Bridge Global Pharmaceutical Services, Inc. (Bridge)
(d/b/a Bridge Laboratories)
610 Professional Drive
Gaithersburg, Maryland 20879
Phone: 240.364.6300
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STUDY PROTOCOL

Study to Evaluate the Effect of Enteral ACN-8337 Administration on Absolute Neutrophil Count (ANC) in Sprague Dawley Rats (Part A)

Bridge Study No. 1803-08628
Acorn Protocol No. P2008-001 (Part A)

APPROVALS

Bridge:

C. Steven Godin, PhD, DABT
Study Director

11/6/08
Date

Kory J. Engelke, PhD, DABT
Test Facility Management

11/6/08
Date

Acorn Biomedical, Inc.:

Ben Rosner, MD, PhD
Sponsor Representative

Date

Stamp:

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Acorn Biomedical, Inc.:

Ben Rosner, MD, PhD — 11-6-08 Date
Sponsor Representative
Alan Jacobs for Ben Rosner

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PROTOCOL

I. Study Title

Study to Evaluate the Effect of Enteral ACN-8337 Administration on Absolute Neutrophil Count (ANC) in Sprague Dawley Rats (Part A)

II. Purpose

The purpose of this study is to determine the ANC response of enterally delivered ACN-8337 in Sprague Dawley rats.

This study is a single dose, placebo controlled proof of concept study with associated positive reference article that is intended to demonstrate efficacy of the test article following intraduodenal delivery.

III. Sponsor Information

A. Name and Address

Acorn Biomedical, Inc.
612 SE 5th Avenue, Ste. 3
Fort Lauderdale, FL 33301

B. Sponsor Representative

Ben Rosner, MD, PhD
Telephone: (650) 529.1429
Cell (760) 969.2857
E-mail: rosne010@gmail.com
Fax: (650) 529.1429

C. Study Monitor

Alan Jacobs, MD, PhD
Telephone: (650) 575.5260
E-mail: ajacobs@acornbio.com
Fax: (650) 230.7130

IV. Bridge Study Director and Alternate Contact

A. Study Director

C. Steven Godin, PhD, DABT
Phone: (240) 364.6436
Email: sgodin@bridgelaboratories.com
FAX: (240) 364.6240

B. Alternate Study Contact

Han S. Seung, MS
Phone: (240) 364.6466
Email: hseung@bridgelaboratories.com
FAX: (240) 364.6240

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V. Test Sites, Archive Facilities, and Contributing Scientist

A. Laboratory Operations

Bridge
620 Professional Drive
Gaithersburg, MD 20879

B. Data Archive Facility

Iron Mountain Incorporated (Non-Electronic Data)
4451 Georgia Pacific Boulevard Suites L & M
Frederick, MD 21704
Phone: (301) 831.8276
FAX: (301) 831.1298

Iron Mountain (Electronic Data)
8928 McGaw Court
Columbia, MD 21045

C. Hematology

Dr. Saroj Das
Ani Lytics
200 Girard St, Ste 200
Gaithersburg, MD 20877
Phone: (301) 921.0168
Fax: (301) 977.0433

VI. Regulatory Information

A. Compliance

This study will be conducted according to the protocol, amendments, and the test facility Standard Operating Procedures (SOPs).

This study is an exploratory study and is not intended to support applications for products regulated by governmental agencies. Therefore, this study will not be conducted in compliance with current U.S. FDA Good Laboratory Practice (GLP) Regulations for Non-clinical Laboratory Studies (21 CFR Part 58)

B. Quality Assurance

The Bridge Quality Assurance Unit (QAU) will not audit the study.

C. Data Collection System

Electronic data collection including randomization, dosing, animal husbandry and environmental enrichment, clinical observations, body weights and body weight changes, food consumption, and gross pathology observations will be performed using Provantis™ NT 2000 (Instem LSS, Limited; Stone, UK) as appropriate.

Hematology data will be analyzed at AniLytics using the ABX Pentra 60 (Horiba). The raw data will be provided to Bridge.

