BARDA CLINICAL STUDY PROTOCOL

A Randomized, Three-Sequence, Three-Period Crossover Study to Assess the Bioavailability and Pharmacokinetics of a Single Dose of Atropine Administered Sublingually in Healthy Adult Volunteers

BP-C-19010

Sponsor: Biomedical Advanced Research and Development Authority (BARDA)

[redacted]

Sponsor Contact: [redacted]

Medical Monitor: [redacted]

Version of Protocol: 3.0

Date of Protocol: November 21, 2019

CONFIDENTIAL

All financial and nonfinancial support for this study will be provided by BARDA. The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of BARDA. The study will be conducted according to the Integrated Addendum to International Conference on Harmonisation (ICH) E6 (R1): Guideline for Good Clinical Practice (GCP) E6 (R2).

Protocol Approval - Sponsor Signatory

Study Title	A Randomized, Three-Sequence, Three-Period Crossover Study to Assess the Bioavailability and Pharmacokinetics of a Single Dose of Atropine Administered Sublingually in Healthy Adult Volunteers
Short Title	Sublingual Atropine PK Study
Protocol Number	BP-C-19010
Protocol Version	Version 3.0
Protocol Date	November 21, 2019

Protocol accepted and approved by:

[redacted]

[redacted]

Signature

[redacted]

Date

Investigator's Agreement

Study Title	A Randomized, Three-Sequence, Three-Period Crossover Study to Assess the Bioavailability and Pharmacokinetics of a Single Dose of Atropine Administered Sublingually in Healthy Adult Volunteers
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Protocol Date	November 21, 2019

I have read and understood all sections of the above referenced protocol, and the provided package inserts for atropine sulfate ophthalmic solution, United States Pharmacopeia (USP) 1% and atropine sulfate injection for intravenous administration.

I agree to supervise all aspects of the protocol at my clinical research site and to conduct the clinical investigation in accordance with the protocol and the International Conference on Harmonisation (ICH) regulations and US Investigational New Drug (IND) regulations in 21 CFR 312. I will not make changes to the protocol before consulting with BARDA or implement protocol changes without institutional review board approval except to eliminate an immediate risk to subjects. I agree to administer study treatment only to subjects under my personal supervision or the supervision of a subinvestigator.

I will not supply the study drug to any person not authorized to receive it. Confidentiality will be protected. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from BARDA.

_____

Printed Name of Investigator

_____ _____

Signature of Investigator Date

Emergency Contact Information

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone Number
Sponsor Contact	[redacted]	[redacted]
Medical Monitor	[redacted]	[redacted]
Principal Investigator	[redacted]	[redacted]

1. SYNOPSIS

Name of Sponsor/Company: Biomedical Advanced Research and Development Authority (BARDA)

Name of Study Drug:

Test Drug: Atropine sulfate ophthalmic solution, USP 1% (NDA 206289) for sublingual administration

Reference Drug: Atropine sulfate injection for intravenous (IV) administration (NDA 209260)

Name of Active Ingredient:

Atropine sulfate

Title of Study: A Randomized, Three-Sequence, Three-Period Crossover Study to Assess the Bioavailability and Pharmacokinetics of a Single Dose of Atropine Administered Sublingually in Healthy Adult Volunteers

Short Title: Sublingual Atropine PK Study

Protocol Number: BP-C-19010

Version Number: 3.0

Study center(s): 1 (one)

Study period (years):	**Phase of development:** 1
Estimated date first subject enrolled: December 2019 Estimated date last subject completed: January 2020	

Study Objectives:

Primary Objective

- To evaluate the bioavailability and pharmacokinetics (PK) of atropine administered sublingually against the reference IV route of administration

Secondary Objectives

- To evaluate the safety of atropine administered sublingually
- To evaluate the tolerability of atropine administered sublingually, as assessed by changes in xerostomia

Methodology:

This is a randomized, three-sequence, three-period crossover study to assess the bioavailability and PK of a single dose of atropine administered sublingually in healthy adult volunteers. At least 15 healthy male and female volunteers will be enrolled to obtain approximately 12 evaluable subjects in the per protocol population. Eligible subjects will be randomized at a 1:1:1 ratio to receive one of three treatment dosing sequences (A, B, or C) as depicted in the table below:

Study Design Scheme by Dosing Sequence

Dosing Sequence	Expected Number of Evaluable Subjects (N)	Period 1 (Visit 1; Day 1)	Period 2 (Visit 2; Day 8)	Period 3 (Visit 3; Day 15)
A	4	Low Dose sublingual	High Dose sublingual	IV
B	4	High Dose sublingual	IV	Low Dose sublingual
C	4	IV	Low Dose sublingual	High Dose sublingual

Low Dose sublingual = 0.5 mg (50 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; High Dose sublingual = 1.0 mg (100 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; IV = 1.0 mg (2.5 mL) of atropine sulfate (0.4 mg/mL) administered via intravenous route

Subjects will be screened for study participation from Days -14 to -1 and enrolled into the study on Day 1. On the morning of each treatment day (Visits 1, 2, and 3), subjects will receive a Low Dose sublingual, High Dose sublingual, or IV atropine sulfate administration, according to the randomly assigned treatment sequence. Blood samples for PK will be collected at time 0 (predose) and at 13 time points postdose at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and 2, 4, 6, and 8 hours after dosing. Site staff will record subjects' reports of their subjective xerostomia predose and every 10 minutes up to the first hour after dosing. Subjects will be discharged from the clinic after the 8 hour blood sample collection.

Meal timing, activity levels, and general conditions in the clinical research unit will be as similar as possible for all study subjects irrespective of dose and day.

On the day of atropine administration, each subject:

1. Will not be allowed food or drink 1 hour prior to drug administration and continuing through 1 hour after drug administration or until the subject reports a 10 (maximum) on the xerostomia assessment or expresses the xerostomia is intolerable, whichever occurs first. After 1 hour or if either of these conditions are met, water will be provided ad libitum.

2. Will be provided a meal/snack no less than 4 hours after drug administration.

3. Will abstain from alcohol 24 hours before each dosing visit and until the last blood sample from each dosing visit is collected.

4. Will report to site staff their subjective sense of xerostomia predose and every 10 minutes up to the first hour after dosing or until maximum/intolerable xerostomia is reached, whichever occurs first, based on the questions in Appendix 3.

5. Will have automated blood pressure and heart rate measurements on the opposite arm from blood collection every 10 minutes for the first hour, every 20 minutes for the second hour, and every 30 minutes for the third and fourth hours and thereafter as deemed clinically necessary by the investigator until the end of each visit.

Blood Sampling

An indwelling venous catheter will be placed before dose administration for blood sample collection for serial determinations at times: 0 (predose) and postdose at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and 2, 4, 6, and 8 hours. Ten (10) mL of whole blood will be collected into a sterile vacutainer containing ethylenediaminetetraacetic acid (EDTA) as a preservative for each sampling. Plasma will be stored frozen for future analysis. For IV atropine administration, serial blood sampling will be performed via indwelling catheter on the opposite arm from the one used for dosing; blood

pressure and heart rate readings will be performed on the arm used for dosing after the IV has been removed.

Number of subjects (planned): Approximately 15 healthy volunteers, with the option to enroll additional healthy volunteers, to obtain approximately 12 evaluable subjects (approximately 4 per dosing sequence) in the per protocol population.

Inclusion and Exclusion Criteria

Subjects will be randomized to study treatment only if they meet all of the applicable inclusion criteria and none of the exclusion criteria. In addition, eligibility criteria must be reviewed just prior to each dose of atropine unless specified below; if the subject no longer meets applicable eligibility criteria, the investigator, in consultation with the Rho medical monitor in cases of uncertainty, must determine whether the subject should receive the atropine dose or be terminated early from the study drug.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study or subject safety. Therefore, adherence to the criteria specified in the protocol is essential.

Subject Inclusion Criteria:

1. Healthy male and nonpregnant female volunteers between the ages of 18 and 55 years at time of randomization
2. Willing and able to provide written informed consent
3. Females who are of childbearing potential and are sexually active with a male partner must have used an acceptable method of birth control for at least 2 months prior to Screening, and must agree to continue using an acceptable method of birth control from Screening through Follow-up (Day 21)
    a. A female of childbearing potential is defined as a postonset menarche and premenopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: menopausal > 2 years, tubal ligation > 1 year, bilateral salpingo-oophorectomy, or hysterectomy.
    b. Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label. Examples include oral contraceptives, injectable progestogen, implants of etonogestrel or levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, intrauterine device or intrauterine system, or male partner sterilization at least 6 months prior to the female subject's Screening Visit.
4. In the judgment of the investigator, the subject is in good health, based on review of medical history and the results of screening evaluation (including vital signs, physical examination, 12-lead electrocardiogram [ECG], and routine clinical laboratory testing, performed no more than 14 days prior to randomization into the study).
5. Able to comply with the dosing instructions and available to complete the study Schedule of Events (Appendix 2)

Subject Exclusion Criteria:

1. Females who have a positive pregnancy test or who are breastfeeding
2. Subjects with thyroid disease as evidenced by a thyroid-stimulating hormone (TSH) < 0.9 × lower limit of normal (LLN) or > 1.2 × upper limit of normal (ULN) at screening. (This test will not be repeated prior to subsequent dosing.)

3. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), or serum creatinine > 1.5 × ULN at screening. (These tests will not be repeated prior to subsequent dosing.)
4. Have known human immunodeficiency virus (HIV), or acute or chronic hepatitis B or hepatitis C infection based on medical history; or test positive for any of these at Screening. Subjects who have been effectively treated for hepatitis C, as evidenced by a negative hepatitis C ribonucleic acid (RNA) confirmation test and who no longer require antiviral therapy, are eligible for participation. (Screening tests will not be repeated prior to subsequent dosing.)
5. Subjects who took any prescription medications (with the exception of oral contraceptives or hormone replacement therapy) within 30 days of screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the subject should be terminated from further dosing.
6. Subjects who took any over-the-counter medication/vitamins/herbal supplements in the last 72 hours prior to screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the subject should be terminated from further dosing.
7. Subjects with glaucoma and/or history of ocular surgery (including Lasik™), ocular trauma, or congenital ocular disorder
8. Subjects with any history of heart disease, including but not limited to coronary artery disease, arrhythmia (treated or untreated), congestive heart failure, pacemaker, history of vasovagal syncope, peripheral vascular disease, or claudication
9. Subjects with clinically significant arrhythmias or abnormal conduction; abnormal conduction is defined as a prolonged PR or QRS, or a QTc ≥ 450 msec for males or ≥ 470 msec for females
10. Subjects with a history of partial organic pyloric stenosis, chronic constipation, or other gastrointestinal motility issues
11. Subjects with a history of xerostomia due to an underlying disease or previous radiation therapy to the head and neck
12. Males with history of symptomatic prostatic hypertrophy; males or females with a history of urinary hesitancy or retention
13. Subjects with a blood pressure > 140/90 mm Hg taken after the subject has been seated and resting for at least five minutes
14. Subjects with a history or current diagnosis of myasthenia gravis
15. Subjects with a history of drug or alcohol abuse in the last two years or evidence of a positive urine drug test at screening. (This screening test will not be repeated prior to subsequent dosing.)
16. Subjects with a known sensitivity or prior adverse reaction to atropine

Subjects cannot be rescreened for exclusionary laboratory test results. Potentially exclusionary vital sign results may be repeated once. If a subject's repeat vitals remain exclusionary or the investigator determines that the repeat vital signs could pose a risk to the subject participating in the study, then the subject will be excluded.

