# Appendix A

# PROTOCOL AND AMENDMENTS

**Cardiovascular Safety Pharmacology Study of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs**

**I. SRI STUDY NUMBER:** M748-10

**II. SPONSOR:**

National Institutes of Health
National Institute of Mental Health, NIH, DHHS
NCS Building, Rm. 7185 (MSC 9641)
6001 Executive Blvd.
Bethesda, Maryland 20892-9641

**Contract and WA Number:** HHSN271200900018C, WA#5

**Sponsor's Representative:**

Jamie Driscoll, Program Analyst, NIMH
Phone: 301-443-5288
Email: jdrisco1@mail.nih.gov

Robert Innis, Ph.D.
National Institute of Mental Health, NIH
Email: Innisr@intra.nimh.nih.gov

**III. TESTING FACILITY:**

SRI International
Biosciences Division
333 Ravenswood Avenue
Menlo Park, CA 94025

**Study Director:**

James Bakke, BS
Phone: 650-859-4573 Fax: 650-859-3444
Email: james.bakke@sri.com

**IV. PROPOSED IN-LIFE SCHEDULE:**
**Start of In-Life (first dose):** May 24, 2010

**V. APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Representative | Date |
| Robert Innis, Ph.D, Sponsor's Representative | Date |
| James Bakke, SRI Study Director | 5/24/10 Date |
| **REVIEWED BY:** SRI Quality Assurance | 5/21/2010 Date |

**Cardiovascular Safety Pharmacology Study**
**of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs**

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| Jamie Driscoll, Sponsor's Representative | Date |
| Robert Innis<br>Robert Innis, Ph.D, Sponsor's Representative | 5/19/10<br>Date |
| James Bakke, SRI Study Director | Date |

**REVIEWED BY:**

| | |
|---|---|
| SRI Quality Assurance | Date |

**Cardiovascular Safety Pharmacology Study of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs**

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| II. | **SPONSOR:** | National Institutes of Health<br>National Institute of Mental Health, NIH, DHHS<br>NCS Building, Rm. 7185 (MSC 9641)<br>6001 Executive Blvd.<br>Bethesda, Maryland 20892-9641 |
| | **Contract and WA Number:** | HHSN271200900018C, WA#5 |
| | **Sponsor's Representative:** | Jamie Driscoll, Program Analyst, NIMH<br>Phone: 301-443-5288<br>Email: jdriscol@mail.nih.gov<br><br>Robert Innis, Ph.D.<br>National Institute of Mental Health, NIH<br>Email: Innisr@intra.nimh.nih.gov |
| III. | **TESTING FACILITY:** | SRI International<br>Biosciences Division<br>333 Ravenswood Avenue<br>Menlo Park, CA 94025 |
| | **Study Director:** | James Bakke, BS<br>Phone: 650-859-4573 Fax: 650-859-3444<br>Email: james.bakke@sri.com |
| IV. | **PROPOSED IN-LIFE SCHEDULE:** | |
| | **Start of In-Life (first dose):** | May 24, 2010 |

V. **APPROVALS**

Jamie Driscoll, Sponsor's Representative — Date: 5/24/10

Robert Innis, Ph.D, Sponsor's Representative — Date

James Bakke, SRI Study Director — Date

**REVIEWED BY:**

SRI Quality Assurance — Date

## VI. PURPOSE OF STUDY

The purpose of this study is to provide data of suitable quality and integrity to support applications to the U.S. Food and Drug Administration (FDA) and other regulatory agencies. Therefore, this study will be performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies" (GLP) as described in 21 CFR Part 58.

## VII. OBJECTIVE OF STUDY

The objective of this safety pharmacology study is to determine any potential cardiovascular or toxicity effects of CUMI in male and female Beagle dogs after a single oral dose administration.

