Date of Approval: February 6, 2009

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-294

Bc6 rDNA Construct in GTC 155-92 Goats

A specific hemizygous diploid line of domestic goats (*Capra aegagrus hircus*), containing five copies of the Bc6 rDNA construct located at the GTC 155-92 site, directing the expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland of goats derived from lineage progenitor 155-92.

Sponsored by:

GTC Biotherapeutics, Inc.

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number:	NADA 141294
B. Sponsor:	GTC Biotherapeutics, Inc. 175 Crossing Blvd. Framingham, MA 01702 Drug Labeler Code: 042976
C. Proprietary Name(s):	Bc6 rDNA construct in GTC 155-92 Goats
D. Dosage Form(s):	Heritable construct
E. Species/Class(es):	Domesticated goat
F. Indication(s):	Expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland

II. PRODUCT DEFINITION

A specific hemizygous diploid line of domestic goats (*Capra aegagrus hircus*), containing five copies of the Bc6 rDNA construct located at the GTC 155-92 site, directing the expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland of goats derived from lineage progenitor 155-92.

III. MOLECULAR CHARACTERIZATION OF THE CONSTRUCT

The Bc6 constructs consists of both caprine and human sequences. The goat sequences include the β casein promoter, translation initiation signal, polyadenylation sequence, RNA splice sequences, and 5' and 3' flanking regions. The open reading frame is comprised of the complementary DNA sequence for human antithrombin III. Other than the addition of DNA endonuclease restriction sites, no other sequences are present in the construct.

The sponsor submitted sequence information for the construct as well as methods and data verifying the methods used for the generation of the Bc6 rDNA construct. These data were evaluated and found to be adequate to support the characterization of the construct used to generate the GTC 155-92 lineage of goats.

IV. MOLECULAR CHARACTERIZATION OF THE GE ANIMAL LINEAGE

The GTC 155-92 goats contain five copies of the Bc6 construct located at a specific insertion site. The sponsor provided data supporting the characterization of the 155-92 lineage. These data included, but were not limited to, PCR sequencing, protein mapping, Southern analysis, Northern analysis, Western analysis, and fluorescence *in situ* hybridization. All data support the product definition's molecular description of the 155-92 lineage.

V. PHENOTYPIC CHARACTERIZATION

A. Farm Overview and Animals

Dairy goats at the sponsor's farm in Massachusetts consist primarily of the Alpine, Saanen, Toggenburg, and Nubian breeds (and their crosses). Initial animal sourcing was from the United States (New England and New York) and New Zealand. Goats sourced from the U.S. were used to produce the first GE goats, including the male founder goat (155-92) for the GTC 155-92 herd. The sponsor imported goats from New Zealand on three separate occasions between 1994 and 1998. The U.S. and New Zealand sourced goats were maintained as separate herds until they were combined in 2000. The sponsor currently maintains their goat herd as a closed herd to further

introduction of outside animals, and is certified scrapie-free by Animal and Plant Health Inspection Service (APHIS) in the USDA Voluntary Scrapie Flock Certification Program.

The GTC 155-92 goats carry a recombinant DNA (rDNA) construct (Bc6) containing the cDNA coding region of recombinant human antithrombin (rhAT) and the goat beta casein promoter. The rDNA construct carries the genetic information directing the specific expression of rhAT in the mammary glands of GTC 155-92 lineage goats. The DNA construct was microinjected into fertilized one-cell goat embryos, which were transferred to female surrogate dams. Kids born from the surrogate dams were tested for presence of the rDNA construct and expression of the human protein. The lineage progenitor was a male goat from which subsequent offspring were generated by natural breeding to give rise to the GTC 155-92 production herd. The production herd consists of qualified female dairy goats with the Bc6 rDNA construct stably integrated into their genome. This phenotypic characterization applies to offspring (F_1 and later generations) in the GTC 155-92 goat lineage.

B. Animal Housing

Animal housing on the sponsor's farm consists predominantly of a large animal barn design with internal penning and feeding areas. Goats have free access from internal pens through doors to adjoining outside paddock areas unless inclement weather dictates internal housing. No free-range pasturing is allowed for any goats. There is a double fence system at the site; internal fencing maintains each group of animals within the adjoining paddocks attached to each barn, while the external fencing system encompasses the entire campus of buildings. Most barns employ passive ventilation through screened ventilation curtains, with active ventilation (i.e., exhaust fans) present in some buildings.

