



Confidential Study

Assessment of the Effectiveness of GlcNBu as a Therapeutic for Osteoarthritis in the  
Beagle dog

Study No. VRI59-13116-CE

Sponsored by

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## **Results Summary**

- T-maze running speed increased under GlcNBu as compared to control (baseline). Values did not improve under treatment with Carprofen.
- Both GlcNBu and Carprofen produced statistically significant improvements in ratings on QOL (Quality of life) questionnaire including pain scores.
- Both GlcNBu and Carprofen produced improved performance on measures indicative of weight bearing lameness on force mat evaluations.

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**1. TITLE**

Assessment of the Effectiveness of GlcNBu as a Therapeutic for Osetoarthritis in the Beagle dog.

**2. PROTOCOL NUMBER**

VRI59-13116-CE

**2.1. Type of Protocol**

This is a single, site-specific protocol. All in-life procedures will be conducted at Vivocore Inc. Analytical procedures will be conducted at an outside reference laboratory.

**3. SPONSOR**

Dr. Tassos Anastassiades, Queen’s University, Dept of Medicine (Rheumatology)

**4. PROTOCOL OBJECTIVE**

The purpose of this study is to assess the effectiveness of N-butyryl glucosamine (GlcNBu) in treatment of Beagle dogs with demonstrated osteoarthritis as compared to Carprofen.

**5. STUDY OBJECTIVE**

The test article is a synthetic analogue of glucosamine and is under development as a therapeutic agent for treatment of osteoarthritis in dogs. This is a non-pivotal, non-GLP study that is not intended for regulatory approval.

## 6. STUDY SCHEDULE

### 6.1. Proposed date of initiation

Study Initiation date: date protocol is signed by Scientific Director  
In-life phase initiation: December 2013

### 6.2. Proposed date(s) of completion

End of study: date the final report is signed

## 7. STUDY DESIGN

### 7.1. Study design summary

Procedure	Study Day														
	-6	-5 to -4	-3	-2	-1	0 to 13	6	11 to 12	13	14 to 18	18	19 to 32	25	30 to 31	32
Twice Daily Observations	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
T-Maze Assessment	X	X	X	X				X						X	
Force mat Assessment			X						X						X
Quality of Life Questionnaire	X			X			X		X				X		X
Blood Collection				X					X						X
Synovial Fluid Collection				X					X						X
Randomization/Allocation					X										
Body Weights					X						X				
Test Article Administration						X	X	X				X	X	X	X

### 7.2. Treatment groups

There will be 2 treatment groups, each containing 6 dogs.

The groups will be as follows:

1. Control group receiving Carprofen
2. Treatment group receiving GlcNBu

### 7.3. Experimental design

The study is a randomized, blinded crossover design. The experimental unit will be one individual dog and the number of dogs per group will be 6.

## 7.4. Randomization procedures

### 7.4.1. *Allocation of animals to groups*

Subjects will be ranked based on combined pain scores from the Quality of Life Questionnaire Assessment and speed of running on the T-maze. The dogs will be ranked in descending order into 2 blocks, so that each group contains 6 animals (e.g. the best performing animal will receive a rank of 1, and the poorest performing animal will receive a rank of 12). In the event that 2 or more animals have the same score, animals with the same score will be ranked alphanumerically (A-Z) by animal identification name. Subsequently, dogs will then be allocated to groups. Ranks 1 and 2 will be placed into Treatment groups 1 and 2. Ranks 3 and 4 will be placed into Treatment groups 2 and 1 respectively. This pattern will continue until all subjects have been placed in a group.

### 7.4.2. *Allocation of treatment groups to experimental units*

Once the dogs are assigned to a group, designated study personnel will randomly assign each group to a treatment condition by the drawing of a lot. For lot drawing, two pieces of identically sized paper will be used. Order 1 Carprofen first and Order 2 GlcNBu first will each be written on one of the pieces of paper, and papers will be placed into an opaque container. The designated non-blinded personnel will remove one of the papers and will assign Group 1 to that condition. The second drawn paper will be used to assign Group 2 to the condition written on the paper. Group assignments will be recorded and this information will be kept in the archive room during the study to ensure blinding of personnel collecting data.