## VIII. EXPERIMENTAL DESIGN

| Group [A] | Target Dose [B] | Dose Level [C] ($\mu g/m^2$) | Dose Level (µg/kg) | Concentration (µg/ml) | No. Dogs Evaluated [C] |
|---|---|---|---|---|---|
| 1 | 100x Human Dose | 264.2 | 13.2 | 26.4 | 1M/1F |
| 2 | 500x Human Dose | 1321.0 | 66.0 | 132 | 1M/1F |
| 3 | 10x Human Dose | 26.42 | 1.32 | 2.64 | 1M/1F |
| 4 | TBD (Confirm NOAEL) | TBD | TBD | TBD | 1M/1F |

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to10x HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 $\mu g/m^2$. Scaling for the dog gives 2.642 $\mu g/m^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Species and Strain:** | Canine, Beagle |
| **Route of Administration:** | Intravenous (iv); slow bolus. |
| **Frequency:** | Single dose per dose level (Group). Day of dose administration will be Study Day 1 for each dose level. |
| **Dosing Volume:** | 0.5 mL/kg. Dose volumes will be calculated based on most recent body weight. The dose volume may be adjusted to achieve the target dose levels based on actual measured concentration of dose solution. Any change will be approved by the Study Director and documented in the study records. |
| **Experimental Duration:** | To be included in the final report |

## IX. MATERIALS AND METHODS

### A. Test and Control Articles

| | | |
|---|---|---|
| **1.** | **Test Article:** | CUMI |
| | **Supplier:** | National Institute of Mental Health, NIH |
| | **Manufacturer:** | Alpha Biopharmaceuticals, Inc. |
| | **Lot Number:** | AB-090505R |
| | **Physical Description:** | White solid |
| | **Storage Conditions:** | Refrigerated at 2-8°C |
| | **Characterization of Test Article:** | Characterization, purity and stability of the test article are the responsibility of the Sponsor. A Certificate of Analysis (CofA), or equivalent documentation, will be provided to SRI for inclusion in the report. The raw data generated by the Sponsor in support of this CofA or its equivalent will not be verified or maintained by SRI. |
| **2.** | **Vehicle Control:** | 5% ethanol in saline |
| | **Component 1:** | Ethanol |
| | **Supplier:** | To be included in the final report |

| | |
|---|---|
| **Manufacturer:** | To be included in the final report |
| **Lot Number:** | To be included in the final report |
| **Physical Description:** | To be included in the final report |
| **Storage Conditions:** | Approximately 15-30°C (Room Temperature) |
| **Component 2:** | Sterile Saline (USP) |
| **Supplier:** | To be included in the final report |
| **Manufacturer:** | To be included in the final report |
| **Lot Number:** | To be included in the final report |
| **Physical Description:** | Clear colorless liquid |
| **Storage Conditions:** | Approximately 15-30°C (Room Temperature) |
| **Characterization of Vehicle Control:** | Information on the identity, purity, and stability of the control article may be obtained by recording all of the pertinent information provided on the container labels, in a CofA or equivalent documents provided by the supplier. |

**3. Preparation of Dose Formulations:**

Dose formulation(s) will be prepared by mixing the appropriate amount of test article in the vehicle to achieve the target concentration. Lower dose formulations may be prepared by diluting the high dose formulation with the vehicle to achieve the target concentrations. If necessary, mixing with a stir bar and or sonication may be used. The pH of the solution will be recorded and if necessary pH will be adjusted to be compatible with intravenous administration. Dose formulations will be used and stored within the window of stability determined at SRI.

**Storage of Dose Formulations:**

Dose formulation(s) will be stored refrigerated at approximately 4–8°C until the day of use. Dose formulations will be used and stored within conditions of stability determined at SRI.

Formulations will be brought to ambient room temperature prior to administration.

**4. Characterization of Dose Formulations:**

Determination of formulation stability and homogeneity over the range of concentrations used in this study will be conducted by SRI prior to, or concurrently with, the study (see Attachment A). Dose formulations will be used within the window of stability determined in the stability study. Results of the stability study will be included in the final report.

Dose formulation concentration will be determined using an analytical method developed for this compound and described in Attachment A. The analytical method will be provided in the final report. Dosing mixtures will fall within +10% of the target concentration.

**5. Test Article Handling:**

At a minimum, the test, reference, and control article formulations will be handled with the use of eye protection, gloves, and a protective smock or laboratory coat.

**6. Disposition:**

At the end of the study any remaining partially used and unused containers of vehicle control and test article will be shipped to the Sponsor unless the Sponsor issues other directions.

Residual dose formulations will be discarded when the final report is submitted or when samples no longer afford evaluation.

Empty control, and test article containers will be destroyed by SRI on submission of the final report to the Sponsor.

See Section X.D for retention of records and study samples.

**7. Method for Assuring Correct Dosing:**

The administration of each dose formulation will be properly documented, and the amount administered to each animal will be recorded.