Concomitant medications:

Contraceptive medications and estrogen or estrogen/progesterone hormone replacement therapy are permitted for female subjects. Nonsteroidal anti-inflammatory drugs and acetaminophen are permitted for all subjects. No other concomitant medications are allowed.

Test drug, dosage and mode of administration:

Each subject will receive a single dose of 0.5 mg (50 µL) of atropine sulfate 1% ophthalmic solution administered via the sublingual route and, on a separate day, a single dose of 1.0 mg (100 µL) of atropine sulfate 1% ophthalmic solution administered via the sublingual route. The order of administration will be based on the dosing sequence to which the subject is assigned.

Reference drug, dosage and mode of administration:

Each subject will receive a single dose of 1.0 mg (2.5 mL) of atropine sulfate (0.4 mg/mL) administered via an indwelling IV cannula. The order of administration of this dose relative to the sublingual doses will be based on the dosing sequence to which the subject is assigned.

Duration of treatment: After a Screening period of up to 14 days, each subject will receive 3 doses of atropine, each dose separated by a washout period of 6 ± 1 days, and will be followed for approximately 6 days after their last dose. The expected study duration is approximately 35 days per subject.

Criteria for evaluation:

Primary Endpoint

- The relative bioavailability of atropine administered sublingually versus intravenously as measured by the following key PK parameters:
  - Area under the analyte concentration versus time curve to infinity (AUC_∞)
  - Area under the analyte concentration versus time curve to time of last quantifiable data point (AUC_t)
  - Maximum measured plasma concentration (C_{max})
  - Time to C_{max} (t_{max})
  - Apparent terminal elimination half-life ($t_{½}$)
  - Apparent total body clearance after extravascular administration (CL/F)
  - Apparent total volume of distribution after extravascular administration (V_d/F)

Secondary Endpoints

- All treatment-emergent adverse events (AEs) and serious adverse events (SAEs) occurring during study participation
- Xerostomia assessment (Appendix 3), completed during screening and each day of dosing starting at predose, and every 10 minutes postdose up to 1 hour postdose

Statistical methods:

This study is descriptive in nature. Bioavailability of the sublingual administration of atropine relative to that of IV atropine will be assessed based on ratios of least-squares means of key plasma PK parameters and associated 90% confidence intervals (CIs). The PK parameters and baseline subject characteristics will be summarized using descriptive statistics. Key PK parameters (e.g., C_{max}, AUC_t, AUC_∞, t_{max}, $t_{½}$, CL/F, and V_d/F) for plasma concentrations will be compared between the study dosages by analysis of variance (ANOVA) including terms for sequence, dose, and period as fixed

effects, and subject nested within sequence as a random effect in the model. The comparisons of interest are:

Low Dose sublingual vs IV

High Dose sublingual vs IV

Low Dose sublingual vs High Dose sublingual

Safety data will be presented as tabular summaries of AEs and SAEs, and descriptive statistics for vital signs. Xerostomia scores will be analyzed descriptively for each of the 3 questions on the xerostomia assessment at each timepoint. Additional information on statistical methods will be described in the statistical analysis plan for the study.

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LIST OF ABBREVIATIONS

Abbreviation or Specialist Term	Explanation
AE	adverse event
ALT	alanine aminotransferase
ASPR	Assistant Secretary for Preparedness and Response
AST	aspartate aminotransferase
AUC	area under the analyte concentration versus time curve
AUC_{∞}	AUC to infinity
AUC_t	AUC to time of last quantifiable data point
BARDA	Biomedical Advanced Research and Development Authority
BMI	body mass index
CFR	Code of Federal Regulations
CI	confidence interval
CL/F	apparent total body clearance after extravascular administration
C_{max}	maximum measured plasma concentration
CNS	central nervous system
CTCAE	Common Terminology Criteria for Adverse Events
DMP	Data Management Plan
eCRF	electronic case report form
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
ET	early termination
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HHS	Department of Health and Human Services
ICF	informed consent form
ICH	International Conference on Harmonisation
IRB	institutional review board
IV	intravenous

Abbreviation or Specialist Term	**Explanation**
LLN	lower limit of normal
MCM	medical countermeasure
MedDRA	Medical Dictionary for Regulatory Activities
NA	nerve agent
OTC	over-the-counter
PK	pharmacokinetics
QA	quality assurance
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAR	suspected adverse reaction
$t_{½}$	apparent terminal elimination half-life
t_{max}	time to C_{max}
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
US	United States
USP	United States Pharmacopeia
UV	unscheduled visit
V_d/F	apparent total volume of distribution after extravascular administration

2. INTRODUCTION

2.1. Background

The Biomedical Advanced Research and Development Authority (BARDA) is tasked with, among other things, promoting and supporting the development of strategic medical countermeasures (MCMs) for the United States. Typically, this effort involves supporting novel drug development up to FDA approval and inclusion into the Strategic National Stockpile. However, in the event of widespread nerve agent (NA) poisoning, predeployed MCMs are likely to be inadequate due to limited availability of community caches (Chempack), logistical challenges in bringing the resource to the site of treatment, depletion of the resources before all patients needing treatment receive it, and expiration or unavailability of approved MCMs and/or preferred delivery devices (auto-injectors)[1].

In response to this challenge, the Department of Health and Human Services (HHS) is investigating the bioavailability and PK of an alternative (sublingual) route of administration of atropine sulfate ophthalmic solution 1% USP, compared to the reference, atropine sulfate injection, administered intravenously (IV)[2].

Atropine is an approved drug indicated for temporary blockade of severe or life-threatening muscarinic effects, symptomatic bradycardia, and to dilate the pupils to facilitate eye exams. Atropine is an antidote for organophosphorus or carbamate chemical warfare agent or pesticide poisoning, as well as the treatment of some (muscarinic) mushroom toxicity. Atropine, available in multiple dosing forms, is commonly administered by intramuscular injection, IV injection, and as ocular drops. The ocular drop formulation is also given off-label sublingually for excessive drooling in multiple contexts including: maintenance of perioral hygiene in hospice patients, to alleviate hypersalivation caused by certain antipsychotic medications, to manage oral secretions/reduce ventilator-associated pneumonia risk in mechanically ventilated patients, and for excessive drooling in developmentally disabled children[3-6].

This randomized, three-sequence, three-period, phase 1 study is designed to assess the bioavailability and pharmacokinetics (PK) of sublingually administered atropine sulfate ophthalmic solution 1% USP (at 0.5 mg and 1.0 mg; test) compared to atropine sulfate injection administered IV (1.0 mg; reference).

2.2. Study Rationale

Nerve agent toxicity requires the rapid administration of antimuscarinic MCMs to mitigate life-threatening bronchorrhea and seizures. Rapid administration of centrally acting doses of atropine and anticholinergic medications are most effective within 10 minutes of administration. Following IV administration of atropine, the heart rate increases within seconds, and the antisialagogue and central nervous system (CNS) effects may take a few minutes to achieve maximum effect. In contrast, the intramuscular route of atropine administration requires a very high dose before central antimuscarinic effects are seen, and the onset of action is delayed. Further, stockpiled NA MCM configurations (Chempack), containing drugs administered by auto-injector, are insufficient to treat large numbers of NA-poisoned patients in a timely manner. Ophthalmic atropine solution is generally available in the community and may provide a rapid, available source of effective medication in the event of an emergency.

Administration of 1% ophthalmic atropine via the sublingual route may be an alternative means of delivering a rapid therapeutic dose of atropine to individuals exposed to NA, and in addition overcome the stockpile supply deficits identified above.

The purpose of the current study is to evaluate the bioavailability and PK of atropine sulfate 1% ophthalmic solution when administered via the sublingual route at two different dose levels.

2.3. Previous Studies

BARDA conducted an extensive literature review and identified 110 published English language articles involving the administration of atropine by sublingual, nasal, and oral routes. Eighteen studies were selected based on their relevance to the proposed clinical study and are summarized in Appendix 1. Included among these reports are 6 wherein hyoscyamine, the levo-enantiomer of atropine, was the active agent. A total of 470 subjects were exposed to varying doses and regimens of sublingual atropine, including the ophthalmic formulation, or hyoscyamine in these reports. In several reports, atropine was administered sublingually on a regular regimen versus the single dose per timepoint as proposed in this study. No severe adverse reactions were reported, and the side effects observed were consistent with known anticholinergic effects previously observed with oral or parenteral administration, e.g., dry mouth. In one study of children in Brazil with cerebral palsy and drooling, side effects occurred in 4 of 33 (12%) patients. These side effects and their respective frequency were as follows: fever and flush (1), irritability (1), flush and irritability (1) and flush and angioedema(1)[5]. Further characterization of these events was not provided. Norderyd et al. prospectively studied the effect of sublingual atropine in children and adolescents 5 through 18 years old with disabilities and excessive salivation (n=23). During the course of the study, 1064 person-doses of atropine were administered to children without any reported adverse events (AEs)[3].

A study of sublingual injection of atropine is included here since it is a useful evaluation of atropine PK, and the atropine dose used was two-fold greater than the 1 mg dose to be used in the current study. Rajpal et al. [7] evaluated 9 healthy male volunteers (mean age 20.8 ± 4.7 years, mean weight 59.67 ± 4.76 kg) administered a single sublingual injection of 2 mg atropine sulfate (in 0.1 mL). Three of the subjects had ^{99m}Tc-labeled diethylene triamine pentaacetic acid (DTPA) added to their injection to monitor the release rate of atropine sulfate. Within 10 minutes, 85% of the injected dose was released from the sublingual site of injection. This compared to only 24% released at 10 minutes from one subject who received intramuscular atropine. The remaining six subjects underwent blood sampling for PK and clinical monitoring for signs of atropinization (heart rate, pupil diameter, and mouth dryness). The peak serum concentration of sublingual atropine was 20 ng/mL at 15 minutes compared to peak serum concentrations of 6 to 8 ng/mL at 30 minutes following intramuscular administration. All volunteers who received atropine by sublingual injection showed signs of atropinization by 10 minutes with peak intensity at 30 minutes, and persistence of symptoms for nearly 60 minutes. The authors concluded that atropine administered by sublingual injection achieves clinically important serum levels more rapidly than by intramuscular administration. Adverse events were not reported in this trial.