Goats are segregated before sexual maturity into age and size cohorts, and where applicable, physiological status (i.e., lactating vs. dry, pregnant, etc.). Young goats (kids) up to approximately two months of age are housed in dedicated nurseries and kid spaces (no access to external paddocks). The GTC 155-92 goats are co-mingled with non- GE herd mates at all times except when rhAT-producing does are moved to separate housing/milking facilities for collection of milk for source material.

C. Animal Identification

All goats at the sponsor's farm have three forms of identification: (1) neck chain; (2) ear tattoo; and (3) subcutaneous electronic transponder.

D. Feeding and Nutrition

Kids are not allowed to nurse from their dams. During the first 24 hours of life, kids receive three colostrum feedings to assure passive transfer of immunity from

colostrum. Colostrum from herd does is heat treated at 57° C (135 ° F) for 30 minutes and frozen until needed to feed newborn kids. Through two months of age, kids are fed pasteurized (63 ° C/145 ° F for 30 minutes) milk from herd does. During this time, kids are provided a pelleted commercial kid starter, specially formulated to be free of ruminant-derived protein and fat, to acclimate them to consuming solid feed. The kid starter contains decoquinate at approved concentrations for control of coccidiosis.

The daily dietary ration for all adult goats is comprised of grass or alfalfa hay, pelleted commercial concentrate, and trace mineral salt blocks (free choice), and goats have free access to water. Goats receive a nutritionally balanced ration (NRC, 1981) with respect to the goat's age, size, and condition. Farm personnel assess body condition (1 to 5 scale; 1 = underweight, 3 = optimum weight, 5 = overweight) of goats and further segregate underweight/thin animals to help them regain condition and meet their nutritional requirements. Personnel evaluate diets every two weeks and make adjustments to diets and feeding instructions as necessary to assure the goats' nutrient requirements continue to be met. Pelleted feed concentrates are specially formulated to be free of animal-derived protein and fat. The sponsor selects hay suppliers based on their ability to provide hay from fields that (1) are not grazed by livestock; (2) are not treated with pesticides (within a specified time of harvest); and (3) have not had animal manure spread as fertilizer.

Samples of all dry feeds (hay, pelleted concentrates) are subjected to proximate analyses for nutrients to assure they are suitable for use. In addition, these feeds are assayed for contaminants. No feedstuffs are released for use until confirmation of (1) acceptable nutrient analyses, and (2) negative contaminant results. The sponsor's herd veterinarian evaluates all feed testing results to assure the feeds meet the nutritional requirements of the goats.

E. Health Management Procedures and Observations

The sponsor's herd health program at their farm represents an integrated approach that encompasses procedures related to animal husbandry, nutrition, preventative health, routine daily health observations, veterinary medical evaluation, and therapy.

1. Preventative Health Procedures

The sponsor maintains a closed (no further live goat introductions since 2000), specific pathogen-free (SPF) herd, that is certified scrapie-free by APHIS in the USDA Voluntary Scrapie Flock Certification Program. To establish and maintain SPF status of the farm, the sponsor employs a strict disease surveillance program, with regularly-scheduled testing of animals for specific disease pathogens as described in Table 1.

Table 1. Specific Pathogen-Free Herd

Disease	Causative Agent	Status of GTC Herd
Caprine Arthritis Encephalitis (CAE)	Retrovirus	Herd is documented free of disease
Caprine Herpes	CapHV-1 Virus	Herd is documented free of disease
Contagious Ecthyma (Orf)	Parapox Virus	Herd is documented free of disease
Bovine Viral Diarrhea (BVD)	Pestivirus	Herd is documented free of disease
Johne's Disease (Paratuberculosis)	*Mycobacterium avium*, subsp *paratuberculosis*	Herd is documented free of disease
Brucellosis	*Brucella spp.*	Massachusetts is free of disease and herd is documented free of disease
Tuberculosis (TB)	*Mycobacterium tuberculosis*	Massachusetts is free of disease and herd is documented free of disease
Neospora	*Neospora caninum*	Herd is documented free of disease

To control further potential disease entry into the herd, the sponsor has a comprehensive and ongoing biosecurity program that encompasses internal and external aspects of farm operations and the overall animal care program. The external aspect of the program covers all sponsor personnel and visitors or service personnel/contractors. The program addresses wildlife that exists in the surrounding environment with appropriate monitoring and population control measures when indicated. The program also includes an integrated pest management program that monitors and controls incursions by birds, rodents, and insects. Internal aspects of the program focus on the herd itself, and addresses herd closure, evaluation of feeds, water, bedding, and other raw materials provided to goats, and the overall clinical health monitoring program.

