

**SINGLE DOSE ESCALATION TOXICITY
OF
IN BEAGLE DOGS**

SPONSOR:

**Toxicology & Pharmacology Branch
Developmental Therapeutics Program
Division of Cancer Treatment, Diagnosis, and Centers
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20852**

COTR:

CONTRACT NUMBER:

CONTRACTOR:

PRINCIPAL INVESTIGATOR:

STUDY DIRECTOR:

PROPOSED IN-LIFE PHASE:

Start:

Finish:

I. OBJECTIVE

The objective of this study is to determine target organ toxicity and its reversibility of a single intravenous dose of TBD in dogs.

II. MATERIALS AND METHODS

A. Test and Control Article:

1. **Name of Test Article:**

2. **Name of Control Article:**

3. **Characterization and Documentation of Methods of Synthesis, Fabrication or Derivation:**

a. **Test Article:**

Compound identity, strength, quality, stability and purity as well as documentation of methods of synthesis, fabrication or derivation are the responsibility of the NCI. Confirmation of identity will be done immediately upon receipt of each shipment of the compound. Sufficient quantity of drug shall be reserved for archiving from each lot and shipment used.

b. **Control Article:**

4. **Stability and Storage:**

a. **Test Article:**

b. **Control Article:**

5. **Formulation Preparation, Stability and Storage:**

a. **Test Article:**

b. **Control Article:**

6. **Dose Concentration and Homogeneity Analyses:**

An adequate quantity of each dosing mixture will be retained for possible analysis until the acceptance of the final report on this compound.

B. Test System:

1. **Species, Strain Supplier and Test System Justification:**

Purebred beagles will be used in this study. This is an accepted species to support studies of compounds used or intended for use in humans.

2. Initial Age, Sex and Weight:

Male and female dogs will be approximately 8 to 12 months of age and approximately 7 to 14 kg at study initiation. Dogs will be assigned to dose groups such that the heaviest males and females are used together.

3. Care and Housing:

General procedures for animal care and housing will be in accordance with DHHS Publication No. (NIH) 86-23 (Revised, 1985) and the U.S. Department of Agriculture through the Animal Welfare Act (7 USC 2131), 1985 and Animal Welfare; Standards incorporated in 9 CFR Part 3, 1991.

4. Diet and Water Supply:

A certified, commercial, dry chow or meal with the following minimum composition will be used.

- Approximately 10% moisture
- At least 20% crude protein
- Approximately 5% fat
- Nutritionally adequate amounts of minerals
- Both water soluble and fat soluble vitamins

Dogs will have exposure to their daily ration for a total period of 1 to 2 hours per day. The quantity of the daily ration will be sufficient to meet nutritional requirements. The water source will be the public supply given ad libitum. No contaminants will be present in the feed or water which could interfere and affect the results of the study.

5. Animal Identification:

Dogs will be uniquely identified by ear tattoo number or letter combination. Positive identification will be required at least after every cage change, blood sampling and dosing.

C. Experimental Design

1. Group Assignments:

Two dogs of each sex will be randomly assigned to receive one of three different levels and to the vehicle control group.

Dose (mg/kg)	Number of Dogs per group	Number of doses	Dosing day (s) ^b
Level 1 (vehicle)	2 ^a	3	1, 3 and 5
Level 2 ^c	2	1	1
Level 3	2	1	3
Level 4	2	1	5

^a 1 male and 1 female.

^b Depending on the results from the measurements taken after a single dose of the two initial dose levels, escalation (or additional doses at the same level) may be used to obtain mild, readily reversible toxicity may be necessary. The need for an additional dosing or escalation will be determined after review of available data.

^c Dose level may remain constant for the second round of dosing

2. Route of Administration and Reason for Choice:

The test article will be given by the intended route of administration of the compound in humans.

3. Dosing Procedure:

The test article will be administered intravenously on day one. Dogs in the vehicle control group (VCTL) will receive a volume of vehicle equivalent to the greatest volume administered on a mL/kg basis.

In order to reduce the possibility of encountering concentration effects, a uniform concentration should be selected and maintained at all dose levels. Appropriate doses will be achieved by varying the volume of dose administered. Dose calculations will be based on the most recent individual body weight. A second individual who will initial and date the verification will check all calculations for amount of drug given to each dog.

Depending on the results from the measurements taken after a single dose of the initial dose level, escalation (or additional dose at the same level) may be used to obtain mild, readily reversible toxicity may be necessary. The need for additional doses will be determined after review of all available clinical data.

4. Measurements:

a. Clinical Signs:

PRETEST - Observe dogs daily and record any abnormal clinical signs. Baseline body temperature will be taken once on day -7 and once on day -3.