Based on the reported experience to date, the administration of atropine via the sublingual route appears to have an acceptable safety profile and does not significantly increase the risk associated with the use of the drug product as proposed.

3. STUDY OBJECTIVES

3.1. Primary Objective

- To evaluate the bioavailability and PK of atropine administered sublingually against the reference intravenous route of administration

3.2. Secondary Objectives

- To evaluate the safety of atropine administered sublingually
- To evaluate the tolerability of atropine administered sublingually, as assessed by changes in xerostomia

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design

This is a randomized, three-sequence, three-period crossover study to assess the bioavailability and PK of a single dose of atropine administered sublingually in healthy adult volunteers. At least 15 healthy male and female volunteers will be enrolled to obtain approximately 12 evaluable subjects in the per protocol population. Eligible subjects will be randomized at a 1:1:1 ratio to receive one of three dosing sequences (A, B, or C) as depicted in Table 2.

Table 2: Study Design Scheme by Dosing Sequence

Dosing Sequence	Expected Number of Evaluable Subjects (N)	Period 1 (Visit 1; Day 1)	Period 2 (Visit 2; Day 8)	Period 3 (Visit 3; Day 15)
A	4	Low Dose sublingual	High Dose sublingual	IV
B	4	High Dose sublingual	IV	Low Dose sublingual
C	4	IV	Low Dose sublingual	High Dose sublingual

Low Dose sublingual = 0.5 mg (50 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; High Dose sublingual = 1.0 mg (100 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; IV = 1.0 mg (2.5 mL) of atropine sulfate (0.4 mg/mL) administered via intravenous route

Figure 1 presents a diagram of the overall study design. After screening, subjects meeting all of the inclusion criteria and none of the exclusion criteria will be randomized to one of three dosing sequences as presented in Table 2. Once randomized, each subject will receive 3 doses of atropine according to their assigned dosing sequence. Each dose will be separated by a washout period of 6 ± 1 days. Blood samples for measurement of atropine plasma concentrations and to determine PK parameters will be collected during each dosing visit at the following time points: time 0 (predose), and postdose at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and 2, 4, 6, and 8 hours. Site staff will record subjects' reports of their subjective xerostomia predose and every 10 minutes up to the first hour after dosing. Subjects will be discharged from the clinic after the 8 hour blood sample collection. Subjects will be followed for approximately 6 days after their last dose.

On the day of atropine administration, each subject:

1. Will not be allowed food or drink 1 hour prior to drug administration and continuing through 1 hour after drug administration or until the subject reports a 10 (maximum) on the xerostomia assessment or expresses the xerostomia is intolerable, whichever occurs first. After 1 hour or if either of these conditions are met, water will be provided ad libitum.
2. Will be provided a meal/snack no less than 4 hours after drug administration.
3. Will abstain from alcohol 24 hours before each dosing visit and until the last blood sample from each dosing visit is collected.

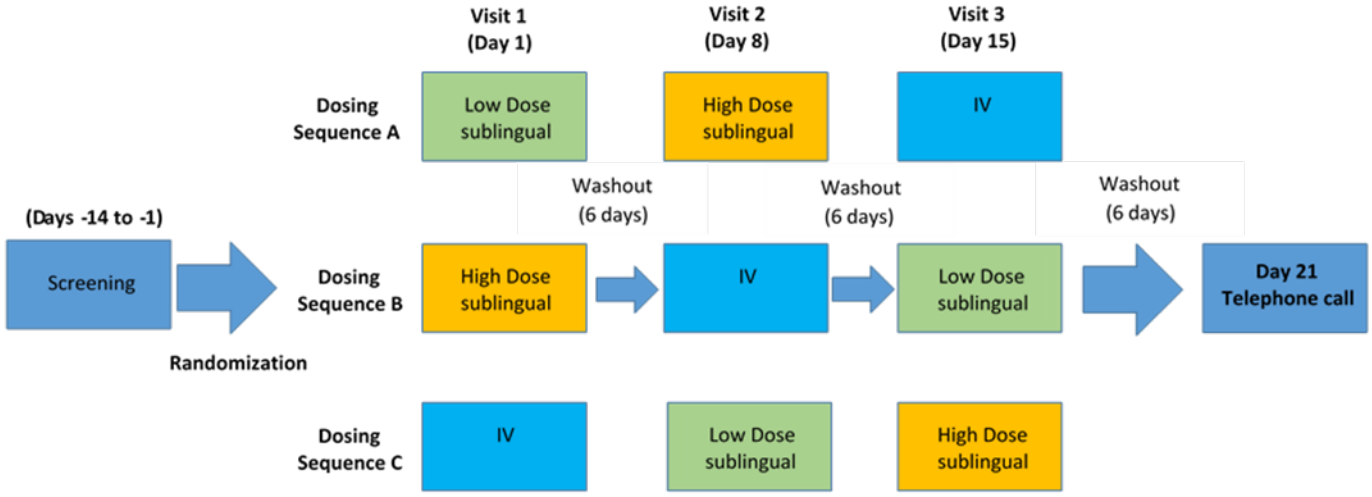
4. Will report to site staff their subjective sense of xerostomia every 10 minutes up to the first hour after dosing or until maximum/intolerable xerostomia is reached, whichever occurs first, based on the questions in Appendix 3.
5. Will have automated blood pressure and heart rate measurements recorded on the opposite arm from blood collection every 10 minutes for the first hour, every 20 minutes for the second hour, and every 30 minutes for the third and fourth hours and thereafter as deemed clinically necessary by the investigator until the end of each visit.

Meal timing, activity levels, and general conditions in the clinical research unit will be as similar as possible for all study subjects irrespective of dose and day.

Safety will be assessed by interval medical history and physical examinations, vital signs, and standard 12-lead electrocardiograms (ECGs) as outlined in the Schedule of Events (Appendix 2).

The expected study duration is approximately 35 days per subject.

Figure 1: Overall Study Design



Low Dose sublingual = 0.5 mg (50 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; High Dose sublingual = 1.0 mg (100 µL) of atropine sulfate 1% ophthalmic solution, administered via sublingual route; IV = 1.0 mg (2.5 mL) of atropine sulfate (0.4 mg/mL) administered via intravenous route

4.2. Number of Subjects

The proposed enrollment for this study is approximately 15 subjects in stable health, aged 18 through 55 years, inclusive (Section 5.1). Additional subjects may be enrolled to obtain approximately 12 evaluable subjects (approximately 4 per dosing sequence) in the per protocol population.

4.3. Treatment Assignment

Each subject will receive 3 doses of atropine according to their assigned dosing sequence: one low dose sublingually, one high dose sublingually, and one dose IV. Each dose will be separated by 7 ± 1 days. Subjects will be randomized 1:1:1 across 3 dosing sequences (approximately

5 subjects per dosing sequence) to achieve approximately the expected number of evaluable subjects (12) in the per protocol population as specified in Table 2.

4.4. Individual Subject Dosing and Study Stopping Rules

4.4.1. Early Termination of Dosing for an Individual Subject

If a subject is confirmed pregnant, she will not receive further dosing of the study drug.

If a subject meets one or more of the criteria below, then the subject's dosing will be paused until the investigator has discussed the subject with the Rho medical monitor and the BARDA Pharmacovigilance Subject Matter Expert:

- Subject experiences any Grade 3 or higher AE (Common Terminology Criteria for Adverse Events [CTCAE] v 5.0) [8] that, in the opinion of the investigator, is at least possibly related to the study drug.
- Subject experiences an AE that, in the opinion of the investigator, makes further dosing inadvisable because the AE causes functional impairment or because the AE requires a medical intervention or observation for safety.
- Subject no longer meets eligibility criteria in such a way that, in the judgment of the investigator, the safety of the subject may be compromised by continued participation or interpretation of the subject's subsequent study data are likely to be significantly compromised.

Once a subject has been reviewed by the Rho medical monitor and the BARDA Pharmacovigilance Subject Matter Expert, they will recommend to either (1) continue dosing per the protocol or (2) discontinue further dosing of the subject. In cases of uncertainty, Rho and BARDA can also request the input of the independent medical monitor.

Subjects who do not complete all study drug doses for any reason will continue to be followed for safety for six days following the last dose.

The reason for early discontinuation of dosing will be captured in the electronic case report form (eCRF).

4.4.2. Study Pausing Rules

If the following criterion is met, further dosing of all subjects will be paused.

- Two or more subjects experience a grade 3 AE in the same Medical Dictionary for Regulatory Activities (MedDRA) system organ class deemed at least possibly related to the study drug by the investigator, BARDA, or Rho medical monitor.

At that time, the Rho medical monitor, the independent medical monitor, and the BARDA Pharmacovigilance Subject Matter Expert will convene to discuss and review these cases. After this review, it may be decided to either continue to pause dosing pending review of additional data, resume dosing as specified in the protocol, modify the study, or terminate the study. If the individuals designated above cannot come to agreement, the independent medical monitor will make the final decision. The clinical principal investigator may be present at these meetings to provide additional information but will not vote on the final decision.

BARDA retains the right to suspend, modify, or end the study at any time. In case of premature termination or suspension and safety review of the study, Rho will promptly inform the investigator of the termination or suspension of the study and the reason for termination or suspension, and the clinical site will inform the institutional review board (IRB).

4.5. Independent Medical Monitor Review

An independent medical monitor will review the protocol, adjudicate individual stopping and study pausing rules if they occur, and provide guidance regarding subject safety, trial conduct, and data integrity as requested by Rho and/or BARDA. The independent medical monitor will perform these functions while protecting the integrity and confidentiality of the trial data.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1. Selection of Study Population

Subjects will be randomized to study treatment only if they meet all of the applicable inclusion criteria and none of the exclusion criteria. In addition, eligibility criteria must be reviewed just prior to each dose of atropine unless specified below; if the subject no longer meets applicable eligibility criteria, the investigator, in consultation with the Rho medical monitor in cases of uncertainty, must determine whether the subject should receive the atropine dose or be terminated early from study drug.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study or subject safety. Therefore, adherence to the criteria specified in the protocol is essential.

