergoing such a high-risk procedure.

Onabotulinum toxin A has been used (and in many cases FDA-approved) as a means of decreasing tone in a number of patients with conditions including spasticity and dystonia, but even hyperkinetic movements such as blepharospasm, hemifacial spasm, essential tremor, and spastic bladder. Although Parkinson's disease is traditionally thought of as a hypokinetic movement disorder, the rest tremor inherently is an adventitious movement considered hyperkinetic in phenomenology.

When choosing the proper targets for injection of botulinum toxins in rest tremor, without proper planningone could easily overdose the muscles and lead to weakness which may outweigh the benefits on reduced tremor amplitude. To date, studies which have evaluated the effect of botulinum toxin on rest tremor in Parkinson’s disease have used typical doses effective for dystonia, later titrating doses higher to effect. Given that statistically-significant effect is based on gross characterization of tremor amplitude into previously-existing nominal scales (namely the Unified Parkinson’s Disease Rating Scale tremor subscore), it would not be suprising that titrating doses “to effect” will subject the participant to higher toxin doses that, in turn, are more likely to produce clinically-evident weakness. Subsequently, these same studies have not been able to correlate reduced tremor amplitude to any clear functional improvement save for a single, recently-published open-label study on the subject. However, functional improvement in the

studies has generally only been assessed through subjective, self-reported measures/scales and not through any objective measures.

We propose there is a lesson to learn from task-specific dystonias: usually we have to utilize much lower doses of botulinum toxin in practice to avoid weakness while still providing benefit on function of the limb as measured by their ability to perform the task. Given that rest tremor inherently is not related to nor produced by increased tone, there is reason to believe that low doses of botulinum toxin may still provide reduction of amplitude without inducing weakness. Previous studies sometimes injected only involve either a set of flexor or extensor muscles while others injected a combination of both muscles involved in creating the oscillatory movement. To help reduce chances of weakness as best possible, we propose that splitting doses of botulinum injections into a combination of both flexor and extensor muscles would be safest. What remains to be seen his whether such low doses of botulinum toxin injected into a combination to both tremor-inducing flexor and extensor muscles might also lead to improvement of tremor amplitude and/or function of the limb.

To achieve the goal of reducing tremor amplitude without inducing weakness, we may require a more sensitive means of measuring distance traveled without relying on previous gross, nominal scales. Over time, a number of measuring instruments in the form of gyroscopic gloves have been created to help study quantitative and qualitative data for adventitious movements such as tremor. However, information derived from gyroscopes and accelerometers has been technically limited by their inability to accurately track distances within a foot, whereas tremor amplitudes are generally characterized and graded at the centimeter level. Further limiting the utility of these instruments is the fact that they add weight onto a participant's hand/limb, artificially suppressing some of the amplitude of the organic tremor. For this reason, most studies have utilized such devices/gloves to grossly characterize tremor amplitudes into the previously-described nominal scales. As such, a device that can measure amplitude changes more accurately and precisely without bearing weight on the affected limb with tremor could also potentially be designed primarily to evaluate the change in tremor amplitude with greater sensitivity. Subsequently, this would allow for more precise measurements of changes in amplitude after an intervention such as botulinum toxin. Such a tool could also provide quantitative data on direction of greatest oscillatory movement such that it may help guide decision-making when choosing which muscle groups are thought to produce the rest tremor.

In an effort to meet the demand for such a device with better ability to measure amplitude and directional information from a tremor, the utilization of motion-capture cameras on multiple other facets of mobility in neurologic disease has been promising. When applied at the appropriate distance from an object, information about trajectory in 3-dimensional space has been achieved with greater accuracy and reliability. For purposes of obtaining more accurate, objective information regarding tremor amplitude, we plan to apply this technology to evaluate tremor characteristics both prior to and after interventions and predetermined time intervals. Hereafter, the stereotactic camera apparatus shall be labeled as the "Px1."

II. Objectives and Hypotheses

i. **Specific Aims**:

1. Evaluate the effect of onabotulinumtoxin A on medically-refractory rest tremor:
    a. Severity/Amplitude: As measured by the MDS-UPDRS tremor subscore.
    b. Limb function: As measured by the Action Research Arm Test (ARAT)
2. Evaluate Px1 versus expert clinical assessment and its sensitivity to detect changes based on the MDS-UPDRS tremor subscore
3. Determine whether methodology for this pilot project can feasibly be applied to a larger subsequent study that could be adequately powered to detect a clinically-meaningful effect of onabotulinumtoxin A on medically-refractory rest tremor.

ii. **Hypotheses**:

1. (A) Onabotulinumtoxin A significantly attenuates the amplitude of medically-refractory rest tremor of the upper limb in Parkinson's patients as compared to sham injections; as measured by reduction in the MDS-UPDRS tremor subscore.
   1. (B) Onabotulinumtoxin A significantly improves the limb function of Parkinson's patients with medically-refractory rest tremor of the upper limb as compared to sham injections; as measured by an increase in ARAT scores.