## B. Test System

| | |
|---|---|
| **Species:** | Canine |
| **Strain:** | Beagle |
| **Supplier:** | Marshall Farms (Rose, NY) or other reputable supplier |
| **Number of Animals:** | 4 assigned to test |
| **Sex:** | 2 males and 2 females |
| **Age at First Dose:** | 8-10 months |
| **Weight Range At First Dose:** | 7-13 kg (males and females)<br><br>Weights should be appropriate for the age and sex of the animals. Animals are dosed by body weight; therefore, weights outside the protocol-specified range will not be considered a protocol deviation as this does not affect the study's integrity. |
| **Animal Care:** | General procedures for animal care and housing will be in accordance with the National Research Council (NRC) *Guide for the Care and Use of Laboratory Animals* (1996) and the Animal Welfare Standards Incorporated in 9 CFR Part 3, 1991. |
| **Quarantine:** | These dogs were previously quarantined and used for another study. However, all dogs will have gone through a wash out period of at least 2 weeks after their final treatment before they are transferred to this study. A physical examination will be performed by the attending veterinarian following the wash out period to verify that animals are healthy before they are assigned onto the study. |
| **Housing:** | 1-2 per enclosure |
| **Cages:** | 4' x 8' wire mesh enclosure (run) with a concrete floor or 4' x 6' stainless steel runs with resting boards and slatted fiberglass flooring. |
| **Light Cycle:** | 12 hr light/12 hr dark |

| | |
|---|---|
| **Temperature:** | 64–84°F |
| **Humidity:** | 30–70%. Brief excursions outside this range may occur; excursions of less than 4 hr/day will not be considered deviations from the protocol. |
| **Ventilation:** | At least 10 room volumes per hour, with no recirculation of air. |
| **Food:** | Harlan Teklad 2025C Certified Global 25% Protein Dog Diet (Hayward, CA) or equivalent. Dogs will be exposed to their daily ration of food, except for periods of fasting required by the study protocol. The quantity of the daily ration is sufficient to meet nutritional requirements. Feed is analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed are not present at levels that would affect the study. Documentation of feed analyses is maintained at SRI for reference. A copy of the lot specific reports provided by the supplier will be maintained in the study records. |
| **Water:** | Water will be provided either from the public water supply or purified, reverse osmosis system, and will be provided *ad libitum*. Based on previous reports, no contaminants that could interfere with and affect the results of the study are expected to be present in the water. Copies of annual analysis reports are maintained at SRI for reference. |

**Assignment of Animals to Study:**

| | |
|---|---|
| **Day:** | No more than 10 days before initiation of treatment. |
| **Randomization:** | Randomly assigned to treatment groups by manual assignment. Animals may be excluded based on health or inappropriate weight. |
| **Identification:** | Animals will be individually identified by a uniquely numbered ear tattoo. |
| **Welfare of the Animals:** | Every effort will be made to minimize, if not eliminate, pain and suffering in all animals in this study. Moribund animals and animals experiencing |

undue pain and suffering will be euthanized at the discretion of the Study Director, attending veterinarian, or other qualified person. The Study Director will, however, make every effort to protect the scientific validity of the study.

## C. Experimental Procedure (In-Life Evaluations)

**1. Dose Administration:** Intravenous (iv); slow bolus. This route of administration is proposed for clinical use in humans.

**2. Mortality/Morbidity:** At least once daily

**3. Clinical Observations:** Recorded prior to dose administration, immediately following and 1-4 hrs postdose on the day of dosing for each dose administration when possible, or more often as clinical signs warrant. Animals will be examined for clinical signs related to the pharmacology and toxicology of the test article, gross motor and behavioral activity, and observable changes in appearance.

**4. Body Weights:** Body weights will be recorded prior to each dose administration for the purpose of dose volume calculation.

Body weights will be recorded for animals found dead and for unscheduled sacrifices, but these weights will not be included in the statistical evaluations.

**5. Body Temperature** Body temperature data will be collected for all animals predose and approximately 1-4 hr postdose for each dose escalation.

**6. Cardiology:** Electrocardiograms (ECG), blood pressure (BP and heart rate (HR) will be collected predose and approximately 0.5, 1, 2, 4 and 24 hrs after each dose administration. Appropriate measurements may be performed at other intervals as warranted by clinical signs.