5.1.1. Subject Inclusion Criteria

1. Healthy male and nonpregnant female volunteers between the ages of 18 and 55 years at time of randomization
2. Willing and able to provide written informed consent
3. Females who are of childbearing potential and are sexually active with a male partner must have used an acceptable method of birth control for at least 2 months prior to Screening, and must agree to continue using an acceptable method of birth control from Screening to Follow-up (Day 21)

   A female of childbearing potential is defined as postonset menarche and premenopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: menopausal > 2 years, tubal ligation > 1 year, bilateral salpingo-oophorectomy, or hysterectomy

   Adequate contraception is defined as a contraceptive method with a failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label. Examples include oral contraceptives, injectable progestogen, implants of etonogestrel or levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, intrauterine device or intrauterine system, or male partner sterilization at least 6 months prior to the female subject's Screening Visit
4. In the judgment of the investigator, the subject is in good health, based on review of medical history and the results of screening evaluation (including vital signs, physical examination, 12-lead ECG, and routine clinical laboratory testing, performed no more than 14 days prior to randomization into the study)
5. Able to comply with the dosing instructions and available to complete the study Schedule of Events (Appendix 2)

5.1.2. Subject Exclusion Criteria

1. Females who have a positive pregnancy test or who are breastfeeding

2. Subjects with thyroid disease as evidenced by a thyroid-stimulating hormone (TSH) < 0.9 × lower limit of normal (LLN) or > 1.2 × upper limit of normal (ULN) at screening. (This test will not be repeated prior to subsequent dosing.)
3. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT), or serum creatinine > 1.5 × ULN at screening. (These tests will not be repeated prior to subsequent dosing.)
4. Have known human immunodeficiency virus (HIV), or acute or chronic hepatitis B or hepatitis C infection based on medical history; or test positive for any of these at Screening. Subjects who have been effectively treated for hepatitis C, as evidenced by a negative hepatitis C ribonucleic acid (RNA) confirmation test and who no longer require antiviral therapy, are eligible for participation. (Screening tests will not be repeated prior to subsequent dosing.)
5. Subjects who took any prescription medications (with the exception of oral contraceptives or hormone replacement therapy) within 30 days of screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the subject should be terminated from further dosing.
6. Subjects who took any over-the-counter medication/vitamins/herbal supplements in the last 72 hours prior to screening. Prior to each dose, the investigator will review prohibited medication use and determine whether the subject should be terminated from further dosing.
7. Subjects with glaucoma and/or history of ocular surgery (including Lasik™), ocular trauma, or congenital ocular disorder
8. Subjects with any history of heart disease including but not limited to coronary artery disease, arrhythmia (treated or untreated), congestive heart failure, pacemaker, history of vasovagal syncope, peripheral vascular disease, or claudication
9. Subjects with clinically significant arrhythmias or abnormal conduction; abnormal conduction is defined as a prolonged PR or QRS, or a QTc ≥ 450 msec for males or ≥ 470 msec for females
10. Subjects with a history of partial organic pyloric stenosis, chronic constipation, or other gastrointestinal motility issue
11. Subjects with a history of xerostomia due to an underlying disease or previous radiation therapy to the head and neck
12. Males with history of symptomatic prostatic hypertrophy; males or females with a history of hesitancy or retention
13. Subjects with a blood pressure > 140/90 mm Hg taken after the subject has been seated and resting for at least five minutes
14. Subjects with a history or current diagnosis of myasthenia gravis
15. Subjects with a history of drug or alcohol abuse in the last two years or evidence of a positive urine drug test at screening. (This screening test will not be repeated prior to subsequent dosing.)

16. Subjects with a known sensitivity or prior adverse reaction to atropine

Subjects cannot be rescreened for exclusionary laboratory test results. Potentially exclusionary vital sign results may be repeated once. If a subject's repeat vitals remain exclusionary or the investigator determines that the repeat vital signs could pose a risk to the subject participating in the study, then the subject will be excluded.

5.2. Subject Withdrawal Criteria

Every subject has the right to refuse participation in the study (i.e., withdraw consent) at any time without providing any reason for withdrawal. A subject's participation must be discontinued immediately upon his/her request, and the reason(s) for discontinuation will be documented accordingly in the corresponding eCRF.

Any subject who meets 1 or more of the following criteria will be removed from the study:

- Subject request
- Subject noncompliance, defined as refusal or inability to adhere to the study protocol or any other instances determined by the investigator or BARDA
- Subject lost to follow-up
- Investigator no longer believes participation is in the best interest of the subject
- At request of BARDA
- At request of the IRB.

For any subject who is withdrawn prior to completion of the Follow-up (Day 21) Visit, the Early Termination (ET) Visit assessments will be performed as outlined in the Schedule of Events (Appendix 2), when possible. The ET Visit may be conducted in clinic or by phone as determined by the investigator. Applicable eCRFs will be completed for each ET.

5.3. Replacements

If a subject is randomized but does not receive the first dose of study drug, the subject will be withdrawn and will not be counted toward the total enrollment goal. Subjects who withdraw before receipt of the first dose of study drug will be replaced.

If a subject is withdrawn from the clinical study after receipt of the first dose of study drug and prior to completing the third dose of study drug, the subject will be removed from the per protocol population. Additional subjects may be randomized to achieve the target number of evaluable subjects within the per protocol analysis population.

6. TREATMENT OF SUBJECTS

6.1. Description of Study Drugs

6.1.1. Atropine Sulfate Ophthalmic Solution, USP 1%

Atropine sulfate ophthalmic solution, USP 1% manufactured by AKORN LABORATORIES, INC, is a sterile topical anti-muscarinic indicated for cyclopegia, mydriasis, and penalization of the healthy eye in the treatment of amblyopia. Each mL of Atropine Sulfate Ophthalmic Solution USP, 1% contains active ingredient: atropine sulfate 10 mg equivalent to 8.3 mg of atropine. Inactive ingredients include benzalkonium chloride 0.1 mg (0.01%), dibasic sodium phosphate, edetate disodium, hypromellose (2910), monobasic sodium phosphate, hydrochloric acid and/or sodium hydroxide may be added to adjust pH (3.5 to 6.0), and water for injection, USP.

Atropine Sulfate Ophthalmic Solution, USP 1% will be supplied in dropper bottles containing 2 mL. Each bottle will only be used to administer a single dose, to a single subject.

6.1.2. Atropine Sulfate Injection

Atropine sulfate injection is indicated for temporary blockade of severe or life-threatening muscarinic effects, e.g., as an antisialagogue, an antivagal agent, an antidote for organophosphorus, carbamate, or muscarinic mushroom poisoning, and to treat symptomatic bradycardia.

Atropine sulfate injection, USP,8mg/20mL (0.4 mg per mL) manufactured by Fresenius Kabi USA, LLC is a sterile, nonpyrogenic, isotonic, clear solution of atropine sulfate in water for injection with sodium chloride sufficient to render the solution isotonic. Each mL contains atropine sulfate, 0.4 mg; benzyl alcohol, 9 mg; sodium chloride 9 mg; and may contain sulfuric acid for pH adjustment, pH 3.5 (3.0 to 3.8).

Atropine sulfate injection will be supplied in multidose vials containing 20 mL. Each vial will only be used to administer a single dose, to a single subject.

6.2. Concomitant Medications

Any treatment, including all prescription drugs, herbal products, vitamins, minerals, and over-the-counter (OTC) medications, administered from the time of consent through the end of study participation is considered a concomitant medication. Concomitant medication use will be recorded in the eCRF and will include the medication name, dose, frequency, route of administration, and the dates of administration. All concomitant medications used by the subject from the time of consent until the Follow-up (Day 21) Visit will be recorded in the subject's eCRF. Any changes or additions to concomitant medications throughout the course of the subject's participation in the study will be recorded in the subject's eCRF.

If a subject is discovered to be using a prohibited concomitant medication after he or she is enrolled in the study, the investigator, in consultation with the Rho medical monitor in cases of uncertainty, should determine the impact on the subject's participation. All instances of use of prohibited concomitant medications must be documented on the appropriate eCRFs.

6.2.1. Permitted and Prohibited Concomitant Medications

Contraceptive medications and estrogen or estrogen/progesterone hormone replacement therapy are permitted for female subjects. Nonsteroidal anti-inflammatory drugs and acetaminophen are permitted for all subjects. No other concomitant medications are allowed.

6.3. Treatment Compliance

The study drug will be administered by an unblinded study staff member and thus is an observed compliance. Subject compliance will be determined by the number and percentage of subjects who receive study drug at Visits 1, 2, and 3 by dosing sequence. Any deviations from the dosing schedule outside the defined visit windows (Appendix 2) must be approved by BARDA and will be recorded on the appropriate eCRF.

7. STUDY DRUG MATERIALS AND MANAGEMENT

7.1. Study Drug

Refer to Section 6.1 for detailed information regarding the atropine sulfate ophthalmic, USP 1% and the atropine sulfate injection.

7.2. Study Drug Packaging and Labeling

Study drug will be packaged and labeled according to applicable local and regulatory requirements.

7.3. Study Drug Storage

Study drug must be stored in a secure area (e.g., a locked room or locked cabinet), and protected from light and moisture. Atropine sulfate ophthalmic, USP 1% and atropine sulfate injection should be kept at controlled room temperature between 20° to 25°C (68° to 77°F), inclusive. The temperature of the storage unit must be monitored and documentation of proper storage must be maintained.

7.4. Study Drug Preparation and Administration

Because atropine sulfate ophthalmic, USP 1% and atropine sulfate injection are distinguishable visually and by route of administration, unblinded study staff will be responsible for preparing and administering study drug.

Study drug will be visually inspected for particulate matter and discoloration prior to administration, whenever solution and container permit; it should not be administered if either condition exists. Study drug will not be administered if the seal is not intact immediately prior to dosing. Each bottle/vial of atropine will only be used to administer a single dose. After initial use, the used bottles/vials should be maintained in a separate location from the unused bottles/vials until drug accountability has been reconciled. Study drug will not be destroyed until authorized in writing by BARDA.

Atropine sulfate ophthalmic, USP 1% will be administered sublingually. For low dose (0.5 mg) sublingual treatment, 50 µL of atropine sulfate ophthalmic, USP 1% will be administered via the sublingual route. For high dose (1.0 mg) sublingual treatment, 100 µL of atropine sulfate ophthalmic, USP 1% will be administered via the sublingual route. Before administration of sublingual atropine, subjects will be asked to swallow. Following administration, subjects will be instructed to attempt not to swallow for 30 seconds and thereafter swallow as they normally would.

For intravenous administration (1.0 mg), 2.5 mL of atropine sulfate injection (0.4 mg/mL) will be administered via an indwelling IV cannula.

7.5. Study Drug Accountability

The investigator is required to maintain adequate records of the disposition of the study drug, including the date and quantity of drug received, to whom the drug was dispensed (subject-by-subject accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A study drug dispensing log will be kept current and will include identification of each subject and the date and quantity of study drug dispensed. All records regarding the disposition of the study product will be available for inspection by the study monitor and BARDA.

7.6. Study Drug Handling and Disposal

After dosing has been completed, to satisfy regulatory requirements regarding accountability, all study drug will be reconciled and destroyed according to applicable regulations. Study drug will not be destroyed until authorized in writing by BARDA.