III. Subjects

i. Pilot group

- **Number** – 16
    - Due to the limitations brought on by internal funding for this pilot project and the overall cost of botulinum toxin, an even number of patients was chosen after a cost analysis was completed. It was determined that we could cover the cost of 16 participants throughout the proposed course of the study.
- **Gender** – male or female
- **Ethnicity** – all, no restrictions
- **Race** – all, no restrictions
- **Age** – 45-80

ii. Eligibility Criteria

Inclusion Criteria:

1. At least 45 years of age, and no more than 80 years of age.
2. Meet UK Parkinson's disease brain bank diagnostic criteria
3. Have clinical evidence of rest tremor of one or both upper extremities defined as involuntary, rhythmic oscillations about any joint within the upper extremities
4. Rest tremor amplitude must be at minimum 3 cm as determined by expert opinion by a movement disorders specialist. Confirmation of amplitude measurement will

be obtained from the Px1 prior to active participation in the study but will not be used for inclusion/exclusion in study participation.

5. Rest tremor must be historically refractory to at least 2 categories of medications typically used as anti-parkinsonian agents including levodopa formulations, dopamine agonists, amantadine, and anticholinergics.
6. **Determining Medication Refractoriness:**

   AFTER patient is consented:

   a. By history, patient reports that they received no subjective benefit from two different categories of anti-Parkinsonian medications, including: levodopa replacement, dopamine agonists, anticholinergic agents, amantadine. If subject is on therapy at present, the current therapy could count towards quantifying the medications which have failed to reduce tremor, so long as tremor amplitude meets inclusion criterion "4". Patient will be asked whether, on average, they believe their tremor has been consistently disabling over the course of the last month.
   b. Medication refractoriness will be confirmed through observation at time of peak-dose effect of one of the reported oral agents which have failed to reduce tremor. Note that within-individual intermittency and variability of rest tremor can be influenced by anxiety, stress, cold temperature, and fatigue. In an effort to reduce this variability, we will examine subjects in a comfortable area of the clinic space, providing up to 20 minutes to allow them to relax in a temperature-neutral location, and reduce anxiety.
   c. If at peak-dose effect the subject is felt to still meet inclusion criterion "4" ,they will remain a possible candidate for the study.
   d. Once the movement disorder specialist considers the subject a possible candidate, the rest tremor amplitude will be assessed using the Px1. This assessment should be completed within 10-20 minutes of the assessment by the rating movement disorder specialist
7. Participants must be able to make no changes to their anti-parkinsonian medications for 150 days (study duration). Ability and safety to do so must also be determined by the participant's treating physician and confirmed in writing prior to participating.
8. Able to provide informed consent

Exclusion criteria:

1. History of having undergone botulinum toxin injections for any other condition previously
2. Allergy to carbidopa or levodopa.
3. Prescreening Montreal Cognitive Assessment (MoCA) score less than 22
4. Prescreening muscle weakness as determined by Medical Research Council grade less than 5/5 on direct testing in the upper limb afflicted with rest tremor.

5. Pregnancy: documentation of non-pregnancy by urine pregnancy test will be obtained from all women of child-bearing potential prior to participation
6. Infection at the proposed injection site
7. Those with a pre-existing, concomitant neuromuscular disorder
8. Compromised respiratory function
9. History of having undergone deep brain stimulation surgery for any condition

IV. Research Plan

i. Recruitment & Consent

Subjects will be recruited from outpatients at UNCH clinics. In order to determine whether a patient meets inclusion or exclusion it will be necessary to review the patient's electronic medical record at UNC Hospitals including: clinic consultation history and physical, other clinic notes, neurological imaging including brain MRI. The information collected will be limited only to that which is necessary to contact the subjects and ask if they are interested in participating in the study.

Prospective subjects will be approached in person by one of the investigators or a designee during a regularly scheduled clinic visit at the UNCH Neurology Movement Disorders Clinic. Alternatively, some prospective participants may have already expressed verbal interest during previous clinic visits for participating in future studies. For those patients, phone calls will be initiated with a script provided to explain the study and the level of their involvement. Prospective subjects will be told basic information about the study. If the prospective subject is interested, a member of the study team will obtain written informed consent. Once consent is obtained, the study team member will go through inclusion/exclusion criteria for the study. Should the consented subject qualify for the study based on these criteria through the screening process, the subject will be scheduled for the first study visit (which may or may not be on the same day as screening).

ii. Clinical Descriptive Data

Clinical descriptive data collected from subjects will include age, gender, race, ethnicity, prior and current medications used for treatment of rest tremor in Parkinson's disease, years of reported Parkinson's symptoms, MoCA score upon enrollment, other significant current or past medical conditions, current medications, and drug allergies. Data recorded from the Px1 will include tremor frequency, amplitude, and suggested joint/muscle groups involved in creating the oscillatory movement.