**Method:** All ECGs will be measured from dogs in right lateral recumbence, when possible, using a 4-lead strip recorded at 25 mm/sec paper speed at standard

sensitivity (10 mm/mV). ECGs will be recorded for at least 5 min or as necessary to collect sufficient chart recordings for evaluation. All selected wave forms that afford evaluation will be retained. A minimum of five recordings will be sent to the cardiologist for evaluation.

HR will be manually calculated by the cardiologist from the ECG printed paper and recorded.

BP will be measured manually (5 replicate measurements), when possible, at each time point using an oscillometric monitor, according to the manufacturer's instructions, with a pediatric cuff on the right or left forelimb.

**Cardiovascular Parameters:**

ECGs, HR and BP recordings will be evaluated by a veterinary cardiologist and study director. The veterinary cardiologist will qualitatively evaluate the ECGs. In the event that abnormalities are observed in any section of ECGs, only that section will be quantitatively evaluated by the veterinary cardiologist. The report of the veterinary cardiologist will be included in its entirety as an appendix to the final report.

The rhythm will be determined subjectively by the cardiologist. If necessary, manual measurements will be made of P wave duration, PR interval, QRS duration, and QT interval as applicable based on the qualitative evaluations. For each of the parameters, measurements will be made from three to five consecutive beats and averaged. The averages will be reported. The beats used for the manual measurements will be identified by the cardiologist by marking those individual beats with an "X" on the ECG printout.

Averages of HR and BP will be calculated from measurements recorded at each time point, when appropriate.

**Statistical Test:**

No statistical analyses will be performed. Groups consisting of less than three dogs per gender are not large enough for statistical comparison.

### D. Necropsy

**Interval:** This is a survival study, and no necropsy will be performed.

### E. Control of Bias

While evaluating the responses of the animals and conducting the analyses, the technical staff will be aware of the treatment history of each animal and sample. Based on the relatively objective endpoints to be examined, however, bias is not expected to influence the results of the study.

## X. REGULATORY COMPLIANCE

### A. Good Laboratory Practice Compliance

This study is intended to be submitted to and reviewed by the U.S. FDA or an equivalent regulatory agency, and this study therefore will be performed in accordance with the U.S. FDA "Good Laboratory Practice for Nonclinical Laboratory Studies," as described in 21 CFR Part 58, with the following exceptions:

- Various pre-initiation study activities (e.g., receipt and physical examination of animals, pre-initiation body weights and randomization) may be performed prior to the approval of the protocol. These activities will be conducted according to testing facility SOPs, but because they may be conducted before the protocol is signed, they may not be considered by the FDA to have been conducted in compliance with GLP requirements.

- Animal water and food analysis will not be performed under GLP compliance by the vendors.

### B. Standard Operating Procedures (SOPs)

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of the laboratory. All deviations from any SOP and the reasons for the deviations will be documented and acknowledged by the Study Director.

### C. Protocol Amendments and Deviations

All changes or revisions made to the approved protocol by any involved party, and the reasons for the changes and revisions, will be documented, signed, and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment as described above will follow.

All unintentional deviations from the protocol and the reasons for the deviations will be documented and acknowledged by the Study Director. The Sponsor's

Representative will be informed of the occurrence of any deviations that might affect the results of the study, and any corrective actions taken.

### D. Retention of Records and Study Samples

The original protocol, amendments, final report, raw data, supporting documents, and records specific to this study will be retained and stored in the Records Center at SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025. All records and materials will be maintained for a period of at least 5 years. At the end of the retention period, the Sponsor will be contacted for instructions regarding disposition of these materials.

## XI. REPORTING

The final report will accurately and completely describe the study design, procedures, and findings. An analysis and summary of the data followed by the conclusions derived from the analyses will also be included. A draft report will be issued prior to submission of the final report.

# Attachment A

## ANALYTICAL CHEMISTRY:

## TEST ARTICLE CHROMATOGRAPHIC PURITY, FORMULATION CONCENTRATION, HOMOGENEITY AND STABILITY ANALYSES

## I. INTRODUCTION

Dose verification analyses will be performed on formulations containing the test article, CUMI, in the selected vehicle, 5% ethanol in saline. Concentration, homogeneity, and stability of test article in the vehicle will be determined by high performance liquid chromatography (HPLC). In addition, test article chromatographic purity will be analyzed using the same HPLC method. The methodology, procedures, and final results will be documented in the Final Report.

## II. EXPERIMENTAL

### A. Reference Standard

The test article will be used as the reference standard in the analyses.