7.7. Randomization

7.7.1. Randomization

Subjects 18 through 55 years of age will be randomized 1:1:1 to receive one of three dosing sequences (A, B, or C) as specified in Table 2. The randomization schedule will be generated by the statistical team at Rho using SAS. Due to the low randomization counts, randomization will not be stratified.

7.7.2. Blinding

This study is not blinded.

8. STUDY ASSESSMENTS

8.1. Pharmacokinetic Assessments

Blood samples will be collected from each subject for PK assessments by a designated, qualified individual from the study research team. An indwelling venous catheter will be placed before dose administration for blood sample collection for serial determinations at the following times: 0 (predose) and postdose at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes, and 2, 4, 6, and 8 hours. For IV atropine administration, serial blood sampling will be performed via indwelling catheter on the opposite arm from the one used for dosing. Ten (10) mL of whole blood will be collected into a sterile vacutainer containing ethylenediaminetetraacetic acid (EDTA) as a preservative for each sampling. Plasma will be stored frozen until analysis.

Pharmacokinetic samples will be analyzed by a bioanalytical facility approved by BARDA.

8.2. Xerostomia Assessment

Subjects will be assessed for xerostomia as described in Appendix 3. Subjects will be asked 3 xerostomia assessment questions predose and after each dose of atropine. Subjects will be asked to rate xerostomia postdose every 10 minutes until one hour has passed, or the subject scores a 10 on one or more of the xerostomia questions, or reports intolerable xerostomia, whichever occurs first.

8.3. Safety Assessments

Safety will be evaluated utilizing the assessments defined in this section. Subjects may experience AEs that necessitate an Unscheduled Visit (UV). There may also be situations in which the investigator asks a subject to report for a UV following the report of an AE. Additional assessments may be conducted at these visits as necessary to ensure the safety and well-being of subjects during the study (Appendix 2). If deemed clinically necessary, additional safety assessments not currently specified in the protocol may be performed at the discretion of the investigator in consultation with the Rho medical monitor and BARDA. Applicable eCRFs will be completed for each UV.

8.3.1. Demographic/Medical History

In order to define a baseline for potential AEs, the demographics and medical history of each subject will be collected at the Screening Visit and recorded on the appropriate eCRFs. Updated medical history will be collected at Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), Visit 3 (Day 15 Predose), UV, and ET Visits.

8.3.2. Tobacco/Vaping History

In order to qualify responses to the xerostomia questionnaire, it will be important to know whether subjects have ever used tobacco products or vaped. At Screening, Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), Visit 3 (Day 15 Predose), UV, and ET Visits, subjects will be asked whether they currently use or have ever used tobacco and whether they currently vape or have ever vaped. Subjects currently using tobacco or vaping will be asked when they last used tobacco or vaped. Subjects will also be asked additional questions regarding the type, frequency,

and duration of tobacco use well as the frequency and duration of vaping. Responses will be recorded in the eCRF.

8.3.3. Vital Signs

Vital sign measurements, including oral temperature, heart rate, respiratory rate, and diastolic and systolic blood pressure (after the subject is seated for at least 5 minutes), will be collected at Screening, Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), and Visit 3 (Day 15 Predose) prior to any blood draws. Postdose automated blood pressure and heart rate measurements will be recorded on the opposite arm from blood collection every 10 minutes for the first hour, every 20 minutes for the second hour, and every 30 minutes for the third and fourth hours and thereafter as deemed clinically necessary by the investigator until the end of each visit. Vital signs may be repeated at UV or ET Visits per the investigator's discretion. Vital signs will be recorded on the appropriate eCRF.

Respiratory rate, if regular, may be assessed over 30 seconds and doubled, but in no case will it be assessed over a period of less than 30 seconds. If the respiratory rate is irregular, it will be assessed over 60 seconds.

8.3.4. Height and Weight

Subject's height and weight will be taken and body mass index (BMI) calculated at the Screening Visit and recorded on the appropriate eCRF. Subject's weight will be taken again at Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), and Visit 3 (Day 15 Predose). Weight may be measured at UV and ET Visits per the investigator's discretion. Measurements will be recorded on the appropriate eCRF.

8.3.5. Physical Examination

A physical examination will be performed at the Screening Visit to assess and confirm eligibility. The examination will include a general assessment of the skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, and musculoskeletal system/extremities, and a neurological (cranial nerve examination, including pupillary diameter, eye movements, and deep tendon reflexes) examination; the results will be recorded on the appropriate eCRF. A symptom-based physical examination may be performed at dosing visits, UV, or ET Visits per the investigator's discretion.

8.3.6. Electrocardiogram

A standard 12-lead ECG will be recorded and assessed at the Screening Visit and results recorded on the appropriate eCRF. Additionally, an ECG will be performed within 5 minutes after IV atropine administration has been completed and repeated as needed at the investigator's discretion during that visit. ECGs may also be performed at UV or ET Visits per the investigator's discretion. ECGs will be reviewed by a medically qualified individual to verify whether any abnormalities are clinically significant. In general, clinically significant abnormal ECGs are expected to be associated with an item recorded in the subject's medical history (when detected at Screening) or as an AE (when detected after Screening).

8.3.7. Clinical Laboratory Assessments

Venous blood will be collected for routine clinical laboratory safety evaluations at Screening. Clinical laboratory assessments may be repeated at UV and ET Visits as clinically indicated per the investigator's discretion. Urine will be collected at the Screening Visit for a drug screen. Sample collection information will be entered on the appropriate eCRFs. Samples will be shipped to the central laboratory for analysis. The details for sample handling, processing, and shipping will be provided in the Laboratory Manual.

Individual results will be sent to the clinical site, and the investigator will perform a clinical assessment of all laboratory safety data to assess eligibility and identify and document AEs as applicable. All results will be transferred electronically directly from the central laboratory to the clinical site and Rho using standard secure data transfer procedures.

The clinical laboratory assessments planned for this study include chemistry, hematology, and a urine drug screen as follows:

8.3.7.1. Chemistry

- ALT
- AST
- blood urea nitrogen
- alkaline phosphatase
- total carbon dioxide
- creatinine
- glucose, random, serum
- direct bilirubin
- total bilirubin (fractionated for values for total bilirubin > ULN)
- sodium
- potassium
- chloride

8.3.7.2. Hematology

Table 3 lists the parameters of the complete blood count with differential.

Table 3: Complete Blood Count with Differential

Red blood cells	**White blood cells**
Hematocrit	Basophils (% and absolute count)
Hemoglobin	Eosinophils (% and absolute count)
Red blood cell count	Lymphocytes (% and absolute count)
Platelets	Monocytes (% and absolute count)
Platelet count	Neutrophils (% and absolute count)
	White blood cell count

8.3.7.3. Urine Drug Screen

The urine drug screen includes tests for amphetamines, cocaine, tetrahydrocannabinol, methylenedioxymethamphetamine, and opiates.

8.3.8. Pregnancy Screen

At the Screening Visit, blood will be collected for a serum pregnancy test for female subjects of childbearing potential. At Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), and Visit 3 (Day 15 Predose), a urine dipstick pregnancy test will be performed onsite for female subjects of childbearing potential.

Additionally, a urine dipstick pregnancy test will be performed at any time during study participation if pregnancy is suspected.

8.3.9. Adverse Event Assessment

Site staff will ask nonleading questions regarding the subject's health status (Section 8.5.1) and document any new or changed AEs on the appropriate eCRF.

8.3.10. Concomitant Medication Assessment

Site staff will review concomitant medications (Section 6.2) and document any new or changed medications on the appropriate eCRF.

8.4. Adverse and Serious Adverse Events

8.4.1. Definition of Adverse Events

8.4.1.1. Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered drug related [21 Code of Federal Regulations (CFR) 312.32(a)]. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (Integrated Addendum to International Conference on Harmonisation [ICH] E6[R1]: Guideline for Good Clinical Practice [GCP] E6[R2]).

Laboratory results and vital sign excursions of any magnitude will be defined as AEs if they are considered clinically significant by the investigator.

Xerostomia will not be considered an AE for this protocol.

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. An SAR implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug [21 CFR 312.32(a)].

An AE or SAR is considered "unexpected" if it is not listed in the package inserts for the study drugs or is not listed at the specificity or severity that has been observed.

8.4.1.2. Serious Adverse Events

An AE or SAR is considered serious if, in the view of either the investigator or BARDA, it results in any of the following outcomes [21 CFR 312.32(a)]:

- Death
- Life-threatening AE that, in the view of the investigator or BARDA, places the subject at immediate risk of death. This does not, however, include an event that might have caused death had it occurred in a more severe form.
- Requires inpatient hospitalization or prolongs existing hospitalization. Planned hospitalizations will **not** be reported as serious adverse events (SAEs) unless categorized as medically important. Emergency room visits and observational admissions of under 24 hours, in themselves, do not qualify as SAEs.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience, when based on appropriate medical judgment, they may jeopardize the subject or the subject may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.5. Collection, Recording, and Grading Severity of Adverse Events

8.5.1. Collection of Adverse Events

Adverse events of any type described above may be discovered through a variety of methods:

- Observing the subject
- Questioning the subject with standard nonleading questions to elicit any medically related changes in their well-being (e.g., Have you been hospitalized, had any medical problems, used any new medications, or changed or stopped any medications [both prescription and OTC]?)
- Receiving an unsolicited complaint from the subject
- An abnormal value or result from a clinical (e.g., vital signs) or laboratory evaluation

8.5.2. Recording of Adverse Events

Throughout the study, the investigator will record AEs on the appropriate eCRF. For the purposes of this study, any detrimental change in the subject's condition after signing informed consent and up to completion of the follow-up period after the last administration of study drug Follow-up (Day 21) will be considered an AE and must be recorded on the eCRF. Treatment-emergent AEs are defined as AEs occurring after the subject receives at least one dose of atropine. AEs will be followed per the below criteria:

- AEs judged related to study drug will be followed to resolution (with or without sequelae) or until considered stable by the investigator (in consultation with the Rho medical monitor in situations of uncertainty).

- AEs judged unrelated to study drug will be followed to resolution (with or without sequelae) or, if not resolving, until considered stable by the investigator (in consultation with the Rho medical monitor in situations of uncertainty), or until the end of the subject's study participation, whichever comes first.

8.5.3. Grading Severity of Adverse Events

The severity of AEs and SAEs will be graded using CTCAE version 5.0.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity, whereas seriousness is defined by the criteria in Section 8.4.1.2.

8.6. Relationship and Attribution to Study Drug

An investigator's causality assessment is the determination of whether a reasonable possibility exists that study drug caused or contributed to an AE, and must be provided for all treatment-emergent AEs (serious and non-serious). The investigator will assess the causality/relationship between the study drug and the AE and record that assessment in the appropriate eCRF.

BARDA's determination of attribution will be used for reporting to the appropriate health and regulatory authorities.

The relation and attribution of an AE to study treatment will be determined using the descriptors and definitions provided in Table 4.