Study Dates and Purpose of Visits					
Visit	1	2	3	4	5
Study Day (relative to start date)	Day 0	Day 30 (+/- 6 days)	Day 90 (+/- 6 days)	Day 120 (+/- 6 days)	Day 150 (+/- 6 days)
Purpose of Visit	Screening Consenting Baseline Assessment Randomization/Blinding	Therapeutic Assessment	Baseline Assessment	Therapeutic Assessment	Assessment

	Intervention Safety Assessment		Intervention Safety Assessment	Safety Assessment	Safety Assessment

iii. Study Design

a. The study will comprise of a double blinded, crossover study where the subjects will serve as their own controls. There will be no medication changes made to Parkinson's disease medications throughout the subjects' participation in the study.

b. Randomization: Determined by the IDS Pharmacy at UNCusing a computer-generated algorithm which will determine whether subject's first intervention will be sham saline injections versus onabotulinum toxin A. The IDS Pharmacy will keep track of which agent was administered first to avoid administering same intervention twice.

c. Blinding: Double-blinded study whereby the subject, the movement disorder specialist injecting neurotoxin, and the movement disorder specialist rating the patient will be unaware of the solution injected and/or planned for injection same day. To ensure that the injecting specialist is blinded to the solution, syringes will be premixed by the IDS Pharmacy and de-identified of any possible labels that would indicate the properties of the solution being administered to the subject.

Injection Sequence	**Visit 1**	**Visit 3**
Sequence AB	Botox	Placebo
Sequence BA	Placebo	Botox

d. Deciding between bilateral tremors: The side to be studied shall be the limb with the tremor of greatest amplitude. This will be determined by a combination of factors: Participant self-report of which tremor is "worse", which tremor is "more disabling", as well direct observation by the examiner and the MDS-UPDRS tremor subscore per limb to determine which limb has the tremor of greatest amplitude. Once that limb is chosen, it shall be utilized throughout the duration of the study.

e. Visit 1: Day 0

a. Screening, as per "Eligibility Criteria"
    i. Participant will be asked to come to clinic at the time they are due for their next dose of anti-Parkinsonian medication. If they are not taking anti-Parkinsonian medication, a single dose of carbidopa/levodopa 25/100mg 1 tablet will be administered (this assumes participant meets exclusion criteria "b"). They will only take medication when instructed. Then at peak-dose effect of the medication taken:
b. Baseline MDS-UPDRS tremor subscore obtained by movement disorder specialist
c. Baseline muscle strength assessment of limb: using Medical Research Council (MRC) muscle testing via physical examination
d. Baseline ARAT score obtained by occupational therapist

e. The Px1 will be used to obtain information regarding the direction of oscillation, average amplitude, peak amplitude, and frequency of the tremor. This will only serve as objective information regarding the tremor
f. Randomization is performed for Visit 1, and after all above measures have been obtained the subject will be injected with either sham normal saline injection using 0.9% normal saline solution (no greater than 2 mL total solution) or predetermined onabotulinum toxin doses based off of affected muscle groups. Refer to table below for information regarding predetermined dosing.
g. Patient will complete a daily diary of symptoms for 30 days starting the day AFTER the study visit, to be collected by study personnel at the following study visit. The diary includes subjective assessments of tremor severity, tremor persistence, arm weakness, and arm dysfunction.

f. Visit 2: Day 30 (+/- 6 days per visit)

a. Participant will be assessed when they are due for their next dose of anti-Parkinsonian medication. If they are not taking anti-Parkinsonian medication, a single dose of carbidopa/levodopa 25/100mg 1 tablet will be administered. They will only take medication when instructed. Then one hour after observing a dose of anti-Parkinsonian medication (i.e. at peak-dose effect of the medication taken):
b. Repeat MDS-UPDRS tremor subscore obtained by movement disorder specialist
c. Repeat muscle strength assessment of limb: using MRC muscle testing via physical examination
d. Repeat ARAT score obtained by occupational therapist
e. The Px1 will be used to obtain information regarding the direction of oscillation, average amplitude, peak amplitude, and frequency of the tremor to then be compared to Visit 1.
f. Patient will complete a daily diary of symptoms for 30 days starting the day AFTER the study visit, to be collected by study personnel at the following study visit. The diary includes subjective assessments of tremor severity, tremor persistence, arm weakness, and arm dysfunction.