## 8. STUDY PROCEDURES

### 8.1. Test animal(s)

#### 8.1.1. *Description*

##### 8.1.1.1. Age

Animals greater than 6 years of age will be included in the study.

##### 8.1.1.2. Sex

Sex of the subjects will be random; however, both sexes will be included.

8.1.1.3. Species/breed  
*Canis familiaris* / Beagle

8.1.1.4. Initial body weight  
Appropriate for the age, sex and breed of animal

*8.1.2. Number of animals*  
Twelve (12) animals will be included in the study.

*8.1.3. Source of animals*  
Vivocore Inc. colony

*8.1.4. Identification Method*  
All animals will be individually identified according to standard operating procedures. Each dog will be uniquely identified with a microchip/tattoo and name. For data collection the animal name will be the primary identifier.

*8.1.5. Animal use justification*  
Procedures are designed to avoid or minimize discomfort, distress and pain to the animals in accordance with the principles of the Animal for Research Act of Ontario and the guidelines of Canadian Council on Animal Care (CCAC). The use of 6 dogs per treatment group is within the acceptable range for osteoarthritis studies of this type.

## **8.2. Inclusion criteria**

Animals in good general health as determined by historical health records, with previous radiographic evidence of osteoarthritis in a minimum of 2 joints will be included in the study. The inclusion of study animals will be approved by the Scientific Director.

## **8.3. Exclusion criteria**

Any animal deemed unsatisfactory for the purpose of the study such as poor health or uncooperative disposition will be eliminated prior to allocation. The rejection of study animals will be approved by the Scientific Director.

## **8.4. Acclimation of test animals**

*8.4.1. Duration*  
All animals involved in this investigation have been housed in the Vivocore animal facility for a period not less than three months; therefore, no acclimation period will be needed.

**8.4.2. Medication and/or vaccination during acclimation period**

Medications will be avoided to the extent possible. Any necessary vaccinations will be administered prior to the start of the study.

**8.5. Blinding of study**

**8.5.1. Extent of blinding**

The treatment given to each animal will not be revealed to people collecting data. The study will be blinded to all personnel in the investigation with the exception of the person(s) involved with preparing and administering the investigational veterinary product and vehicle, the person responsible for performing allocation, and the bioanalytical scientists. Non-blinded personnel will not collect data other than that at the time of treatment.

**8.5.2. Blinding method(s) and procedure(s)**

Treatment conditions will be assigned to 1 of 2 treatment groups, and this information will be kept in the archive room over the duration of the study.

**8.5.3. Treatment code access**

The code assignments, treatment records and any other documents that would reveal treatments to people collecting data will remain in the archive room until all data are collected. The removal of these documents will be noted in the archive room activity log.

**8.6. Analytical methods**

**8.6.1. Health Observations**

The dogs will be observed for any signs that would not be expected in normal dogs, including but not limited to muscle tremors, coughing, nasal discharge, sedation, rapid or laboured breathing and convulsions. An observed abnormality will be checked off as “abnormal” and briefly described under “comments” on the Daily Animal Observation Record by the person making the observation.

**8.6.2. Body weights**

Dogs will be weighed on Day -1 and Day 18. The most recent body weights will be used to calculate dose amounts. Animals will be weighed according to standard operating procedures. The scales will be operated and maintained as per standard operating procedures. On days that dogs are weighed, the scale will be verified before the



first dog is weighed and after the last dog is weighed using reference weights that bracket dog weights. Scale verifications on the day of use and the dog weights will be recorded on the Animal Weight Record.

**8.6.3. Adverse Event**

An AE is any undesirable experience occurring to an animal, whether or not related to the investigational product. Thus, any post-treatment indication of abnormal (or other than normal) any place in the data is an adverse event. All adverse events, as well as all similar findings pre-treatment, will be tabulated in the final study report. The Scientific Director will report all serious AEs to the sponsor as soon as practical, even if the event does not appear to be drug-related. Adverse Events will be recorded on the Adverse Event Report.