Table 4: Attribution of Adverse Events

Unrelated Categories	
Not Related	The AE is clearly not related to study drug.
Unlikely Related	The AE is unlikely related to study drug.
Related Categories	
Possibly Related	The AE has a reasonable possibility to be related to study drug; evidence exists to suggest a causal relationship.
Probably Related	The AE is likely related to study drug.
Related	The AE is clearly related to study drug.

AE = adverse event

8.7. Reporting Safety Events to IRB

The investigator shall report adverse events, including SAEs, in a timely fashion to the IRB in accordance with applicable regulations and guidelines.

8.8. Reporting of Serious Adverse Events

The following process for reporting an SAE ensures compliance with ICH guidelines. Investigators and other study center staff must inform Rho pharmacovigilance personnel of any SAE that occurs (whether or not attributable to the study drug) in the study within 24 hours of when he or she becomes aware of the SAE. It is the investigator's responsibility to ensure that SAE reporting procedures are followed appropriately. In addition, the investigator must ensure

that these events are entered on the SAE eCRF via Rave EDC or via paper form, if Rave EDC is unavailable or if reported after database lock. The form will be entered into Rave EDC or faxed to the Rho pharmacovigilance personnel within 24 hours. The investigator is responsible for informing the IRB of the SAE as per local requirements. Upon notification of any SAE, Rho will inform BARDA within 1 business day.

The following variables will be recorded for each AE: verbatim/AE description, time and date for AE start and stop, maximum intensity, seriousness, causality rating, whether the AE caused the subject to discontinue, and the outcome.

Serious Adverse Event Reporting Contact Information:

Rho, Inc. Safety Group Email: rho_productsafety@rhoworld.com

Serious Adverse Event Help Line: 1-888-746-7231

Serious Adverse Event Fax Line: 1-888-746-3293

Follow-up information on SAEs must also be reported by the investigator by the same process, and within the same time frames. If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to Rho within 24 hours as described above. SAEs will be followed until resolution or until they become stable in the opinion of the investigator.

8.9. Pregnancy Reporting

This study includes pregnancy as safety data. Although pregnancy is not an SAE, information about any pregnancy in a female study subject will be reported promptly to Rho as an SAE for tracking purposes (Section 8.8).

Study treatment will be discontinued for the pregnant subject. The investigator shall counsel the pregnant subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The pregnancy will be documented on the appropriate eCRF when identified and at its conclusion.

Should pregnancy result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion, an SAE must be submitted to Rho using the SAE reporting procedures described in Section 8.8.

8.10. Reporting Other Safety Information

Investigators will promptly notify Rho, BARDA, and the IRB when an unanticipated problem involving risks to subjects or others is identified, which is not otherwise reportable as an AE.

9. STATISTICS

9.1. Endpoints

9.1.1. Primary Endpoints

The relative bioavailability of atropine administered sublingually versus intravenously as measured by the following key PK parameters:

- Area under the analyte concentration versus time curve to infinity (AUC_{∞})
- Area under the analyte concentration versus time curve to time of last quantifiable data point (AUC_t)
- Maximum measured plasma concentration (C_{max})
- Time to C_{max} (t_{max})
- Apparent terminal elimination half-life ($t_{½}$)
- Apparent total body clearance after extravascular administration (CL/F)
- Apparent total volume of distribution after extravascular administration (V_d/F)

9.1.2. Secondary Endpoints

- All treatment-emergent AEs and SAEs occurring during study participation
- Xerostomia assessment (Appendix 3), completed during screening and each day of dosing starting at predose, and every 10 minutes postdose up to 1 hour postdose

9.1.3. Measures to Minimize Bias

Permuted block randomization will be performed centrally by designated Rho staff and will balance enrollment between treatment dosing sequence (see Section 7.7.1 for more details).

9.1.4. Analysis Plan

Statistical analyses will be performed using SAS® software Version 9.3 or later. Phoenix WinNonLin Version 6.4 software will be used to generate PK estimates.

Due to the descriptive nature of the primary objective, inferential analyses are not planned.

Descriptive statistics (such as mean, standard errors, medians, quartiles, and ranges for continuous data, and percentages for categorical data) will be used to summarize subject characteristics and safety. These summaries will be presented overall and separately for each study dosage received.

Details of the statistical analyses, methods, and data conventions will be described in the Statistical Analysis Plan (SAP).

9.1.4.1. Analysis Populations

9.1.4.1.1. Safety Population

The safety population will include all subjects who are randomized and receive at least 1 study drug dose. Each subject's data will be analyzed according to the corresponding study dosage received at that time or randomized dosing sequence, as appropriate. The safety population will be used for all safety analyses, including xerostomia analyses.

9.1.4.1.2. PK Analysis Population

The PK analysis population will include all subjects who are randomized, receive at least 1 study drug dose, and have PK samples collected for that period. Each subject's data will be analyzed according to the corresponding study dosage received for the applicable period. The PK analysis population will be used for all descriptive PK analyses. Subjects in the PK analysis population will be considered evaluable for comparison between study dosages if they receive at least two study drug doses and have evaluable time-concentration profiles for the 2 applicable periods.

9.1.4.1.3. Per Protocol Population

The per protocol population will include all subjects who are randomized, receive all 3 study drug doses according to the randomized dosing sequence, and have PK samples collected for all 3 periods. In addition, subjects with major protocol deviations may be excluded if it is determined that the deviations affect the integrity of PK data. Each subject's data will be analyzed according to the corresponding study dosage received at that time. Primary and secondary analysis plans will be repeated in the per protocol population if it differs from the PK analysis population.

9.1.4.2. Primary Analyses

Pharmacokinetic parameters (e.g., C_{max}, AUC_t, AUC_∞, t_{max} and $t_{½}$) will be estimated for each subject and study dosage using the noncompartmental method, and will be summarized by study dosage using descriptive statistics. CL/F and V_d/F will be estimated and summarized similarly for both sublingual doses only. Each parameter will be compared between study dosages by analysis of variance (ANOVA), including terms for dosing sequence, dose, and period as fixed effects, and subject nested within sequence as a random effect in the model. Ratios of least-squares means of key plasma PK parameters and associated 90% confidence intervals (CIs) will be presented for the following comparisons of interest:

- Low Dose sublingual vs. IV
- High Dose sublingual vs IV
- Low Dose sublingual vs High Dose sublingual

Additional information on statistical methods will be described in the SAP for the study.

9.1.4.3. Secondary Analyses

The frequency of AEs and SAEs will be summarized by system organ class and preferred term. The risk of each event will be described using event rates and corresponding 95% CIs.

Xerostomia scores will be analyzed descriptively for each of the 3 questions on the xerostomia assessment at each timepoint, including mean, median, standard error, and range.

9.1.4.4. Interim Analysis

No interim analysis is planned for this study.

9.1.4.5. Final Analysis

A clinical study report will be written to include all PK, safety, and tolerability data throughout the study. For the final analysis, the study database will be monitored, cleaned, and locked per the Data Management Plan (DMP). Further details will be specified in the SAP.

9.1.4.6. Exploratory Analyses

Exploratory analyses will be defined in the SAP, if applicable.

9.2. Sample Size Considerations

The target sample size for this study is 15 subjects, allowing for a 20% dropout rate that would result in approximately 12 evaluable subjects (approximately 4 per dosing sequence) within the per protocol population. This study is descriptive in nature.

9.3. Statistical Considerations

9.3.1. Covariates

Due to the small sample size and cross-over design of this study, analyses adjusted for additional covariates beyond those specified for the primary analysis are not planned.

9.3.2. Subgroup Analyses

All exploratory analyses, including any subgroups that require additional consideration, will be discussed in the SAP, if necessary.

9.3.3. Missing Data

Standard procedures will be used to ensure that the data are as complete and accurate as possible. Due to the exploratory nature of this study, all descriptive summaries will be based upon all available data, and no imputation will be done.

9.4. Procedure for Documenting Deviations from the Planned Analyses

The principal features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol. Additional details will be included in the SAP before initiating analyses. Any changes to that plan will be documented in the final clinical study report and will be approved by BARDA before implementing the changed analysis.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

10.1. Inspection of Records

BARDA, Rho, and the IRB will be allowed to conduct site visits to the clinical facility for the purpose of monitoring, inspecting, or auditing any aspect of the study.

The investigator agrees to allow BARDA, Rho, and the IRB to inspect the clinical facility, including the study drug storage area, and all documentation relating to the study, including but not limited to all source documents, eCRFs, IRB submissions and approvals, study drug accountability logs, study drug temperature monitoring logs, regulatory documents, and correspondence.

10.2. Institutional Review Board

A copy of the protocol, informed consent form (ICF), any other subject-facing documents, and any proposed advertising/recruitment materials will be submitted to the IRB for written approval. Initial IRB approval and all materials approved by the IRB for this study, including the ICF and recruitment materials, must be maintained by the investigator and made available for inspection.

11. QUALITY CONTROL AND QUALITY ASSURANCE

Quality assurance (QA) includes all the planned and systematic actions that are established to ensure that the clinical study is performed and the data are generated, documented (recorded), and reported according to ICH GCP and local/regional regulatory standards.

A QA representative from Rho, who is independent of and separated from routine monitoring, may periodically arrange inspections/audits of the clinical study by reviewing the data obtained and procedural aspects. BARDA quality representatives may attend the audit with Rho QA. These inspections may include on-site inspections/audits and source data checks. Direct access to source documents is required for the purpose of these periodic inspections/audits.

11.1. Data Quality Assurance

Before enrolling any subjects in this study, BARDA personnel and the investigator will review the protocol, study drug package inserts, the eCRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs.

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate source documents for the subjects treated as part of the research under this protocol. In addition, the investigator agrees to provide access to those records to BARDA's study monitor and auditor. Study monitors will verify information in the eCRFs against the source documents.

Data collected at the study site will be entered accurately and contemporaneously by study staff into Medidata RAVE, a 21CFR11-compliant, internet-based, remote data entry system, which is backed up nightly with backup tapes saved in a secure, off-site location. Data will be provided using the subject's unique identification number, not name or initials; Rho and BARDA will not collect personally identifying information such as the subject's name or social security number. Subjects will provide demographic information such as race, ethnicity, and birth date. All elements of data entry (e.g., time, date, verbatim text, and the person performing the data entry) will be recorded within the RAVE system's audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations. Data collected by the laboratories will be transferred electronically directly from the laboratory to Rho using standard secure data transfer procedures. The analysis datasets will incorporate data from both sources. Data collected by Rho will be held in the strictest confidence, and are protected from access that could reveal personally identifying information about any subject in the study.

Clinical data management and data cleaning procedures (i.e., resolving errors and inconsistencies in the data) will be performed in accordance with applicable Rho and/or BARDA standards and validation plans to ensure the integrity of the data. Adverse events (including SAEs), medical history conditions, and concomitant medication terms will be coded using MedDRA and the World Health Organization Drug Dictionary, respectively.