D. Record Retention

All study data and any communications concerning the conduct of the study will be retained in a Bridge-designated archive for a period of 5 years following issuance of the final report.

Study data generated by the Sponsor or subcontractors will be archived by the Sponsor or subcontractor(s).

Following the 5-year period (or before at Sponsor's request), the Sponsor will be contacted to determine the disposition of these materials. Bridge will maintain all electronic data and records regarding disposition of data and specimens at a designated archive facility.

VII. Proposed Study Timetable

A. Study Initiation Date[a]	See footnote
B. Experimental Start Date	November 13, 2008
C. Dosing	November 18, 2008
D. Animals Returned to Stock	November 21, 2008
E. Submission of Draft Letter Report	December 7, 2008
F. Study Completion Date[b]	See footnote

[a] = The date Study Director signs protocol (Protocol must be signed before any study specific procedures are performed).
[b] = The date the Study Director signs the final report. (The Study Director may finalize the report 60 days after submission of audited draft final report, if no Sponsor's comments are received).

VIII. Test Materials

A. Identification and Supplier

1. Test Article

ACN-8337 will be supplied by the Sponsor as a pre-formulated stock solution in Test Article Vehicle (see below) at a concentration to be supplied by the Sponsor. The lot number will be provided. The stock solution will be diluted with Test Article Vehicle to achieve desired dosing concentrations.

2. Test Article Vehicle Components

The vehicle will consist of 50 mg/mL mannitol and 0.1 mg/mL Tween 80 formulated in 50 mM phosphate buffer with the final pH adjusted to a value specified by the Sponsor. The vehicle components will be USP grade or higher (except Tween 80 which may be NF grade or higher) and endotoxin tested and minimized as applicable The vehicle components will be provided by Bridge.

3. Reference Article

G-CSF (Neupogen®; 600 μg/1mL vial) will be supplied by Bridge.

4. 5% dextrose in water (D5W)

D5W will be supplied by Bridge.

B. Purity and Stability

1. Test Article

The purity or other characteristics that appropriately define the test article are on file with the Sponsor and/or will be provided by the Sponsor. The test article will be used as received and will not be adjusted based on stated purity. Stability of the formulation under conditions of storage and use will be provided by the Sponsor.

2. Test Article Vehicle Components

The purity or other characteristics that appropriately define the vehicle components will be provided by the manufacturer or supplier.

3. Reference Article

The purity or other characteristics that appropriately define the reference article are on file with the manufacturer. The reference article will be characterized by its product label.

4. D5W

The purity or other characteristics that appropriately define D5W will be provided by the manufacturer or supplier

C. Storage Conditions

1. Test Article

Test article will be shipped frozen as multiple aliquots and will be stored at -75 ± 15°C immediately upon receipt and maintained frozen until the time of use. Test article will not undergo more than two freeze/thaw cycles, with minimization of these cycles to the greatest extent possible.

2. Test Article Vehicle Components

The vehicle components are to be supplied by Bridge and stored under temperature and humidity conditions as specified by the manufacturer or supplier.

3. Reference Article

G-CSF (Neupogen®) is to be stored as specified by the manufacturer or supplier.

4. D5W

D5W is to be stored as specified by the manufacturer or supplier.

D. Reserve Samples and Disposition

1. Reserve Samples

Reserve samples will not be required.

2. **Disposition**

Excess test article not used in this study will be stored frozen, and may be pooled with test article for Part B of the study if needed.

Any other unused materials will be retained by Bridge for use on subsequent studies as needed. Any empty test material containers will be disposed of after the completion of the in-life phase of the study.

IX. Dose Formulations, Sampling, and Analysis

A. Formulation

1. **Frequency and Procedure**

The vehicle will consist of 50 mg/mL mannitol and 0.1 mg/mL Tween 80 formulated in 50 mM phosphate buffer with the final pH adjusted to a pH specified by the Sponsor. The final pH will be within ± 0.1 units of the Sponsor-specified pH. The vehicle will be sterile-filtered using a 0.22-μm filter and stored at 2-8°C following preparation. Formulations containing ACN-8337 are NOT to be filtered.