### B. Analytical Method

The analytical method provided by the Sponsor has been verified to be operational in SRI's laboratory. The Sponsor's method has been modified for its suitability for the dose formulation analyses.

The method conditions after modification are as follows:

| | |
|---|---|
| Instrument: | Hewlett-Packard Model 1100 series liquid chromatography system |
| Column: | Phenomenex Luna C18 (2), 5µm, 4.6 x 250 mm or equivalent |
| Mobile Phase: | A: 25 mM Aqueous ammonium formate, 60%<br>B: Acetonitrile (ACN), 40% |
| Flow rate: | 1.2 ml/min |
| Run time: | 10 minutes |
| Injection Volume: | 75 µl or as needed |
| Detection: | 254 nm |
| Column Temp.: | 25$^{o}$C |
| Data System: | HP Chemstation Software; version A. |

Note: An equivalent column or instrument may be used. Minor adjustments in the method, e.g. amounts of the mobile phase ingredients or injection volume, may be

made by the analyst, only if necessary, to achieve desired chromatographic characteristics.

## C. Procedures

### 1. Preparation of Standard Solutions

**Standard Stock Solutions:** A test article sample will be weighed and dissolved in 60% water: 40% ACN (the diluent) as standard stock solution Sa. To ensure the accuracy of the standard stock solution, a second standard stock solution (Sb) will be prepared in the same way as for Sa.

**Calibration Standard Solutions:** With each experiment, a minimum of five calibration standard solutions at different concentrations will be prepared from the stock solution using the diluent. Calibration standards will be chromatographed to demonstrate the linearity of the calibration curve over the concentration range of interest.

For test article purity evaluation, one of the standard solutions prepared above will be used.

### 2. Preparation of Sample Solution from Dose Formulation

For dose verification, a minimum of duplicate aliquots will be sampled for each dose concentration. Aliquots will be accurately transferred into separate volumetric flasks or selected container and diluted to the desired concentration with the diluent.

For homogeneity testing, triplicate aliquots will be sampled from the lowest and the highest concentration of formulations. Aliquots will be taken from the top, middle, and bottom of a stirring solution to demonstrate homogeneity.

For stability determination, a minimum of duplicate aliquots will be sampled and analyzed. Stability will be established over a concentration range that covers the dose levels used in the study.

## D. Analysis

### 1. System Suitability

A single standard solution will be chromatographed at least six consecutive times to determine the system repeatability (reported as relative standard deviation, % RSD, of peak area or response factor). The % RSD should be $\leq$ 3.0% for the six injections.

The response factors of the calibration standards and the standards used during the run (e.g. bracketing standards) will be calculated. The %RSD of these response factors should be ≤ 10.0%.

The response factor of the accuracy check standard Sb will be compared to the response factor of the standard stock solution Sa. The percent accuracy of the two, when compared, should be within 100.0 ± 5.0%.

**2. Linearity**

Each of the calibration standards will be chromatographed, and a linear regression calibration curve will be generated. The correlation coefficient (r) should be ≥ 0.990.

**3. Test Article Chromatographic Purity**

SRI will perform chromatographic purity analyses for the test article both prior to and following in-life.

**4. Dose Verification**

At a minimum, duplicate samples from each dose level will be analyzed. The verified concentration, reported as a percentage of the nominal concentration, should be within 100.0 ± 10.0% of the nominal concentration.

**5. Homogeneity Determination**

Triplicate samples each from the lowest and highest concentrations of the formulations will be analyzed. The individual values, reported as percentages of nominal, from each set of triplicate samples will be averaged and the %RSD for each set should be ≤5.0%. Homogeneity needs to be determined only once for the study.

**6. Stability Determination**

The stability of the formulations over a range of concentrations bracketing those used in this study will be established prior to or concurrently with the study. Stability results should be within 100.0 ± 10.0% of the initial concentration. Stability determination will be established only once for the study under the given concentration range and set of storage conditions.

### E. Calculations

Concentration of the test article in the dose formulations will be determined by HPLC using an external standard method. The linear regression curve obtained from the calibration standards will be used to calculate the concentration of test article in each formulation. Results from each dose concentration level will be averaged as a percentage of the nominal concentration. Homogeneity will be measured as RSD% of the triplicate samplings at the lowest and highest concentration levels. Stability results will be calculated using an approach similar to that for concentration determination. Chromatographic purity will be calculated by normalized peak area percentage.