g. Visit 3: Day 90 (+/- 6 days)

a. Participant will be asked to come to clinic at the time they are due for their next dose of anti-Parkinsonian medication. If they are not taking antni-Parkinsonian medication, a single dose of carbidopa/levodopa 25/100mg 1 tablet will be administered. They will only take medication when instructed. Then at peak-dose effect of the medication taken:
b. Repeat MDS-UPDRS tremor subscore obtained by movement disorder specialist
c. Repeat muscle strength assessment of limb: using MRC muscle testing via physical examination
d. Repeat ARAT score obtained by occupational therapist
e. The Px1 will be used to obtain information regarding the direction of oscillation, average amplitude, peak amplitude, and frequency of the tremor.

f. Based on enrollment randomization from Visit 1, and after all above measures have been obtained:
    i. If sham injection was performed at Visit 1, then onabotulinum toxin injection is performed at Visit 3 and vice versa. The same injection paradigm used in Visit 1 will be used for Visit 3 to ensure greatest level of consistency between injections.
g. Patient will complete a daily diary of symptoms for 30 days starting the day AFTER the study visit, to be collected by study personnel at the following study visit. The diary includes subjective assessments of tremor severity, tremor persistence, arm weakness, and arm dysfunction.

h. Visit 4: Day 120 (+/- 6 days per visit)

a. Participant will be asked to come to clinic at the time they are due for their next dose of anti-Parkinsonian medication. If they are not taking antni-Parkinsonian medication, a single dose of carbidopa/levodopa 25/100mg 1 tablet will be administered. They will only take medication when instructed. Then at peak-dose effect of the medication taken:
b. Repeat MDS-UPDRS tremor subscore obtained by movement disorder specialist
c. Repeat muscle strength assessment of limb: using MRC muscle testing via physical examination
d. Repeat ARAT score obtained by occupational therapist
e. The Px1 will be used to obtain information regarding the direction of oscillation, average amplitude, peak amplitude, and frequency of the tremor to then be compared to Visit 3.
f. Patient will complete a daily diary of symptoms for 30 days starting the day AFTER the study visit, to be collected by study personnel at the following study visit. The diary includes subjective assessments of tremor severity, tremor persistence, arm weakness, and arm dysfunction.

i. Visit 5: Day 150 (+/- 6 days)

a. Participant will be asked to come to clinic at the time they are due for their next dose of anti-Parkinsonian medication. If they are not taking antni-Parkinsonian medication, a single dose of carbidopa/levodopa 25/100mg 1 tablet will be administered. They will only take medication when instructed. Then at peak-dose effect of the medication taken:
b. Repeat MDS-UPDRS score obtained by movement disorder specialist
c. Repeat muscle strength assessment of limb: using MRC muscle testing via physical examination
d. Repeat ARAT score obtained by occupational therapist
e. Last safety review performed, ensuring that the toxin effect has worn off as expected without deficits.

j. Predetermined onabotulinum toxin dosing

a. In an effort to prevent undue weakness to an otherwise strong limb that is affected by rest tremor, the following paradigm was predetermined based off of typical safety measures and initial dosing recommendations for other limb conditions amenable to onabotulinum toxin A.

i. Choice of doses per muscle are based off of clinical practice experience and typical doses utilized for task-specific dystonia where perceived and measurable weakness based on MRC (Medical Research Council) muscle testing have been found to be, on average, undetectable.

b. Decision of which muscles to inject will be based off of clinical expertise by the injecting physician. The following paradigms will be adhered to:

Specific Injection Paradigm		
Joint Involvement	**Muscles to Inject**	**Botox Dose Per Muscle**
Elbow flexion/extension	Biceps brachii; Triceps	10 units
Forearm rotation	Supinator; Pronator teres	5 units
Wrist flexion/extension	Flexor carpi ulnaris; Flexor carpi radialis; Extensor carpi ulnaris; Extensor carpi radialis	5 units
Digit 1-4 flexion/extension (DIP;distal interphalangeal)	Flexor digitorum profundus; Lumbricals	2.5 units
Digit 1-4 flexion/extension (PIP;proximal interphalangeal)	Flexor digitorum superficialis; Extensor digitorum	2.5 units
Thumb flexion/extension*	Flexor pollicis brevis OR adductor pollicis brevis OR opponens pollicis; Abductor pollicis brevis	2.5 units

*Only 1 of the 3 flexor compartment muscle groups for the thumb will be injected, whereas the abductor pollicis brevis will always be injected

c. A maximum of 50 units of onabotulinum toxin would be allowed per upper extremity.