The test article formulations will be prepared from the pre-formulated material on the day of use. On the day of dosing, a sufficient number of frozen aliquots of test article shall be allowed to thaw at room temperature. Any aliquots not needed for this study shall remain frozen for use in Study Part B, if needed. The pre-formulated test article stock solution will be diluted with vehicle as needed to achieve dosing concentrations appropriate for a 20 mg/kg dose for each animal receiving test article in a total dosing volume of 13.3 mL/kg. All formulations shall be carried out in sterile polypropylene containers. The dosing formulations containing test article ACN-8337 will NOT be sterile-filtered prior to use, and they will be maintained at room temperature for no less than 30 minutes and no longer than 90 minutes before dose administration. Unused formulated material will be stored frozen at -75 ± 15°C following use.

The reference article will be prepared on the day of dosing. Neupogen will be diluted in sterile amber glass containers from a concentration of 600 μg/mL to 25 μg/mL using D5W. The reference article will be stored at 2-8°C following use for possible return to the Sponsor.

2. **Disposition**

Any remaining solutions containing test article that were prepared for dosing but which remain following completion of dosing will be labeled and stored in polypropylene tubes, in 2mL aliquots or smaller, at -75 ± 15°C until the Sponsor provides disposition in writing. Unused vehicle will be discarded following SOP.

B. Dose Formulation Analysis

No analysis of the formulations will be required.

X. Test System and Husbandry

A. Animals

1. Strain/Source

Sprague Dawley

Charles River Breeding Labs, Raleigh, NC

2. Age at First Dose

Approximately 11-13 weeks

3. Weight at First Dose

Females will weigh 200-250 grams.

4. Number/Sex

Six females with intraduodenal cannulas + four extra female animals with cannulas as spares; 3 females without cannulas + 2 female animals as spares

5. Identification

Each animal will be identified by individual ear tags, and each cage will be identified with a cage card.

6. Animal Welfare

Bridge's Institutional Animal Care and Use Committee (IACUC) will review this protocol for accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals and the U.S. Interagency Research Animal Committee Principals for the Utilization and Care of Research Animals prior to authorizing its execution. In the event of severe toxicity in which decisions are to be made regarding treatment or euthanasia of a study animal, the Bridge GPD veterinarian and the Study Director retain the right for subsequent action.

B. Husbandry

1. Housing

Animals will be housed individually in polycarbonate cages that contain SaniChip® certified hardwood bedding.

Bedding is analyzed by the manufacturer for acceptable limits of heavy metals, aflatoxins, bacteria, yeasts, molds and organophosphates prior to certification. Certification specifications are on file at Bridge.

2. Food

Animals will be fed Certified Global Harlan Teklad Laboratory 2018 Diet (Pellets), *ad libitum* except when noted otherwise. Prior to dosing, animals will be food-fasted for a minimum of 8 hours. Water will be available *ad libitum* until 1 hour prior to dosing. Following dosing, animals will be fasted (food and water) for at least 4 hours. Feeders will be changed at least biweekly.

Feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates.

Certificates of nutrient and contaminant analyses are on file at Bridge.

3. **Water**

Water is provided *ad libitum* (except as specified in Section X.B.2) via an automatic watering system and/or water bottles. The water is routinely analyzed by Bridge for contaminants and specific microbes. The results of these analyses are on file at Bridge.

4. **Contaminants**

Available information indicates that no substance is present in the diet or drinking water, or bedding at a concentration likely to influence the outcome of this study.

5. **Environment**

Animals will be housed in a controlled environment (18-26°C and 30-70% relative humidity). Temperature and humidity will be monitored and recorded continuously in each animal room by an environmental monitoring system. In the event of a system failure, manual recording will be performed (once daily) as defined in the Standard Operating Procedures. A 12-hour light/12-hour dark cycle will be maintained except when interrupted by study-related events. These cycle interruptions will be documented in the study data. At least 10 animal room air changes/hour will be maintained.

6. **Environmental Enrichment**

Environmental enrichment will be provided.