## III. RESULTS/DISCUSSION

Experimental results will be discussed and compared to the criteria in the Study Protocol. Deviations from the criteria will be documented and reported to the Study Director. Analytical methods, procedures, and raw data will be archived with the study records, and an analytical report will be included as an appendix to the study report.

# Protocol Amendment No. 1

**PROTOCOL TITLE:** Cardiovascular Safety Pharmacology Study of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs

**SRI Study Number:** M748-10

**Sponsor:** National Institutes of Health

**Sponsor's Representatives:** Jamie Driscoll, Program Analyst, NIMH
Robert Innis, Ph.D.

**SRI Study Director:** James Bakke, B.S.
**SRI Principal Investigator:** Hanna Ng, Ph.D., DABT

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This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section VII. OBJECTIVE OF STUDY, page 2:**

The objective of this safety pharmacology study is to determine any potential cardiovascular or toxicity effects of CUMI in male and female Beagle dogs after a single ~~oral~~ ***intravenous (iv)*** dose administration.

**Reason for Change:** The typographical error was corrected to reflect the actual dose route.

**Section VIII. EXPERIMENTAL DESIGN, page 2:**

| Group [A] | Target Dose [B] | Dose Level [C] ($\mu g/m^2$) | Dose Level (µg/kg) | Concentration (µg/ml) | No. Dogs Evaluated [C] |
|---|---|---|---|---|---|
| 1 | 100x Human Dose | 264.2 | 13.2 | 26.4 | 1M/1F |
| 2 | ~~500x~~ ***50x*** Human Dose | ~~1321.0~~ ***132.1*** | ~~66.0~~ ***6.6*** | ~~132~~ ***13.2*** | 1M/1F |
| 3 | 10x Human Dose | 26.42 | 1.32 | 2.64 | 1M/1F |
| 4 | TBD (Confirm NOAEL) | TBD | TBD | TBD | 1M/1F |

SRI Study No. M748-10

# Protocol Amendment No. 1

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to10x HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 100x HD dose. |
| **Effect on the Study:** | None. Dose route described in the Study Purpose was a typographical error and counter to the route described in the protocol title and Experimental Design.<br><br>The dose level for Group 2 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | 5/28/10<br>Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | Date |

# Protocol Amendment No. 1

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to10x HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[C] Maximum human dose is 5 μg per 70 kg person. 5 μg/70 kg = 0.0714 μg/kg x 37 (human surface area conversion) = 2.642 μg/m$^2$. Scaling for the dog gives 2.642 μg/m$^2$ / 20 (dog surface area conversion) = 0.132 μg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 100x HD dose. |
| **Effect on the Study:** | None. Dose route described in the Study Purpose was a typographical error and counter to the route described in the protocol title and Experimental Design.<br><br>The dose level for Group 2 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis | 6-1-10 |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | Date |

# Protocol Amendment No. 1

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to10x HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/$m^2$. Scaling for the dog gives 2.642 µg/$m^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 100x HD dose. |
| **Effect on the Study:** | None. Dose route described in the Study Purpose was a typographical error and counter to the route described in the protocol title and Experimental Design.<br><br>The dose level for Group 2 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | 6/7/10<br>Date |

# Protocol Amendment No. 2

**PROTOCOL TITLE:** Cardiovascular Safety Pharmacology Study of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs

**SRI Study Number:** M748-10

**Sponsor:** National Institutes of Health

**Sponsor's Representatives:** Jamie Driscoll, Program Analyst, NIMH
Robert Innis, Ph.D.

**SRI Study Director:** James Bakke, B.S.
**SRI Principal Investigator:** Hanna Ng, Ph.D., DABT

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This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section VIII. EXPERIMENTAL DESIGN, page 2:**

| Group [A] | Target Dose [B] | Dose Level [C] ($\mu g/m^2$) | Dose Level ($\mu g/kg$) | Concentration ($\mu g/ml$) | No. Dogs Evaluated [C] |
|---|---|---|---|---|---|
| 1 | 100x Human Dose | 264.2 | 13.2 | 26.4 | 1M/1F |
| 2 | 50x Human Dose | 132.1 | 6.6 | 13.2 | 1M/1F |
| 3 | ~~10x~~ ***25x*** Human Dose | ~~26.42~~ ***66.1*** | ~~1.32~~ ***3.3*** | ~~2.64~~ ***6.6*** | 1M/1F |
| 4 | TBD (Confirm NOAEL) | TBD | TBD | TBD | 1M/1F |