k. **EMG-guided injections**

a. For purposes of properly identifying muscles intended for injection, a portable EMG (Myoguide system from Intronix Technology Corp., or equivalent) will be attached to an appropriate gauge EMG-guided botulinum toxin needle (Myoject by Optima, or equivalent), which in turn will be used to hear/see motor evoked potentials (MEPs). Subjects will be asked to activate the muscle while needle is inserted to ensure proper placement of the needle in the desired muscle prior to injection of study solution.

l. Obtaining measurement with the Px1

a. Duration employed for tremor assessment: 15 seconds per assessment. We will plan on obtaining 3 different measures prior to any of the interventions (saline or botulinum toxin) per visit, essentially over the course of 30 minutes. Every measurement/assessment is inherently an average, but the plan will be to average the 3 measurements to account for some variability in tremor severity as might otherwise naturally fluctuate for the participant. To help account for the effect that any pharmacotherapy has on the tremor, aim will be to assess the tremor at the expected peak dose effect time of their antiparkinsonian medication (using known, typical pharmacodynamic data) with every visit. This will require coordination with the participant to ensure timely oral delivery, as delineated above for each visit.

V. Benefits and Risks/Minimization of Risk

Benefits

There could be direct benefits to subjects who agree to participate. During the course of the study, the investigational agent (onabotulinumtoxin A) may reduce the amplitude of the participants' rest tremor. Given the nature of neurotoxins, however, this clinical effect is expected to be transient and last for no longer than three months. There will be no travel expense reimbursement and no payment for participation.

The benefits to society: given that currently rest tremor in Parkinson's disease can be disabling and refractory to oral medication options, this pilot study looks into the feasibility of injecting affecting limbs with onabotulinumtoxin A to minimize the amplitude of tremor and return prior function to affected upper extremities. Thereafter, those individuals whose tremors might be positively affected could also contribute to the workforce for longer (as rest tremor could be highly influential in making decisions to either apply for early disability or retirement).

Risks and Minimization of Risk

The Px1

The Px1 is an optical measurement system that aims to capture data on digital oscillation in 3-space. The Px1 is physically comprised of Intel's RealSense SR300 peripheral camera / projector, with software integration which identifies structures of the hand (primarily joints) reliably without the need of obstructing elements such as soft rubber finger "caps" or "cones". To use the device, the subject need only extend or rest their arm in proximity of the SR300 camera (i.e., within 1 meter). Instructions on how participants should position the hand in front of the camera will be provided at the time of assessment. In total, the hand will be assessed in 3 different postures:

1) With the participant sitting in an armchair with elbow rested on the armrest while the wrist entirely hangs over the front edge of the armrest for proper assessment by the camera
2) With the participant sitting in an armchair, arm outstretched in the forward position (arm flexed 90°, elbow extended fully and wrist at midplane)

3) With the participant sitting in an armchair with shoulder abducted 90°, elbow flexed 90°, forearm rotated 90°, and wrist flexed 90° from midplane (a known, common posture that can exacerbate rest tremor)

   The patient will make no contact with the device. There are no conceivable risks anticipated for participants exposed to this device. .

   The Px1's function is achieved almost entirely in software developed for this purpose. The SR300 peripheral camera should present no conceivable risk to the subject and has been rigorously tested as a commercial product that is featured in new HP, Dell, and Lenovo computers.

   Output from the Px1's recordings will come in 2 separate forms. First, there will be a graphical representation of an articulated hand with concentric circles surrounding each joint of varying sizes depending on the total distance traveled by that joint in comparison to its adjacent joints. These amplitudes will be further quantified in a tabular fashion (Excel spreadsheet) with distance traveled measured in centimeters to a total of 2 decimal points. Similar graphical representation and tabular quantification will be obtained for frequency of oscillatory movement measured in hertz.

   Onabotulinumtoxin A
   There are two potential categories of risks associated with neurotoxin injections: procedural risks and medication side effects.

   a. Procedural risks:
      a. Pain/discomfort/local site reaction: (approximate incidence 1-10%) could be related to either the puncture through the dermis, or through the muscle itself. Frequently self-limiting, with the option to use over-the-counter analgesics as needed until pain resolves. Plans to minimize risk involve using commonly-used injection approaches when injecting the planned muscle groups of the hand, forearm, and arm.
      b. Injury: given the minimally-invasive nature of this procedure, there are risks including bleeding and infection/inflammation. To minimize risk, will utilize the smallest needle gauges possible to obtain access to the individually-injected muscles while also providing smaller dilution to minimize total volume delivered (0.1 mL: 5 units of onabotulinum toxin). Sterile technique will be kept during the entirety of the procedure, with instructions provided to the subject on how to address any evidence of infection in a timely fashion.
   b. Medication side effect:
      a. Weakness: (approximate incidence 1-10%) The predetermined neurotoxin dosing regimen per muscle group was designed to minimize the likelihood of developing weakness while still potentially providing benefit to involuntary oscillations of movement of the muscle groups. However, weakness would be a transient effect and will wear off as any medication benefit also wears off.