The study database will be monitored and cleaned on an ongoing basis per the DMP. For the final analysis, the study database will be monitored, cleaned, and locked per the DMP.

After the end of study database lock, the study site will receive an electronic copy of all of their site-specific eCRF data as entered into Medidata RAVE for the study, including full discrepancy

and audit history. Additionally, all study data (STDM and ADaM) will be sent to BARDA electronically for storage. Rho will maintain a duplicate copy for its records.

11.2. Study Monitoring

According to ICH GCP guidelines, the sponsor of the study is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of data recorded on the eCRFs. This study will be monitored to ensure the maintenance of complete, accurate, legible, well-organized, and easily retrievable data. In addition, the study monitor will explain and interpret for the investigator all regulations applicable to the clinical evaluation of the study drug as documented in ICH guidelines.

It is the study monitor's responsibility to inspect the eCRFs and source documentation directly throughout the study to protect the rights of the subjects; to verify adherence to the protocol; to verify completeness, accuracy, and consistency of the data; to perform study drug accountability; and to confirm adherence of study conduct to any local regulations. Details will be outlined in the Clinical Site Monitoring Plan.

11.3. Protocol Deviations

11.3.1. Protocol Deviation Definition

A protocol deviation is any noncompliance with the IRB-approved study protocol, ICH GCP guidelines, protocol-specific operational documents, or applicable regulatory requirements. Protocol deviations will be captured during the study. BARDA will determine how the deviations will impact the study analysis populations. Prospective permission to deviate from protocol requirements (i.e., "study waivers") will not be granted for this study.

11.3.2. Reporting and Managing Deviations

The investigator has the responsibility to identify, document, and report deviations. Protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review. All deviations, regardless of the cause, must be documented on the appropriate eCRF, which will document at a minimum the date the deviation occurred, the date it was identified, a description of the deviation, and documentation of a corrective/preventative action. In addition, the investigator will report noncompliance to the IRB, as applicable.

Rho and/or BARDA may request discussion with the site investigator to determine the effect of any protocol deviation on a study subject and his/her further study participation, the effect of the deviation on the overall study, and corrective actions.

12. ETHICS

12.1. Ethics Review

Before study initiation, the protocol, the informed consent documents, any other subject-facing documents, and any proposed advertising/recruitment materials will be reviewed and approved by the IRB. Prior to study initiation, the site will procure the BARDA-approved study drug from their drug depot. The site will be activated when all approvals are in place and study drug has been received. Any amendments to those documents will be submitted to the IRB (following approval by BARDA) and must be approved by the IRB before they are implemented at the site. Protocol documents must be re-approved by the IRB annually. Only institutions holding a current US Federal Wide Assurance issued by the Office for Human Research Protections at HHS may participate in the study.

The investigator will promptly report all unanticipated problems involving risks to subjects to the IRB, as applicable. The investigator will not make any changes to the research conduct without BARDA and IRB approval, except where necessary to eliminate apparent immediate hazards to the subjects.

The investigator will provide progress reports to the IRB as required by the IRB. The investigator will provide a final report to the IRB after completion of participation in the study.

12.2. Ethical Conduct of the Study

The investigator will conduct the study in accordance with this protocol, the Declaration of Helsinki, current ICH GCP guidelines, and 21 CFR Part 50 (Protection of Human Subjects) and Part 56 (IRBs).

12.3. Written Informed Consent

The IRB's informed consent document template will be used to write the site-specific consent document prior to IRB approval. The investigator is responsible for ensuring that the subject fully understands the nature and purpose of the study during the informed consent process. Information will be given in both oral and written form. No subject should be obligated to participate in the study. The information must make clear that refusal to participate in the study or withdrawal from the study at any stage is without any prejudice to the subject's subsequent care. Subjects must be allowed sufficient time to inquire about the details of the study and to decide whether they wish to participate. Written informed consent will be obtained before the subject undergoes any study procedures.

The subject must be made aware of and give consent to direct access to his/her source medical records by study monitors, auditors, and the IRB. The subject will be informed that such access will not violate subject confidentiality or any applicable regulations. The subject will also be informed that he/she is authorizing such access by signing the ICF.

The investigator will retain the original signed informed consent, and each subject will be given a signed copy to keep for his/her records.

13. DATA HANDLING AND RECORDKEEPING

13.1. Confidentiality

Each subject will be assigned a unique identification number and these numbers, rather than names, will be used to collect, store, and report subject information. All biological samples will be labeled with a unique identification number rather than names. Site staff will not transmit documents containing personal health identifiers to BARDA or its representatives. Data reported in medical journals or scientific meetings will be presented in aggregate for subjects as a whole. No individual subject will be identified in any way.

13.2. Retention of Records

The investigator will retain all documentation relating to the study (including but not limited to ICFs, source documentation, study drug records, eCRFs, and essential documents) for a period of at least 2 years after completion of the study.

If this requirement differs from any local regulations, the local regulations will take precedence unless the local retention policy is less than 2 years.

At study closure, the investigator must inform BARDA, or designee, of the long-term storage location of the study's records. Following study closure, the investigator must inform BARDA if that location changes (e.g., the investigator leaves the institution where the study was conducted).

No study records will be destroyed without prior authorization from BARDA.

14. PUBLICATION POLICY

BARDA will be responsible for publication activities and will work with the investigators to define the manuscript/presentation development process, the number and order of authors, the publication/scientific meeting to which it will be submitted, and other related issues. BARDA has final approval authority over all such issues.

Data are the property of BARDA and cannot be published without prior authorization from BARDA, but data and publication thereof will not be unduly withheld.

The Assistant Secretary for Preparedness and Response (ASPR) Public Access Plan[9] and the National Institutes of Health Public Access Policy[10] will apply to this study. The ASPR-funded investigators will be required to submit an electronic version of final, peer-reviewed manuscripts resulting from this study to the National Library of Medicine's PubMed Central upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication.

15. REFERENCES

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APPENDIX 1. PUBLICATION REVIEW OF SUBLINGUAL ADMINISTRATION OF ATROPINE

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Antonello C, Tessier P. (1999). "Clozapine and sialorrhea: a new intervention for this bothersome and potentially dangerous side effect." J Psychiatry Neurosci. May;24 (3):250.	Adults	Chronic schizophrenia	Case reports	Treat clozapine-induced Hypersaliva-tion	1% ophthalmic solution	0.5 mg once daily	Not reported	3	3	"None of the patients reported any side effects."
Chaptini, L. A., et al. (2008). "Sublingual hyoscyamine spray as premedication for colonoscopy: a randomized double-blinded placebo-controlled trial." Am J Surg 196(1): 51-55.	Adults	Elective colonoscopy	Randomized double-blind, placebo controlled	Effect on colonoscopy quality and comfort	Hyoscyamine spray	0.25 mg	Single dose	100	50	"No significant difference in occurrence of side effects between the 2 groups." Vital signs comparable before, during and after procedure between the 2 groups.

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Comley C, Galletly C, Ash D. (2000). "Use of atropine eye drops for clozapine induced hypersalivation." Aust N Z J Psych. Dec;34(6):1033-4.	44 years	Chronic schizophrenia and associated depression	Case report	Treat clozapine-induced Hypersaliva-tion	1% ophthalmic solution	0.5 – 1.0mg once daily	Not reported	1	1	"There have been no adverse effects from this treatment."
De Simone, G. G., et al. (2006). "Atropine drops for drooling: a randomized controlled trial." Palliat Med 20(7): 665-671.	Adults	Esophageal and gastric cancer and drooling	Double-blind, cross-over, randomized, placebo controlled	Effectiveness for drooling	0.5% ophthalmic solution	0.5mg q6h	48 hours	22	21	No severe toxicity - no blurred vision, palpitations, heartburn, hesitancy, urinary retention. One patient discontinued for cognitive impairment thought due to severe chest infection.
Dias, B. L. Set al. (2017). Treatment of drooling with sublingual atropine sulfate in children and adolescents with cerebral palsy. Arq Neuropsiquiatr, 75(5), 282-287.	2 – 17 years	Cerebral palsy, drooling	Open-label, non-controlled	Effectiveness for drooling	0.5% ophthalmic solution	0.125mg thrice daily for weight 10-19 kg; 0.5mg thrice daily for weight >= 20 kg	30 days	33	25	Side effects in 4/33 (12%): fever, flush, irritability, angioedema

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Dumot, J. A., et al. (1998). Sublingual hyoscyamine for patient comfort during screening sigmoidoscopy: a randomized, double-blind, placebo-controlled clinical trial. Gastrointest Endosc, 48(3), 283-286.	25 – 83 years	Patients undergoing screening sigmoidoscopy	Double-blind, placebo controlled	Efficacy for comfort during screening sigmoidoscopy	Hyoscyamine 0.125 mg tablets	0.5 mg (2 tablets 10 min prior to sigmoidoscopy)	1 day	150	75	No treatment related adverse effects reported
Ghobrial, P. M., et al. (2014). Cine MR enterography grading of small bowel peristalsis: evaluation of the antiperistaltic effectiveness of sublingual hyoscyamine sulfate. Acad Radiol, 21(1), 86-91.	Not Described	Patients undergoing magnetic resonance enterography (MRE)	Open-label, non-controlled	Effect on peristalsis	Hyoscyamine 0.125 mg tablets	0.5mg 1 hour before MRE	Single dose	92	92	Safety was not discussed

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Hyson, H. C., et al. (2002). "Sublingual atropine for sialorrhea secondary to parkinsonism: a pilot study." Mov Disord 17(6): 1318-1320.	62-82 years	Parkinson's disease/ Parkinsonism and drooling	Open-label, non-controlled	Effectiveness for drooling	1% ophthalmic solution	0.5 mg twice daily	1 week	7	7	No complaints of worsening constipation, blurred vision, dizziness, confusion;
Jones, J. B., & Dula, D. J. (1998). The efficacy of sublingual hyoscyamine sulfate and intravenous ketorolac tromethamine in the relief of ureteral colic. Am J Emerg Med, 16(6), 557-559.	18 years or older	Ureteral colic due to calculi	Randomized open-label, two-arm study	Effect on ureteral colic	Hyoscyamine sulfate	0.125 mg	Single dose	49	24	Safety was not discussed

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Jones, J., et al. (2001). Sublingual hyoscyamine sulfate in combination with ketorolac tromethamine for ureteral colic: a randomized, double-blind, controlled trial. Ann Emerg Med, 37(2), 141-146.	18 years or older (44 years mean)	Ureteral colic	Double-blind, two-arm study	Effect on ureteral colic	Hyoscyamine sulfate	0.125 mg	Single dose	43	23	Safety was not discussed
Lynch, C. R., et al. (2007). "Sublingual L-hyoscyamine for duodenal antimotility during ERCP: a prospective randomized double-blinded study." Gastrointest Endosc 66(4): 748-752.	Adults	Patients undergoing ERCP	Randomized double-blind, placebo controlled	Effect on glucagon needed during ERCP	Hyoscyamine	0.5 mg	Single dose	200	101	No significant difference between the two groups in incidence of adverse drug effects including nausea, vomiting, and xerostomia at 2 and 24 hours, tachycardia, hypotension, hypoxemia.