All goats in the sponsor's herd are on a regular vaccination schedule based on known diseases in the area of the farm, and no live or modified-live vaccines are used. Rabies, tetanus, and *Clostridium* C and D vaccinations are administered semiannually or annually. A separate vaccination schedule is implemented for pregnant does and kids to provide additional protection for these specific groups of animals.

2. General Health and Mastitis Observations

Goats are formally observed for health status three times daily. They are observed during routine management procedures such as feeding or milking. Personnel

document the health observations and provide documentation to on-site veterinary medical staff for follow-up veterinary care and treatment.

With respect to mastitis observations made on lactating does, personnel use milk strip cups at every milking to observe presence or absence of abnormal milk. In addition, for the rhAT-producing does, milk samples are collected periodically to perform mastitis cultures. Milk samples from GTC 155-92 does are sent to a Dairy Herd Improvement Association (DHIA) laboratory for determination of somatic cell counts (SCC). The DHIA laboratory determines SCC on the same samples submitted for milk composition evaluation. For purposes of data summary and evaluation, does were considered to have mastitis if they had abnormal milk, abnormal mammary gland(s), or positive mastitis culture results.

3. Veterinary Medical Evaluation

Assessment of the health of the goats at the sponsor's farm and subsequent veterinary medical intervention includes identification of health abnormalities through routine testing procedures or regular health observations by farm personnel. Once identified, abnormalities or illnesses are brought to the attention of on-site veterinary medical staff. The veterinary medical staff make follow-up veterinary medical observations and diagnoses, and as appropriate, request additional diagnostic tests, administer therapeutic treatments, and follow the abnormality or illness to resolution.

The sponsor uses post-mortem examination (necropsy) as a diagnostic tool. On-site veterinary medical staff perform post-mortem examinations on all animals that die unexpectedly, are euthanized for medical reasons (but not for culling), and as a part of their Johne's disease surveillance program. Because of its use as a diagnostic and not a screening tool, the sponsor conducts necropsies to the level necessary to make a diagnosis and thus they are generally not comprehensive.

4. Results of Health and Veterinary Observations

Table 2 contains a summary of health observations/diagnoses (summarized as percentage of total cases, by year – 2000-2006), for major conditions. Incidences of dermatitis, diarrhea, mastitis, musculoskeletal lameness, and pneumonia were similar between GTC 155-92 goats and their non-GE herd mates. Yearly fluctuations in disease incidence between two groups were also similar. Mastitis results reflect observation of abnormal milk, abnormal mammary gland(s), and positive mastitis culture. When examining only clinical mastitis (based solely on observation of abnormal milk), incidence by year (2000 to 2006) ranged from 0.7 to 2.1%, indicating the maintenance of good mammary health in the sponsor's herd does.

Similar to the results in Table 2, there were no differences between GTC 155-92 goats and their non-GE herd mates in reproductive (hydrometra, stillbirth, dystocia, vaginal discharge), ocular (conjunctivitis, corneal ulcer, ocular discharge, uveitis), respiratory (cough, nasal discharge), digestive (colic, bloat, ketosis), skin and integument (alopecia, broken/infected horns, ectoparasites, laceration), and general (abscess, fever, infection, swelling, trauma, unthrifty) abnormalities (data not shown).

There are no concerns with respect to the health of GTC 155-92 goats, based on the similarities between the GE goats and their non-GE herd mates for the type and incidence of health abnormalities observed.