Difficulty swallowing/breathing: (approximate incidence < 1%) mostly hypothetical adverse effect of onabotulinum toxin which is within the warnings typically given to any patient

receiving onabotulinum toxin for FDA-approved purposes. Theoretically, the neurotoxin could make its way through the vascular supply to a site distant from the intended target (and hence, could develop swallowing/breathing difficulties related to the weakness of muscle groups required for these activities)

Carbidopa/Levodopa:

This medication is prototypically used in almost all patients with Parkinson's disease at some point during their disease course. It would be standard of care that at least at some point patients will have been attempted on this therapy throughout their disease course, whether early or advanced disease. In fact, typically this medication is the only tolerated medication in Parkinson's disease in advanced stages of disease (when patients would be thought to be most susceptible to side effects).

Note that approximate incidences for carbidopa/levodopa medication side effects are primarily published as common versus uncommon. Rare adverse effects are also listed in the package insert with an understanding that a correlation between use of medication and the symptom in question might exist:

1. Common side effects: Dyskinesias, nausea
2. Uncommon side effects: hypotension, orthostatism, hallucinations
3. Rare side effects: Chest pain, , diarrhea, hallucinations, hypersexuality, impulse control disorders, headache, dizziness, confusion, neuroleptic malignant syndrome, rash, abdominal pain

Sham normal saline injections

Procedural risks associated with these injections would be similar to those identified above for onabotulinumtoxin A injections. Medication side effects would not be expected in this group.

Medical follow-up:

in the event that a subject were found during the course of the study to need medical or psychological follow-up related to their study involvement, the following procedures will be followed to address their issues.

1. Medical: For urgent issues while on site, we will direct subjects to the UNC emergency room. For urgent issues that arise outside of the study site but as a potential result of the intervention, we will direct subjects to their nearest emergency room versus urgent care center for evaluation, based off of the described difficulties and symptoms. For nonurgent (subacute, chronic) issues related to Parkinson's disease, a follow-up appointment within our department will be scheduled for within 72 hours of the communication. If the issue is unrelated to their Parkinson's but also nonurgent, will communicate with the subject's primary care physician and, when appropriate, disclose necessary information regarding the study protocol should it be relevant to the complaints at hand.
2. Psychological: for urgent issues, will direct subjects to the nearest crisis unit if necessary (including but not limited to suicidal ideations or intentions, severe and rapid depression/anxiety). For nonurgent (subacute/chronic) issues, will supply

patient with local mental health care specialists near them as well as contact information for those within the university system to obtain the next available appointment.

VI. Data Monitoring and Statistical Analysis

Power Calculation

No power calculation can be performed. This is a pilot feasibility study to determine if onabotulinumtoxin A can be utilized to reduce resting tremor amplitude and improve arm function.

Data Analysis (Performed by Dr. Kevin Robertson, PhD)

Note that all statistical analysis, and as such any missing data/values/dropouts, will be performed assuming an intention-to-treat principle. All statistical estimates will be tabulated along with corresponding confidence intervals (CIs). All hypothesis tests that are observed to be not statistically significant will be reported as being inconclusive. Lastly, all data acquired for Visit 5 will be utilized for review of safety. This is to ensure that the expected washout effect is occurring and that there is expected normalization of values trending towards or back to previous baseline values. For this reason, there is an absence of any testing with the Px1 on Visit 5 (since data will not be utilized to compare to previous visits for purposes of statistical analysis).

Specific Aim 1a. The change in tremor severity effected by onabotulinumtoxin Awill be compared to the change effected by sham saline. Tremor severity will be measured via the MDS-UPDRS tremor subscore. A Student's t-test will be utilized to compare postintervention measures within subjects.

Specific Aim 1b. The change in limb function as measured by the ARAT created by onabotulinumtoxin A will be compared to the change created by sham saline. A Student's t-test analysis will be used to compare change in ARAT scores within subjects.

Specific Aim 2. The strength of the correlation between MDS-UPDRS tremor subscore and tremor characteristics as assessed by the Px1 will be obtained. Will then evaluate the strength of the correlation between the change in MDS-UPDRS score and change in the ARAT score. This latter correlation will then be compared to the correlation between the change in tremor characteristics as assessed by the Px1 and the change in the ARAT score.

Specific Aim 3. Pilot data will be reviewed and trends analyzed. Subsequent power analysis will be performed to determine size of study population that is necessary to adequately measure statistically significant effect. Data regarding dropout rates will also be reviewed to determine if study methodology needs to be adjusted for a larger study population.