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to~~10x~~ ***25x*** HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the

# Protocol Amendment No. 2

NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 50x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 3 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | 06/11/2010 Date |

# Protocol Amendment No. 2

NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[c] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 50x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 3 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis | 6-10-10 |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | Date |

# Protocol Amendment No. 2

NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

[c] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The next dose level had to be adjusted down based on clinical observations findings and ECG results observed after treatment with the initial 50x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 3 was set in advance of treatment and as determined in accordance to the protocol's de-escalation criteria. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | 6/9/2010<br>Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | Date |

# Protocol Amendment No. 3

**PROTOCOL TITLE:** Cardiovascular Safety Pharmacology Study of a Single Intravenous Dose of CUMI in Conscious Beagle Dogs

**SRI Study Number:** M748-10

**Sponsor:** National Institutes of Health

**Sponsor's Representatives:** Jamie Driscoll, Program Analyst, NIMH
Robert Innis, Ph.D.

**SRI Study Director:** James Bakke, B.S.
**SRI Principal Investigator:** Hanna Ng, Ph.D., DABT

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This amendment modifies the following lines/sections of the study protocol. Additions are in bold and italics: ***addition***. Deleted text has been struck through: ~~deleted~~.

**Section VIII. EXPERIMENTAL DESIGN, page 2:**

| Group [A] | Target Dose [B] | Dose Level [C] ($\mu g/m^2$) | Dose Level ($\mu g/kg$) | Concentration ($\mu g/ml$) | No. Dogs Evaluated [C] |
|---|---|---|---|---|---|
| 1 | 100x Human Dose | 264.2 | 13.2 | 26.4 | 1M/1F |
| 2 | 50x Human Dose | 132.1 | 6.6 | 13.2 | 1M/1F |
| 3 | 25x Human Dose | 66.1 | 3.3 | 6.6 | 1M/1F |
| 4 | ~~TBD~~ ***25x*** (Confirm NOAEL) | ~~TBD~~ ***66.1*** | ~~TBD~~ ***3.3*** | ~~TBD~~ ***6.6*** | 1M/1F |

[A] Each group will be stagger with each starting on a new Day 1.

[B] One male and one female dog will initially be treated at a dose of 100x human dose (x HD). If no effect is observed at 100x HD, the dose level will be escalated to 500x HD for Group 2. No further escalation is required after testing 500x HD. If an effect is observed at 100x HD, the dose level will be de-escalated to 50x HD for Group 2. If an effect is observed at 50x HD then further de-escalation to 25x HD will be tested for Group 3. However, if no effect is observed at 50x HD, then further de-escalation is not required. Testing will conclude by proceeding to confirm the NOAEL. Two animals (1/sex) previously not used for the escalation/de-escalation groups will be used to confirm NOAEL.

# Protocol Amendment No. 3

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The NOAEL confirmatory dose level has been determined based on clinical observation findings and ECG results observed after treatment with the initial 25x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 4 was set in advance of treatment and as determined in accordance with the protocol. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | June 25, 2010<br>Date |

SRI Study No. M748-10

# Protocol Amendment No. 3

[c] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The NOAEL confirmatory dose level has been determined based on clinical observation findings and ECG results observed after treatment with the initial 25x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 4 was set in advance of treatment and as determined in accordance with the protocol. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | 6/23/2010 Date |
| Robert Innis, Ph.D, Sponsor's Authorized Representative | Date |
| SRI Study Director | Date |

# Protocol Amendment No. 3

[C] Maximum human dose is 5 µg per 70 kg person. 5 µg/70 kg = 0.0714 µg/kg x 37 (human surface area conversion) = 2.642 µg/m$^2$. Scaling for the dog gives 2.642 µg/m$^2$ / 20 (dog surface area conversion) = 0.132 µg/kg for the equivalent human dose in dog.

| | |
|---|---|
| **Reason for Change:** | The NOAEL confirmatory dose level has been determined based on clinical observation findings and ECG results observed after treatment with the initial 25x HD dose. |
| **Effect on the Study:** | None. The dose level for Group 4 was set in advance of treatment and as determined in accordance with the protocol. |

**APPROVALS**

| | |
|---|---|
| Jamie Driscoll, Sponsor's Authorized Representative | Date |
| Robert Innis<br>Robert Innis, Ph.D, Sponsor's Authorized Representative | 6/21/2010<br>Date |
| SRI Study Director | Date |