**8.6.4. Blood Collection**

**8.6.4.1. Whole blood collection for serum separation**

Whole blood collections will be conducted on Days -2, 13 and 32. Approximately 4mL of blood will be collected from a suitable vein as per Vivocore standard operating procedure. On each collection day, blood will be drawn immediately prior to sedation for synovial fluid collections. Serum will be separated within 60 minutes of collection by centrifugation at 2800-3300 RPM for 10 minutes at room temperature. The serum will then be placed into at least 2 cryovials, each containing a minimum of 0.5ml. The serum samples will be frozen in an upright position and stored at -80°C ( $\pm 4^{\circ}\text{C}$ ); samples will be stored for a minimum of 6 months. One cryovial of serum from each dog will be shipped to the Sponsor. Once the first aliquot is received, the second will be shipped.

**8.6.5. Synovial Fluid Collection**

Synovial fluid will be collected from subjects on study Days -2, 13, and 32. Synovial fluid will be collected under sedation. Subjects will be sedated with medetomidine (0.01-0.03mg/kg) and butorphenol (0.1-0.3 mg/kg) IV. Sedation will be reversed with atipamazole (0.1-0.2mg/kg IM). The fluid will be collected by arthrocentesis into the joint following surgical preparation of the area. Baseline and treatment collections will occur from a combination of 4 joints in each subject and will include both shoulder and stifle joints. The goal will be to collect a minimum of 0.4ml of fluid combined across the 4 joints. The fluid from each joint will be collected into separate vials. Collections will be repeated the following day if the minimum volume

is not obtained. If collection volumes are not adequate on treatment days, repeat collections will occur on the first day assigned to washout following treatment administration if applicable. Synovial fluid will be collected 3 hours post dose ( $\pm 30$  minutes). Samples will be stored at  $-80^{\circ}\text{C}$  ( $\pm 4^{\circ}\text{C}$ ).

#### 7.6.5 T-Maze

7.6.6 *The T-maze consists of a large apparatus containing a start box, alleyway and two arms, leading to a right and left runway. In this study, entrance into either of the arms will lead to a food reward. The dependent variable will be running speed. Subjects will be tested on the T-Maze on Days -6, -5, -4, -3, -2, 11, 12, 30, and 31 according to standard operating procedures. The assumption is that running speed is slowed in dogs showing OA because of the pain produced at specific joints. Only latencies from valid trials (i.e. completed trials without any overt distraction) will be included. On treatment days, subjects will be tested 1.5 hours post dose ( $\pm 15$  minutes). Quality of Life Questionnaire (QOL)*

The QOL is a questionnaire adapted from the canine brief pain inventory (CBPI), which was developed based on clinical questionnaires in which owners score the function and pain level of their pets with existing pain conditions. A two-stage procedure will be used. In the first phases, one experimenter will attempt to elicit objective behavior-specific measures of function and pain level across a variety of normal canine behaviors (e.g. walking, trotting, galloping, rearing, stair-climbing, etc). For this stage, one technician was responsible for soliciting the relevant behaviors from a dog using encouragement and/or food rewards, while the evaluating technician scored function and observable pain using the “daily questionnaire for evaluation of function and pain.” For the second phase, the evaluating technician will use the “CBPI Summary” questionnaire to (subjectively) score each dog’s pain level, impact of pain on function and quality of life based on their findings in the first stage. Subjects will be assessed using the QOL on Days -6, -2, 6, 13, 25, and 32. The same technician will be used on each of the assessment days for evaluating scored function and observable pain. On each assessment Day that the T-Maze is also used, subjects will be tested on the QOL immediately following completion of the T-Maze assessment. On all other treatment days, subjects will be tested 1h 45min post dose ( $\pm 15$  minutes).