TEST - Adverse clinical signs will be observed and recorded during the dosing period at least once daily or more often as clinical signs warrant. Body temperatures will be measured and recorded immediately before dosing and 3 hours after dosing, on study days 1, 2, 3, 5 and 7.

b. Body Weight:

PRETEST - Body weights will be recorded on days -10 and -3. Food intake will be quantitatively evaluated and water intake will be qualitatively evaluated on days -7 and -3.

TEST - Body weights will be recorded on the dosing day, study day 7. Food intake will be quantitatively recorded daily for the first week and weekly thereafter. Water intake will be qualitatively evaluated daily for the first week and weekly thereafter.

c. Ophthalmology:

PRETEST - Ocular examinations will be performed once during the pretest period (day -3) before the dogs are placed on study.

TEST - Ocular examinations will be performed on all dogs on day 7.

d. Clinical Pathology:

PRETEST - All dogs will be fasted overnight and bled for clinical pathology on day -3. These baseline samples will be taken after the quarantine period is completed.

TEST - All dogs will be fasted overnight and blood drawn for clinical pathology on study days 1, 2, 4, 6 and 14. Blood drawn on dosing day 2, 4 and 6 will be taken 24 hours after treatment. Blood will be drawn on study day 3, 4 hours post dosing. The procedures will be performed according to the laboratory's SOP but in no case will dogs be bled from the treatment site.

Hematology:

Erythrocyte count (RBC) - $10^6/\text{mm}^3$

Hemoglobin (HGB) - g/do

Hematocrit (HCT) - %

Mean corpuscular volume (MCV) - fl

Mean corpuscular hemoglobin (MCH) - pg

Mean corpuscular hemoglobin concentration (MCHC) -g/dL

Platelet count (Plate) - $10^3/\text{mm}^3$

Reticulocyte count (RETIC) - % RBC

Total leukocyte count (WBC) - $10^3/\text{mm}^3$

Differential leukocyte count - %

Nucleated red blood cell count (nRBC) - nRBC/100 WBC

Clinical Chemistry:

Blood urea nitrogen (BUN) - mg/dL

Serum aspartate aminotransferase (AST) - I.U./L

Serum alanine aminotransferase (ALT) - I.U./L

Alkaline phosphatase (Alk. Phos.) - I.U./L

Serum glucose (BS) - mg/dL

Creatinine (CREAT) - mg/dL

Prothrombin time (PT) - sec

Total protein (T PROTEIN) - g/dl

Sodium (Na) - meq/L

Potassium (K) - meq/L

Chloride (Cl) - meq/L

Troponin I (special handling required)

e. Plasma Drug Level: Blood Draws only:

Blood samples will be drawn from all dogs at 0 (immediately before dosing) 10, 30, 60 and 240 minutes, 8, and 24 hours after the first dosing. An aliquot of each sample

(approximately 2 ml) will be mixed with EDTA. The samples will be centrifuged and the plasma frozen at least at -20°C until analyzed.

III. QUALITY ASSURANCE

A. Type of Study

This is a nonclinical laboratory study and that will not require compliance with the FDA Good Laboratory Practice Regulations.

B. Standard Operating Procedures

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the standard operating procedures of the laboratory and any deviations will be documented.

C. Protocol Amendments

All changes in or revisions of an approved protocol and the reasons therefore will be documented, signed, and dated by the Principal Investigator, Study Director and the NCI COTR. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the NCI COTR, but a written amendment will follow.

D. Records

Data will be verified by the laboratory's Quality Assurance Unit.

IV. REPORTING AND DISCUSSION OF DATA

A. Reports

Initial summarized data should be reported immediately to the COTR for the study so that decisions about subsequent dosing can be made. Any problems encountered and proposed means of resolution should be discussed immediately.

B. Final Report

The data and results of this study will be submitted as a separate draft report, due 30 working days after the last measurement. The final report will be due 15 working days after return of the draft report for revision.

The final report shall accurately and completely describe the study design, procedures and findings, present an analysis and summary of the data followed by the conclusions derived from the analyses. The report will also include: (a) a cover page which will include the title, contract number, authors, laboratory address, dates of initiation and completion, and sponsor; (b) the NTIS Report Documentation Page, to be placed at the beginning of the final report; (c) a comprehensive summary to be placed after the NTIS page; and (d) the signature of the Study Director and any others deemed necessary; (e) a table of contents.

Protocol Approvals:

Study Director: _____
(Date)

Principal Investigator: _____
(Date)

COTR: _____
(Date)