C. Procedures for All Animals during Acclimation

Upon arrival a harness will be placed on all cannulated animals. Animals will be acclimated to the facility for 5 days prior to dosing. During acclimation, rats will be evaluated/treated as shown in **Table 1**. Based on these evaluations, animals considered unsuitable for the study will be excluded from randomization to study groups. Catheters will be exteriorized on the day following arrival (SD -4) and flushed with 0.1 to 0.25 mL of Sterile Saline Solution, 0.9% USP on SD -4 and SD -2. The exposed portion of the cannula will be wiped with an alcohol pad once daily. Wound clips will be removed once healing has been determined by the facility veterinarian.

Table 1: Evaluations/Treatment During Acclimation

Procedure	Frequency
Cageside Observations	≥ 2 daily
Clinical Observations	Once daily starting on day of arrival
Body Weight	Once daily starting on day of arrival (if pre-dose weight gain for age is abnormal, Sponsor Representative or Study Monitor will be contacted and the animal may be replaced by a spare)
Food Consumption	Daily (if quantitative food consumption is outside of normal range, Sponsor Representative or Study Monitor will be contacted and the animal may be replaced by a spare)
Rectal Temperature	Daily (if temperature exceeds 100.5°F the Sponsor Representative or Study Monitor will be contacted and the animal may be replaced by a spare)
Flushing of cannula with saline	On SD -4 and SD -2
External cleaning of cannula	Daily

D. Randomization

All animals considered suitable for study will be assigned to study by random card draw. Three animals with cannulas will each be assigned to Groups 1 and 2 and three animals without cannulas will be assigned to Group 3. Permanent animal numbers will be assigned after randomization.

XI. Study Design

A. Justification Rationale for Species, Number of Animals, Route of Administration and Dose Rationale

Sprague Dawley rats have been selected for this study as their response to our positive reference comparator, G-CSF (Neupogen®), is well characterized, and the species has been used extensively in the pre-clinical development of G-CSF. The number of animals selected for the study is the minimum necessary to generate statistically meaningful results in the context of a proof of concept, non-GLP study. The test article is an enteral formulation, delivered in this proof of concept study to the duodenum to bypass potential hydrolytic degradation in the stomach. Dose is selected to match that reported in the literature for the same test article in mice (Bai, Shen, Pharm Res. 2006 Sep;23(9):2116-21. Epub 2006 Aug 9).

B. Group Designation and Dosages

Animals will be assigned to groups as shown in **Table 2**.

Table 2: Group Designation and Dosage Levels

Group	Treatment	Route of Administration	Dose Volume	Nominal Dose	Number of Animals (Females)
1	Test Article	Intraduodenal	13.3 mL/kg	20 mg/kg	3
2	Test Article Vehicle	Intraduodenal	13.3 mL/kg	0 mg/kg	3
3	Reference Article	Subcutaneous	0.2 mL/kg	5 µg/kg	3

C. Dosing Information

1. Method of Administration

Animals in Groups 1 and 2 will be connected to a swivel/tether and dosed through the intraduodenal cannula using a calibrated infusion pump at a constant rate over a 30-minute interval. Animals will not be anesthetized. The dose start and stop times will be recorded. Upon completion of dosing, a volume of the vehicle equivalent to the dead space inside the cannula will be delivered to evacuate test article or vehicle remaining in the cannula. Animals will be disconnected from the swivel/tether following this procedure. Harnesses will be worn throughout the study to prevent damage to the cannula. Animals in Group 3 will be dosed by subcutaneous injection between the skin and underlying layers of tissue in the interscapular region of the back using a needle and syringe. The injection site will be shaved and swabbed with alcohol prior to injection. The time of dosing will be recorded.

2. Frequency

Animals will be dosed once on SD 1.

D. Observation of Animals during the Study

Animals will be observed and measurements will be collected as shown in **Table 3**.