Interpretation of Results

Specific Aim 1a. A reduction in severity/amplitude of the rest tremor by onabotulinumtoxin A greater than the change in severity/amplitude from sham saline injections suggests that onabotulinumtoxin A may be superior to sham saline injections in reducing severity of medically-refractory rest tremor and will be used as preliminary data for future grant proposals for larger, more rigorous studies. Failure to demonstrate this difference would suggest one or more of several possibilities: (1) that the doses of onabotulinumtoxin A were too low to lead to a measurable effect, and/or (2) onabotulinumtoxin A does not show promise as an intervention to reduce rest tremor amplitude compared to sham injections and should not be further studied for this purpose. The information gathered from this pilot project could then be utilized to (1) postulate higher dose delivery to reach an effect, and/or (2) choose alternative botulinum toxins that might produce an effect on tremor as compared to onabotulinumtoxin A. Using these variables, a subsequent study could be designed.

Specific Aim 1b. A greater increase in score on the ARAT after onabotulinum toxin injection compared to after sham saline injections suggests that onabotulinumtoxin A may provide clinically-meaningful improvement on the interruption caused by rest tremor on activities of daily living, and this preliminary data will be used for future grant proposals for larger, more rigorous studies. Failure to demonstrate this difference would suggest one or more of several possibilities: (1) that the doses of onabotulinumtoxin A were too low to lead to a measurable effect, and/or (2) onabotulinumtoxin A does not show promise as an intervention to improve arm function in the setting of rest tremor as measured by the ARAT, and thus it should not be further studied for this purpose. The information gathered from this pilot project could then be utilized to (1) postulate higher or lower dose delivery to reach an effect, and/or (2) choose alternative botulinum toxins that might produce an effect on tremor as compared to onabotulinumtoxin A. Using these variables, a subsequent study could be designed.

Specific Aim 2. A statistically-significant correlation between the MDS-UPDRS tremor subscores and the tremor amplitude measurements derived from the Px1 could serve to validate this tool against the current gold standard (i.e. the MDS-UPDRS) for measuring severity of tremor in Parkinson's disease both in research and clinical practice. This preliminary data would be used for future grant proposals for larger, more rigorous studies; including potentially additional studies using the Px1 as a potentially more-sensitive measure of tremor severity/amplitude in place of the MDS-UPDRS tremor subscore. Failure to demonstrate this correlation would suggest one or more of several possibilities: (1) that the Px1 is not sufficiently accurate in its measurement acquisition algorithm, and/or (2) the MDS-UPDRS tremor subscore is subject to too much subjectivity from the rater's perception on direct visualization and has less intrarater reliability than was previously perceived. The information gathered from this pilot project could then be utilized to (1) adjust the measurement algorithm for the Px1 to improve accuracy when measuring hand movements in real-life subjects versus calibrated limbs, and/or (2) increase the number of MDS-UPDRS raters assessing a limb tremor. Using these variables, a subsequent study could be designed.

Data Storage & Security/Confidentiality/Sharing

In order to maintain privacy and confidentiality regarding subject participation, there will only be one printed copy of a spreadsheet with codes linked to their identifiers (which will only include name and date of birth). This spreadsheet will be kept in the Dr. Roque’s office within a locked

cabinet with multiple layers of physical security to enter this office including necessary UNC employee badge access. Once the study has ended, only codes shall remain, and the aforementioned spreadsheet shall be destroyed in a HIPAA-compliant fashion. This will not directly affect any UNC medical records.

All baseline clinical and experimental data will be recorded on paper case report forms and then transferred by our research coordinator Diana Drazheva into computerized data forms, entering into the secure database REDCap @ UNC. Paper case report forms will be stored in double-locked storage. All computerized records will be password protected and HIPAA compliant. Access to all records will be restricted to the project investigators unless approved by the participant and the UNC IRB. Confidentiality will be maintained by storing all information under anonymous study numbers. No individual identifying information will be included in any reports on this study.

Given the design of the Px1, data regarding tremor direction, amplitude, frequency, etc. will be obtained automatically by software that integrates with the device. This will be copied into the central spread sheet with all further data collected at pretreatment visit and with subsequent study visits.

VII. Safety Monitoring Plan

In case symptoms occur outside of business hours, the subject will be provided the telephone number of the hospital page operator and the operator will be asked to page the on-call neurologist who subsequently will contact Dr. Roque directly with information. For non-urgent issues, the subjects will be asked to call Dr. Roque's office number that is listed on the consent form of which the subject will receive a copy.