#### 7.6.7 Force mat

On Days -3, 13, and 32, subjects' gait will be assessed using the force mat in the runway set-up according to standard operating procedures. This will measure maximum force, impulse, maximum peak pressure, stance time, and swing time, which is related to the degree of pain in each joint. On each assessment day, 10 passes over the mat will be recorded for each subject. On days in which the QOL and force mat both occur, the force mat will be used to assess the walk/trot portion of the Questionnaire. On days in which the T-Maze is also used, subjects will complete the force mat assessment immediately following completion of the maze.

### 8.7. Study facilities

#### 8.7.1. Housing

Dogs will be kept at Vivocore's animal facility and group housed in pens. Environmental management including temperature regulation, ventilation and humidity regulation and lighting will be provided, maintained and controlled according to standard operating procedures.

##### 8.7.1.1. Containment Equipment

The animal containment room is approximately 40 x 80 ft with a 10-foot ceiling and consists of a cement floor with steel walls and ceiling. The room contains pens, with approximate dimensions of either 2.5'x16' or 5'x16', with 2'x4' perches. Pens are constructed from galvanized steel with open sided mesh walls, epoxy-sealed cement floors and a raised resting platform.

##### 8.7.1.2. Lighting Equipment

Commercially acceptable fluorescent and natural lighting will be provided for the dogs. The photoperiod will be 12 hours and will be maintained on a timer; however, light may vary according to natural light cycle.

##### 8.7.1.3. Heating/Cooling Equipment

Heating and cooling is electronically controlled and will be set to maintain the animal room in a temperature range from 15°C – 28°C.

##### 8.7.1.4. Feeding Equipment

Feed will be provided in stainless steel feeding bowls.

**8.7.1.5. Watering Equipment**

Water will be provided to the animals via stainless steel bowls. The water source is an on-site well.

**8.7.1.6. Ventilation Equipment**

The test room ventilation is designed to provide approximately 15 to 18 air changes per hour.

**8.7.2. *Husbandry***

Inspection of the animals in regards to general health and behaviour will be performed daily throughout the study by the research technicians as per standard operating procedures.

General animal husbandry practices such as grooming and bathing will be conducted according to standard operating procedures. These activities will not be recorded as part of the study data.

All animal housing areas will be cleaned daily and disinfected as per standard operating procedures.

**8.7.3. *Feed and water***

Feeding of the dogs will be conducted according to standard operating procedures. All animals will be fed a standard commercial dry dog diet *ad lib* or to maintain body condition. The manufacturer, identifying name of feed, and lot number will be included in the study file from each lot of feed used. Feed will be provided in stainless steel bowls. Feed consumption will not be recorded.

Water will be provided *ad lib* via stainless steel bowls.

Contaminants of feed and water that could affect the result of the study are not anticipated.

**8.8. Drug administration**

**8.8.1. *Dosing regime and route of administration***

Beginning on Day 0 and continuing through Day 13, half of the subjects on study will be administered the test compound GlcNBu. This will be given orally as powder in gelatine covered capsules. Dosing will occur once a day at a dose of 60 mg/kg. The remaining 6 subjects on study will receive the control compound Carprofen in 25, 75 or 100 mg tablets. Administration will be aimed at achieving a dose level as close as possible to 4.4 mg/kg, with the closest available amount always rounded up. Dosing with Carprofen will also be once a day. Following a washout period from Days 14 to 18, a treatment crossover will begin on Day 19 through to Day 34, such that each animal will be assigned to the opposite treatment condition that it was on Day 0.

## **8.9. Removal of subject(s) from the study**

### *8.9.1. Criteria for removal of subjects from the study*

An animal can be removed from study prior to treatment if it shows severe sign(s) of illness, requires medical or surgical treatment non-compatible with the purpose of the study, is victim of extensive trauma, or dies. Animals removed prior to treatment can be replaced with another suitable animal. After treatment an animal cannot be removed from study for any reason other than death. Investigational veterinary product administration can be discontinued by protocol amendment, but health observations will still be conducted.

### *8.9.2. Procedures for removal of subjects from the study*

The decision to remove prior to treatment may be based on a health status assessment made by the attending veterinarian. The conclusion as to what degree the clinical symptoms are related to the drug administration does not need to be made at the time of removal, but must be discussed in the final study report. The animal's identity, date of the removal, reason for the removal and the prescribed therapy or fate of the animal will be recorded in the study file.