Table 2. Morbidity Summary, 2000-2006 (% of total cases)

	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Diagnosis:							
Dermatitis							
GTC 155-92 does	15	9	8	13	4	18	15
Non-GE does	7	6	8	6	10	14	10
Diarrhea							
GTC 155-92 does	4	10	4	13	23	23	17
Non-GE does	8	13	15	25	20	23	20
Mastitis							
GTC 155-92 does	4	1	2	7	3	3	10
Non-GE does	7	6	5	5	5	4	11
Musculoskeletal Lameness							
GTC 155-92 does	6	9	10	6	6	2	8
Non-GE does	5	4	4	3	3	4	4
Pneumonia							
GTC 155-92 does	2	3	2	1	8	0	6
Non-GE does	10	3	2	1	2	1	9

F. Reproductive Management Procedures/Observations

Once the lineage progenitor buck was identified, the GTC 155-92 herd was established by breeding him with female goats. Mendelian inheritance of the rDNA construct resulted in GE male and female offspring to use for breeding purposes to expand the herd, with GTC 155-92 females capable of producing rhAT in their milk. The sponsor uses marker bucks to identify does in heat, which are subsequently mated to selected bucks via natural service or artificial insemination. Along with natural mating of GE does, the sponsor occasionally introduces superior dairy genetics into the GTC-155-92 lineage and non-GE herds with semen (via artificial insemination) imported from New Zealand sources.

Reproductive performance of the GTC 155-92 does was similar to their non-GE herd mates. Over several generations, pregnancy rates were typically over 80 percent and litter sizes were two kids per doe, with occasional litter sizes of one or three kids per doe. In addition, there were no differences between GTC 155-92 goats and their non-GE herd mates for reproductive abnormalities identified in general health observations (see Section III.E.4).

Examination of the reproductive information revealed no concerns relative to reproductive safety in the GTC 155-92 lineage.

G. Milk Production and Composition Procedures/Observations

Does in the GTC 155-92 herd are milked once or twice daily in a milking parlor using standard milking and sanitation procedures as per recommendations from the distributor/manufacturer for the milking equipment in the parlor. Milk production is recorded for each doe at every milking. Milk samples are collected monthly and sent to the DHIA milk testing laboratory for milk composition determinations. The sponsor also performs analyses for milk protein profiles on monthly samples from individual does.

Does in the GTC 155-92 herd produced approximately two liters of milk per day over lactations of 150-170 days duration. This is in contrast to their non-GE herd mates that produced approximately four liters of milk per day over lactations of 200-220 days duration. This difference is attributed to the difference in dairy genetics between the GTC 155-92 and non-GE herds. The GTC 155-92 herd traces its origin to a single founder male, while the non-GE herd has access to more diverse and superior dairy production traits. The sponsor is steadily improving production in the GTC 155-92 herd through selection and use of artificial insemination with sires of superior dairy genetics.

To further confirm that lactation of GTC 155-92 does is normal, their milk composition values fall within ranges considered normal for the dairy breeds that comprise the GTC 155-92 herd. Milk fat percentages generally ranged from 2.0 to

3.5%, with an isolated number of milk samples collected in summer months (June, July, and August) that fell below 2.0%. The reduction in milk fat percentage during summer months in GTC 155-92 goats is typical of what occurs in normal dairy goat practice. Milk protein percentages generally ranged from approximately 2.0 to 3.5%, and did not vary with season of the year. Milk lactose percentage was generally 4-5% for GTC 155-92 does.

With respect to milk protein profiles, the amounts of caseins, lactalbumins, transferrin, albumin, and lactoferrin in GTC 155-92 doe milk was within the range normally seen in non-GE does. This is of particular importance for beta casein, given the Bc6 rDNA construct was inserted into the genome linked to the goat beta casein promoter.

Milk production and composition results raise no concern with respect to the safety of Bc6 rDNA construct incorporation into the genome of GTC 155-92 goats.

H. Production of rhAT Protein in Milk

To determine the presence of rhAT protein in samples of individual goats and of composited milk pools, the sponsor utilizes western analyses, reverse phase high pressure liquid chromatography, and peptide mapping. The results of these tests confirmed the presence of rhAT protein in milk of the GTC 155-92 goat lineage. These findings support the phenotypic durability aspect of “Genotypic and Phenotypic Durability” and “Claim Validation.”

I. Conclusions on Phenotypic Characterization

There were no apparent differences in the health, mastitis, nutrition, milk composition, and reproductive status of GTC 155-92 goats vs. their non-GE herd mates. Other than the presence of rhAT in the milk of the GTC 155-92 goats (which is present by design) the only difference noted was that GTC 155-92 does had lower daily milk production and shorter lactations than their non-GE herd mates. This is attributable to genetics that originated from a single founder (Buck 155-92) vs. their non-GE herd mates that had a more diverse genetic background with increased opportunity to introduce superior dairy genetics vs. the GTC 155-92 population.