The PI will monitor the study for any adverse and serious adverse events. All serious adverse events will be reported to the IRB. Should there be a serious adverse event that occurs that increases the risks to the participants, the study will be stopped and an investigation will be conducted and a findings report generated before the study is resumed. At the time of enrollment, we will ensure we have an active and accurate phone number on file to contact the subject. Thereafter, weekly phone calls will be made by the PI or a designee for the first four weeks after injection visits to ask questions including but not limited to significant weakness, difficulty breathing, difficulty swallowing, injection site reactions, or other self-reported concerns.

VIII. Potential Problems and Limitations

This is a pilot study aimed at determining the feasibility of utilizing onabotulinumtoxin A therapy to reduce the amplitude of rest tremor and improve arm function. There are two novel components to the study design that have been utilized in similar studies but not directly aimed at measuring and improving rest tremor in Parkinson's. These include the ARAT (which has been utilized for arm function pre-and post-botulinum toxin injections to the upper limb, but not to assess rest tremor) and the introduction of a new measurement device (the Px1) that will provide the objective measures of tremor amplitude, frequency, and direction of movement. Although validation of the data acquired from the Px1 will occur, more traditional methods of measuring

tremor (the MDS-UPDRS tremor subscore) will be performed during the visit intervals of the study to account for the novel applications of these assessment tools.

IX. References

1. Pandyan A, Vuadens P, van Wijck FMJ, Stark S, Johnson G, et al. Are we underestimating the clinical efficacy of botulinum toxin (type A)? Quantifying changes in spasticity, strength and upper limb function after injections of Botox to the elbow flexors in a unilateral stroke population. Clin Rehabil 2002;16:654-660.
2. Hallett M, Albanese A, Dressler D, Segal KR, Simpson DM, Truong D, Jankovic J. Evidence-based review and assessment of botulinum neurotoxin for the treatment of movement disorders. Toxicon 2013; 67:94-114.
3. Brin MF, Lyons KE, Doucette J, Adler CH, Caviness JN, Comella CL, Dubinsky RM, et al. A randomized, double masked, controlled trial of botulinum toxin type A in essential hand tremor. Neurology 2001; 56:1523-1528.
4. Mills R, Bahroo L, Pagan F. An update on the use of botulinum toxin therapy in Parkinson's disease. Curr Neurol Neurosci Rep. 2015; 15:511-522.
5. Marjama-Lyons J & Koller W. Tremor-predominant Parkinon's disease: Approaches to treatment. Drugs & Aging. 2000; 16 (4): 273-278.
6. Rahimi F, et al. Effectiveness of BoNT A in Parkinson's disease upper limb tremor managament. Can J Neurol Sci. 2013; 40: 663-669.
7. Sheffield J & Jankovic J. Botulinum toxin in the treatment of tremors, dystonia, sialorrhea and other symptoms associated with Parkinson's disease. Expert Rev Neurotherapeutics. 2007; 7(6):637-647.
8. Trosch RM & Pullman SL. Botulinum toxin A injections for the treatment of hand tremors. Movement Disorders. 1994; 9(6):601-609.
9. Brin MF, et al. A randomized, double masked, controlled trial of botulinum toxin type A in essential hand tremor. Neurology. 2001; 56:1523-1528.
10. Volkmann J, et al. Central motor loop oscillations in parkinsonian resting tremor revealed by magnetoencephalography. Neurology. 1996; 46:1359-1370.
11. Bevan MD, et al. Move to the rhythm: oscillations in the subthalamic nucleus-external globus pallidus network TRENS in Neurosciences. 2002; 25(10):525-531.
12. Jankovic J & Schwartz PA. Botulinum toxin treatment of tremors. Neurology. 1991; 41:1185-1188.
13. Shuurman PR, et al. A comparison of continuous thalamic stimulation and thalamotomy for suppression of severe tremor. N Engl J Med. 2000; 342(7):461-468.
14. McAuley JH & Marsden CD. Physiological and pathological tremors and rhythmic central motor control. Brain. 2000. 123:1545-1567.
15. Ahmed A & Taylor NRW. The analysis of drug-induced tremor in mice. Brit J Pharmacol. 1959; 14:350-354.
16. Lenz FA, et al. Single unit analysis of the human ventral thalamic nuclear group: Tremor-related activity in functionally identified cells. Brain. 1994;117:531-543.
17. Bergman H, et al. Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. TINS. 1998; 21(1): 32-38.

18. Britton TC et al. Modulation of postural wrist tremors by magnetic stimulation of the motor cortex in patients with Parkinson's disease or essential tremor and in normal subjects mimicking tremor. Ann Neurol. 1993; 33(5):473-479.
19. Samotus, O, et al. Long-term tremor therapy for Parkinson and essential tremor with sensor-guided botulinum toxin type A injections. PLoS ONE. 2017; 12(6): e0178670. https://doi.org/10.1371/journal.pone.0178670