### *8.9.3. Fate of removed study animals*

Living animals removed from the study prior to treatment will be retained by Vivocore Inc. For animals that die or are euthanized, the carcass will be disposed of as per standard operating procedures.

## **8.10. Concurrent/concomitant medications/therapies**

Concurrent medical treatment during the study is not allowed unless necessary for the health of the animal and only if the drug is compatible with the purpose of the study. All treatments will be authorized in advance by the Scientific Director, or if not available, the attending veterinarian. The reason for the treatment, identification of treatment given, manufacturer, expiration date, dose, route, and any other relevant information will be summarized in the Concurrent Treatment Record and fully described in the final study report.

## **8.11. Other key personnel**

Research Technicians  
See Personnel List

## **8.12. Archiving**

Data and specimens will be archived at Vivocore Inc.

## 9. COLLECTION AND RETENTION OF SOURCE DATA

### 9.1. Records

The following records will be maintained in the study file:

#### 9.1.1. *Study documents*

##### 9.1.1.1. Personnel:

- 1) Personnel list of individuals involved in the study

##### 9.1.1.2. Animals and Facilities:

- 1) Animal Identification Form
- 2) Animal disposition records
- 3) Institutional Animal Care and Use Committee (IACUC) approval

##### 9.1.1.3. Procedures:

- 1) Protocol, protocol amendment(s) and protocol deviation(s)

##### 9.1.1.4. Test Article and other medications:

- 1) Test Article receiving and shipping logs, documentation of storage conditions (i.e., temperature logs or charts).
- 2) Information on vaccination or drug treatments used during the conditioning and experimental periods, including product labels (if available).

#### 9.1.2. *Raw Data*

The Sponsor will be provided with original raw data for the following:

- 1) Treatment administration
- 2) General health observations
- 3) Animal weights
- 4) Any other data necessary to interpret study results

#### 9.1.3. *Data Corrections*

Data corrections will be made as per standard operating procedures using the correct error codes.

### 9.2. Reports/Statements

#### 9.2.1. *Adverse Events*

Adverse Events will be fully documented and reported as described in sections 8.6.1 and 8.6.3.

#### 9.2.2. *Final Study Report*

The Scientific Director is solely responsible for the final study report and will be the only one to sign it.

### **9.3. Archives**

Date and Records: Raw data will be maintained in a secure location at the study site until data collection is complete. Certified true copies of raw data forms and study documents in Sections 13.1, 13.2 and 13.3 will be made and the originals submitted to the sponsor as per written and approved standard operating procedures. After the final study report is signed, the original will be sent to the sponsor and a certified true copy will be maintained by Vivocore Inc. The sponsor will archive all study documents.

Specimens: At the conclusion of the study any specimens generated will be sent to the sponsor for archiving.

## **10. ADDENDUMS, AMENDMENTS, DEVIATIONS TO THE PROTOCOL**

### **10.1. Protocol addendums/amendments**

Protocol addendums/amendments will be prepared as per standard operating procedures.

### **10.2. Protocol deviations**

Protocol deviations will be handled as per standard operating procedures.

## **11. INVESTIGATIONAL DRUG AND CONTROL**

Complete details of the test article(s) used in the study will be filed in the study binder.

## **12. DRUG DISPOSITION AND ACCOUNTABILITY/ANIMAL DISPOSITION AND ACCOUNTABILITY/FEED DISPOSITION AND ACCOUNTABILITY**

### **12.1. Disposition of test animals**

Dogs are expected to survive the study and thus will be returned to the Vivocore colony at the conclusion of this study. Carcasses and tissues from euthanized or deceased animals, if any, will be disposed of according to standard operating procedures. Final animal disposition will be documented in the study file.

### **12.2. Disposition of investigational veterinary products**

All test and control article containers sent by the Sponsor, whether used, unused or partially used will be returned to the study Sponsor.