We also conclude that rhAT protein is present in the milk of the 155-92 goat lineage over all generations to date. This conclusion also supports the phenotypic durability aspect of “Genotypic and Phenotypic Durability Assessment,” and “Effectiveness/Claim Validation.”

VI. GENOTYPIC AND PHENOTYPIC DURABILITY

Data were provided that demonstrate that both the phenotype and genotype of the GTC 155-92 lineage are conserved over multiple generations. The sponsor also provided a plan to ensure that future animals in the GTC 155-92 lineage will continue to meet the product definition. In the unlikely event of a failure to meet these specifications, the sponsor has developed standard operating procedures to ensure that these animals are neither used for the production herd nor are they to be introduced into the food or feed supply. Together, these data provide assurances that the GTC 155-92 lineage will continue to be equivalent to those animals evaluated for animal health and safety prior to market approval.

VII. FOOD AND FEED SAFETY

A. Food and Feed Safety

The sponsor has attested that GTC 155-92 animals will not enter the food or feed supply. Control measures include the following:

- Secure locked fencing around the entire facility, with double fencing around animal paddocks;
- Well maintained secure barns leading to fenced paddock and exercise yards;
- Round-the-clock staffing of the facility;
- Active on-site security with personnel supplemented with video surveillance;
- SOPs for animal identification and disposal that include procedures to ensure that
    - all GE animals are identified by ear tag, tattoo, and implanted identification chip;
    - all animals are incinerated at termination use.
- All milk derived from the GTC 155-92 animals is either used for additional research or spread on an organic bed disposal field (no products from this field leave the site). Any other waste material is disposed of through commercial medical waste companies.

We conclude that there is a reasonable certainty that GTC 155-92 goats will not be introduced into the human or animal food supply.

GTC 155-92 goats containing the Bc6 rDNA construct are not intended to be used for human or animal food. Goats containing the Bc6 rDNA construct may not be used for food or feed.

B. Regulatory Method

1. Determinative and Confirmatory Method

A satisfactory method has been provided to detect the Bc6 rDNA construct in blood in the event of an inadvertent release of GTC 155-92 GE goats or their edible tissues.

The regulatory analytical method for the presence of the Bc6 rDNA construct in tissue is a polymerase chain reaction (PCR) method which provides acceptable sensitivity for routine monitoring to identify and confirm tissue from the genetically engineered goats. The method detects the presence of genes for the production of human antithrombin and goat β-casein.

2. Availability of the Method

The validated regulatory method for detection of the Bc6 rDNA construct is available from CVM, FDA, 7500 Standish Place, Rockville, MD 20855.

VIII. ENVIRONMENTAL SAFETY

After reviewing the sponsor's submissions and the CVM site visit report, an environmental assessment (EA) document was prepared which summarizes relevant information from these documents and addresses the potential hazards and risks of the Bc6 rDNA construct in the lineage of GTC 155-92 goats to the environment (EA dated January 29, 2009). Based on the analyses in the EA, it is concluded that the approval of a new animal drug application (NADA) for the Bc6 rDNA construct in the 155-92 lineage of GE goats will not have a significant effect on the quality of the human environment, therefore, an environmental impact statement will not be prepared. A finding of no significant impact (FONSI) has been prepared summarizing the basis for this decision. The EA and FONSI may be seen in the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

IX. CLAIM VALIDATION

To determine the presence of rhAT protein in samples of individual goats and of composited milk pools, the sponsor uses western analyses, reverse phase high pressure liquid chromatography, and peptide mapping. The results of these tests confirmed the presence of rhAT protein in milk of the GTC 155-92 goat lineage. These findings support the phenotypic durability aspect of "Genotypic and Phenotypic Durability" and "Claim Validation."

X. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514, and reflect the recommendations in Guidance 187. The data demonstrate that the Bc6 rDNA construct in GTC 155-92 goats is safe and effective for the expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland.

XI. LABEL AND LABELING

The Label:

A specific hemizygous diploid line of domestic goats (*Capra aegagrus hircus*), containing five copies of the Bc6 rDNA construct located at the GTC 155-92 site, directing the expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland of goats derived from lineage progenitor 155-92.

Food or feed from GTC-155-92 goats is not permitted in the food or feed supply.

Labeling:

The labeling consists of the Label (above) and Standard Operating Procedures that link identification of the GTC 155-92 GE animals with the label.