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Matos Santana, T. E., et al. (2017). "Sublingual atropine in the treatment of clozapine-induced sialorrhea." Schizophr Res 182: 144-145.	Adults	Schizophrenia or schizoaffective disorder	Case reports	Effectiveness for clozapine-induced sialorrhea	1% ophthalmic solution	0.5-1 mg once to twice daily	> 1 week	3	3	"None of the patients reported any systemic side effects"
Mustafa, F. A., et al. (2013). "Sublingual atropine for the treatment of severe and hyoscine-resistant clozapine-induced sialorrhea." Afr J Psychiatry (Johannesbg) 16(4): 242.	46 years	Adult with schizophrenia	Case report	Effectiveness for clozapine-induced sialorrhea	1% ophthalmic solution	0.5 mg up to three times daily	7 days	1	1	Safety was not discussed
Norderyd, J., et al. (2017). "Sublingual administration of atropine eyedrops in children with excessive drooling - a pilot study." Int J Paediatr Dent 27(1): 22-29.	5 - 18 years	Non-medically induced drooling associated with disability (CP, ASD, Down syndrome, etc.)	Open label, non-controlled	Effectiveness for drooling	1% ophthalmic solution	0.5 mg once to twice daily	8 weeks	26	19	Miction problems (3), obstipation (3), changed behavior (3)

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Protus, B. M., et al. (2012). Evaluation of atropine 1% ophthalmic solution administered sublingually for the management of terminal respiratory secretions. Am J Hosp Palliat Care, 30(4), 388-392.	42 - 94 years	Adults, hospice terminal care	Retrospective chart review	Effect on respiratory secretions	1% ophthalmic solution	0.5mg every 2 hours until effect	Varied by patient; range 1 hour to 6 days	22	22	No adverse effects reported
Rapoport, A, (2010). "Sublingual Atropine Drops for the Treatment of Pediatric Sialorrhea.", J. of Pain and Symptom Management 40(5): 783-788.	14 years	Metachromatic leukodystrophy (MLD) and excessive drooling	Case report	Effect on sialorrhea	0.5% ophthalmic solution	0.25 mg up to three times daily	2 weeks	1	1	Tongue fasciculation and dystonia developed after 2 weeks, determined to be due to metoclopramide. Sublingual atropine restarted without recurrence. No facial flushing, change in behavior, or tachycardia.

Citation	Age of Population	Population type	Study Design	Primary Objective	Atropine Formulation	Atropine Dose Regimen (mg)	Duration of Atropine Rx.	Total N	Atropine Exposed N	Major Safety Findings
Rothrock, S.G, et al. (1993) Successful Resuscitation From Cardiac Arrest Using Sublingual Injection for Medication Delivery. Ann Emerg Med. 22(4):751-3.	7 months	Infant with cardio-pulmonary arrest	Case report	Effect in cardiac arrest	Not stated	0.15 mg (estimated 0.02 mg/kg)	Single dose	1	1	Safety was not discussed
Sharma, A., et al. (2004). Intraoral application of atropine sulfate ophthalmic solution for clozapine-induced sialorrhea. Ann Pharmacother, 38(9), 1538.	55 years	Patient taking clozapine	Case report	Effectiveness for clozapine-induced sialorrhea	1% ophthalmic solution	1.0 mg twice daily	2 weeks	1	1	Clinician reported no adverse effects

ASD = autism spectrum disorder; CP = cerebral palsy; ERCP = endoscopic retrograde cholangiopancreatography; Rx = treatment

APPENDIX 2. SCHEDULE OF EVENTS

Study Visit	Screening	Visit 1[a]		Visit 2[a]		Visit 3[a]		Telephone Follow-up	ET[b]	UV
Study Day	**Day -14 to -1**	**Day 1 Predose**	**Day 1 Dosing**	**Day 8 (±1 Day) Predose**	**Day 8 (±1 Day) Dosing**	**Day 15 (±1 Day) Predose**	**Day 15 (±1 Day) Dosing**	**Day 21 -1 Day/ +7 Days**	**N/A**	**N/A**
Visit window based on actual dosing day	**N/A**	**N/A**	**N/A**	**Visit 1 + 7 ±1 Day**	**Visit 1 + 7 ±1 Day**	**Visit 2 + 7 ±1 Day**	**Visit 2 +7 ±1 Day**	**Visit 3 + 6 -1 Day/ +7 Days**	**NA**	**N/A**
Procedures										
Informed consent	X									
Randomization		X								
Physical exam	X[c]	X[c]		X[c]		X[c]			X[c]	X[c]
Medical history	X	X[d]		X[d]		X[d]			X[d]	X[d]
Tobacco/Vaping Questionnaire	X	X		X		X			X	X
Height, weight, BMI	X	X[e]		X[e]		X[e]			X[e]	X[e]
Vital signs	X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]		X[f]	X[f]
12-lead ECG	X		X[g]		X[g]		X[g]		X[g]	X[g]
Clinical laboratories - CBC and chemistry	X								X[h]	X[h]
HIV antibody, HBsAg, anti-HCV	X									
TSH	X									
Serum pregnancy test[i]	X									
Urine pregnancy test[j]		X		X		X				
Urine drug screen[k]	X									

Study Visit	Screening	Visit 1[a]		Visit 2[a]		Visit 3[a]		Telephone Follow-up	ET[b]	UV
Study Day	**Day -14 to -1**	**Day 1 Predose**	**Day 1 Dosing**	**Day 8 (±1 Day) Predose**	**Day 8 (±1 Day) Dosing**	**Day 15 (±1 Day) Predose**	**Day 15 (±1 Day) Dosing**	**Day 21 -1 Day/ +7 Days**	**N/A**	**N/A**
Visit window based on actual dosing day	**N/A**	**N/A**	**N/A**	**Visit 1 + 7 ±1 Day**	**Visit 1 + 7 ±1 Day**	**Visit 2 + 7 ±1 Day**	**Visit 2 +7 ±1 Day**	**Visit 3 + 6 -1 Day/ +7 Days**	**NA**	**N/A**
CRU admission		X		X		X				
Study drug administration			X		X		X			
PK blood collection[l]			X		X		X			
Xerostomia assessment	X	X	X[m]	X	X[m]	X	X[m]			
AEs	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X
Discharge from CRU			X		X		X			

AE = adverse event; BMI = body mass index; CBC = complete blood count; CRU = contract research unit; ECG = electrocardiogram; ET = early termination; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IV = intravenous; N/A = not applicable; PK = pharmacokinetic; TSH = thyroid-stimulating hormone; UV = unscheduled visit

[a] Washouts will be conducted between visits. Washouts will be approximately 6 (±1) days; AEs and concomitant medications will be recorded for all 3 washout periods.

[b] The ET Visit may be conducted in clinic or by phone as determined by the investigator.

[c] All subjects undergo a full physical examination at screening. At subsequent visits, including Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), Visit 3 (Day 15 Predose), ET and UV visits, the investigator may perform symptom-based physical examinations at their discretion.

[d] Medical history updates will be collected at Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), Visit 3 (Day 15 Predose), UV, and ET Visits.

[e] Weight only will be measured at Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), Visit 3 (Day 15 Predose). Weight may also be measured at UV or ET Visits per the investigator's discretion.

[f] Vital signs (heart rate, blood pressure, temperature, and respiratory rate) will be obtained prior to each dose of study drug administration. Postdose automated blood pressure and heart rate measurements will be recorded on the opposite arm from blood collection every 10 minutes for the first hour, every 20 minutes for the second hour, and every 30 minutes for the third and fourth hours and thereafter as deemed clinically necessary by the investigator until the end of each visit. Vital signs may also be performed at UV or ET Visits per the investigator's discretion.

[g] For the IV dose only, an ECG will be performed within 5 minutes after atropine has been administered; the ECG may be repeated as needed at the investigator's discretion. ECGs may also be performed at UV or ET Visits per the investigator's discretion.

[h] CBC (red blood cell, total and differential white blood cell, hemoglobin, hematocrit, and platelet count) and serum chemistry (creatinine, blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, direct bilirubin, total carbon dioxide, glucose, sodium, potassium, and chloride) will be obtained at Screening. Additional laboratory studies may also be performed at UV or ET Visits per the investigator's discretion.

[i] A serum pregnancy test will be conducted for all women of childbearing potential at Screening.

[j] A urine dipstick pregnancy test will be conducted for all women of childbearing potential at Visit 1 (Day 1 Predose), Visit 2 (Day 8 Predose), and Visit 3 (Day 15 Predose). A urine dipstick pregnancy test will also be performed at any time during study participation if pregnancy is suspected. Results from all tests must be negative in order to proceed with dosing.

[k] Urine drug screen includes tests for amphetamines, cocaine, tetrahydrocannabinol, methylenedioxymethamphetamine, and opiates.

[l] Blood samples taken at time 0 (predose) and postdose at 2, 4, 6, 10, 15, 20, 30, 45, and 60 minutes and 2, 4, 6, and 8 hours.

[m] Subjects will be asked to rate xerostomia postdose every 10 minutes until one hour has passed, or the subject scores a 10 on one or more of the xerostomia questions, or reports intolerable xerostomia, whichever occurs first.

APPENDIX 3. XEROSTOMIA ASSESSMENT

Subjective self-reporting of degree of xerostomia in patients without salivary gland dysfunction has been successfully demonstrated in an antimuscarinic-induced (glycopyrrolate) xerostomia model.[11] Select questions were adapted from this study and are noted below. Please note these questions are not validated in their current form but are included to assess anticholinergic effects in trial subjects.

The following questions will be used in the assessment of xerostomia:

1) On a scale of 0-10, with 0 being not difficult at all and 10 being very difficult, rate the difficulty you are currently having swallowing due to your mouth dryness.
2) On a scale of 0-10, with 0 being not dry at all and 10 being very dry, how would you currently rate the dryness of your lips?
3) On a scale of 0-10, with 0 being not dry at all and 10 being very dry, how would you currently rate the dryness of your tongue?

The subject will be asked these questions before dosing and then every 10 minutes after dosing for 1 hour or until one of the following conditions are met: (1) The subject scores a 10 on one or more of the questions or, (2) the subject expresses (either verbally or nonverbally) that he/she cannot tolerate the oral dryness any further.

When either of these conditions are met, the assessment will be terminated, and the subject will be provided water ad libitum. The time when the assessment is terminated will define when a subject experienced their maximum xerostomia.