### **13. ANIMAL WELFARE**

The Study Facility is committed to complying with all local regulations governing the care and use of laboratory animals. Procedures are designed to avoid or minimize discomfort, distress and pain to the animals in accordance with the principles of The Animal for Research Act of Ontario and the guidelines of Canadian Council on Animal Care (CCAC) The CCAC *Guide for the Care and Use of Experimental Animals* and related policies will be regarded as guidelines to follow.

The Study Facility has a certificate of registration of Research Facility under the Animals For Research Act issued by the Ontario Ministry of Agriculture, Food and Rural Affairs.

In order to ensure compliance, this protocol will be reviewed and approved by the Study Facility's Institutional Animal Care and Use Committee (IACUC) before the start of the trial as per IACUC standard operating procedures.

### **14. RESULTS**

#### **T-MAZE**

Mean time (in seconds) to reach goal box was longer under control (baseline) conditions than under either treatment condition for GlcNBu or Carprofen. The difference between GlcNBu and control was marginally significant, suggesting efficacy in reducing discomfort associated with running. Carprofen did not statistically differ from control.

#### **Quality of Life**

Total score for each animal was calculated by summing the score for each item  
Statistical analysis considered both order of testing (GlcNBu first vs Carprofen first), and treatment condition.

(Repeated measures analysis of variance with treatment & then Dunnet multiple comparisons)

The treatments were also compared on associated pain scores.

#### **Score on QOL questionnaire as a function of treatment and order of treatment**

Both GlcNBu and Carprofen improved ratings on QOL questionnaire. GlcNBu showed greater effectiveness when tested in second arm, following administration of Carprofen.



**Pain Score on QOL questionnaire as a function of treatment and order of treatment.**

Both GlcNBu and Carprofen produced statistically significant reductions in pain scores associated with questions in the QOL questionnaire.

Question #	Questionnaire Score			Pain Score			Combined Score		
	Treatment effect	Car vs Ctl	Glu vs Ctl	Treatment effect	Car vs Ctl	Glu vs Ctl	Treatment effect	Car vs Ctl	Glu vs Ctl
3	0.2697	na	na	na	na	na	0.0468	0.0734	0.0450
4	0.0021	0.0026	0.0051	0.5747	na	na	0.0150	0.0207	0.0207
5	0.0166	0.0101	0.0900	0.1337	na	na	0.0033	0.0054	0.0054
6	0.0713	na	na	0.3986	na	na	0.5798	na	na
7	0.0350	0.0356	0.0551	0.0327	0.0333	0.0520	0.0093	0.0048	0.1446
8	0.0672	na	na	0.0298	0.0306	0.0482	0.0321	0.0178	0.2805
9	0.7116	na	na	0.7462	na	na	0.9697	na	na
10	0.1209	na	na	0.2418	na	na	0.1774	na	na
11	0.1521	na	na	0.1219	na	na	0.0443	0.0840	0.0377
12	0.0192	0.0109	0.1267	0.4151	na	na	0.0643	na	na
13	0.1344	na	na	0.1719	na	na	0.1424	na	na
14	0.0346	0.1383	0.0216						
15	0.0034	0.0032	0.0105						

**Item Analysis of Questions Data:** On combined pain and questionnaire score, GlcNBu produced statistically significant effects on 4 of 10 questions, and Carprofen had statistically significant benefits on 5 questions.

Parameter	GlcNBu				Carprofen			
	Left Fore	Right Fore	Left Hind	Right Hind	Left Fore	Right Fore	Left Hind	Right Hind
Maximum Force (kg)	0.066		0.004	0.020	0.035	0.009	<0.0001	0.023
Maximum Force (%BW)	0.040		0.005	0.020	0.032	0.010	<0.0001	0.026
Maximum Peak Pressure (kPa)	0.006	0.051	0.032	0.001	0.002	<0.0001	0.006	0.003
Stance Time (sec)		0.047	0.053		0.053	0.067	0.045	0.097

**Force-mat Data**

T-values comparing two test compounds with control on force mat measures. Empty slots indicate values with  $p > 0.10$

Both GlcNBu and Carprofen produced improved performance on measures indicative of weight bearing lameness.