Table 3: Observations of Toxicity in Study Animals during the Study

Procedure	Frequency of Testing
Cageside Observations[a]	≥ 2 Daily
Clinical Observations[b]	Daily
Body Weight	Prior to dosing on SD 1 and daily thereafter
Food Consumption	Daily
Rectal Temperature	Daily (if temperature exceeds 100.5°F the Sponsor Representative or Study Monitor will be contacted and the animal may be replaced by a spare)
Flushing of cannula with saline	Every other day beginning on SD 2
External cleaning of cannula	Daily

[a] = Cageside observations will include mortality, moribundity, general health and signs of toxicity. Observations will be at least 6 hours apart. If needed, observations may be made more frequently.

[b] = Clinical observations will include skin and fur characteristics, catheter/injection sites, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns. Observations may be made more frequently.

E. Clinical Pathology

Samples will be collected as shown in **Table 4**. All blood samples will be maintained at room temperature and transferred to Ani Lytics on cold packs or wet ice. Samples collected predose will be collected from all animals including spare animals, and analyzed for complete blood cell count and absolute neutrophil count within 2-4 hours of collection. Animals with total leukocyte count > 13.6 X $10^3/\mu L$ or an absolute neutrophil count > 5.1 X $10^3/\mu L$ predose will be replaced with a spare animal if available.

Table 4: Samples for Clinical Pathology

Procedure	Group	Time Point	Blood Volume	Collection Device
Hematology[a]	1 and 2	Predose and 12, 24, 36, 48, 60, 72, and 84 hours post dose	0.2 mL	Tubes containing K2EDTA
	3	Predose and 6, 12, 24, 36, 48, 60 and 72 hours post dose		

[a] = Blood will be collected from the lateral tail vein within ± 10 minutes with the exception of the predose sample

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1. **Required Tests**

Hematology	
Complete blood count	Absolute neutrophil count (ANC)

F. Termination

1. **Unscheduled**

Gross necropsies will be conducted on all moribund animals and all animals not surviving to termination. Moribund animals will be euthanized via CO_2 inhalation followed by exsanguination prior to necropsy (gross necropsy with examination of the gastrointestinal tract only). Found dead animals will be subjected to gross necropsies (examination of the gastrointestinal tract only).

2. **Scheduled**

Following the conclusion of the post-study husbandry period (between Part A and B), and at Sponsor's instruction not to continue with Part B, all surviving animals will be euthanized by CO_2 inhalation and discarded without necropsy. Otherwise, if Sponsor instructs Study Director to proceed with Part B, animals will not be euthanized until completion of Part B, as indicated in Part B protocol.

G. Continuation with Study Phase B

If Study Part A yields sufficient results, Sponsor may direct Study Director to terminate the study without running any portion of Study Part B. Such decision will be made by Study Monitor and communicated to Study Director no later than 7 days following receipt of complete raw data from Study Part A. Animal husbandry will continue as specified in Section X, but will not exceed the 10 day washout period.

H. Contact Sponsor Representative

Study Director will contact Sponsor Representative or Study Monitor by phone to receive input,prior to proceeding with study in the event of, but not limited to the following:

1. Any animal in the pre-dose phase, meeting exclusion criteria for temperature, leukocyte count, ANC, food consumption, or weight gain.
2. Any animal in the post-dose phase, meeting abnormal criteria for temperature, or for any signs of infection.
3. Complications with intraduodenal cannula such as clogging, disruption or loosening of cannula from surgical site, leakage or reflux of test article or vehicle from or adjacent to cannula.
4. Any animal mortality.
5. Any other reason that Study Director believes warrants a reason to exclude an animal from the ongoing study.

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XII. Proposed Statistical Analyses

Descriptive statistics (mean, standard deviations, and N) will be presented for all measurement data.

XIII. Final Report

Electronic data will be provided to Sponsor within 1 day of the completion of each part of the study. A draft letter report will be submitted within two weeks of completion of the study. The report will be finalized four weeks following receipt of Sponsor comments if no further revisions are provided. The report will include, but will not be limited to, the following information:

EXPERIMENTAL DESIGN AND METHODS

RESULTS

- Clinical observations
- Rectal temperatures
- Body weights/body weight changes
- Food consumption
- Complete blood count and absolute neutrophil count data

CONCLUSIONS