CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125460Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM **DEPARTMENT OF HEALTH AND HUMAN SERVICES**
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

FROM: David B. Joseph
Pharmacology Team Leader

DATE: October 31, 2013

SUBJECT: BLA 125,460 (SD # 1 dated March 29, 2013)

Sponsor: BioMarin Pharmaceutical Inc.

Drug Product: VIMIZIM™ (elosulfase alfa)

Comments:

1. VIMIZIM™ (elosulfase alfa; recombinant human N-acetylgalactosamine-6-sulfatase; rhGALNS) is an enzyme replacement therapy developed for the treatment of Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome).

2. The major finding in the toxicology studies in rats and monkeys was the rapid development of neutralizing antibodies. In the chronic toxicity studies, almost all animals in the elosulfase alfa treatment groups tested positive for neutralizing antibodies. In rats, co-administration of diphenhydramine with elosulfase alfa was necessary to prevent or minimize anaphylactic reactions. No target organs of toxicity or major adverse effects, other than signs of anaphylaxis in rats, were observed in the toxicology studies. Because the study results from both species are confounded by the presence of neutralizing antibodies, the value of these studies for characterizing the toxicity of elosulfase alfa is highly uncertain.

3. Juvenile animal toxicity testing was limited to the 52-week study in cynomolgus monkeys (age 2-3 years). This study was deemed sufficient to assure safety in the proposed Phase 3 study (MOR-004) that was included in the initial submission of IND 101,234 (submitted May 20, 2011). This study included patients age 5 years and older.

4. Following the initial IND submission, the Sponsor submitted a protocol for a study (MOR-007) in patients less than age 5 years, with no lower age limit stated (protocol submitted on September 2, 2011). The youngest patient enrolled in this study, which is still ongoing, was 9 months old. This study was allowed to proceed based on the existing juvenile animal data in monkeys age 2-3 years. It should be noted that the overall developmental stage of monkeys in this age range is more advanced relative to humans less than 5 years old, especially infants. However, given the

confounding factor of neutralizing antibodies in both the rat and monkey toxicity studies, it is reasonable to assume that the same problem will occur in a juvenile animal study with either species. Also, a study in juvenile rats would require the use of diphenhydramine, which could be an additional confounding factor. Therefore, there was no reasonable justification for requiring an additional juvenile animal study using animals that are better matched to the developmental stage of children younger than 5 years of age (including infants), to support the conduct of clinical study MOR-007.

Recommendations:

There are no nonclinical issues which preclude the approval of VIMIZIM™. I concur with Dr. Cai's recommendation for approval, and her recommendations for labeling revisions.

_____ _____

David B. Joseph, Ph.D. Date
Pharmacology Team Leader
Division of Gastroenterology and Inborn Errors Products

cc:
BLA 125,460
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/F. Cai
DGIEP/J. Lee
OND IO/A. Jacobs

Reference ID: 3399836

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/s/

DAVID B JOSEPH
10/31/2013

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125,460
Supporting document/s:	000
Applicant's letter date:	March 29, 2013
CDER stamp date:	March 29, 2013
Product:	VIMIZIM™ (elosulfase alfa; recombinant human N-acetylgalactosamine 6-sulfatase; rhGALNS)
Indication:	Mucopolysaccharidosis Type IVA (MPS IVA; Morquio A Syndrome)
Applicant:	BioMarin Pharmaceutical Inc. Novato, CA
Review Division:	Division of Gastroenterology and Inborn Errors Products
Reviewer:	Fang Cai, Ph.D.
Supervisor/Team Leader:	David Joseph, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Elizabeth Ford

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1 Executive Summary

1.1 Introduction

VIMIZIM™ (elosulfase alfa) contains recombinant human N-acetylgalactosamine-6-sulfatase (rhGALNS) expressed by and purified from Chinese Hamster Ovary (CHO) cells. Elosulfase alfa is taken up by cells via the cation-independent mannose-6-phosphate receptor (CI-M6PR) followed by transfer and localization to lysosomes. GALNS hydrolyzes the sulfate ester bonds in N-acetyl-galactosamine-6-sulfate or galactose-6-sulfate on the non-reducing ends of keratan sulfate (KS) and chondroitin-6-sulfate (C6S). KS and C6S are glycosaminoglycans (GAGs) present in lysosomes.

Morquio A syndrome, also known as Mucopolysaccharidosis type IVA (MPS IVA) is a rare, devastating, inherited autosomal recessive disorder caused by mutations in the gene that encodes the lysosomal enzyme N-acetylgalactosamine-6-sulfatase (GALNS), which degrades GAGs including KS and C6S. With insufficient GALNS activity, GAGs progressively accumulate in multiple organs and tissues. The persistent and progressive accumulation of GAGs leads to significant morbidities and multi-systemic clinical impairments resulting in diminished functional capacity, decreased endurance, impaired quality of life, and early mortality.

BioMarin Pharmaceutical Inc. is developing elosulfase alfa (BMN 110) as an enzyme replacement therapy (ERT) for the treatment of Mucopolysaccharidosis type IVA.

1.2 Brief Discussion of Nonclinical Findings

The sponsor has conducted a full battery of nonclinical studies, which included pharmacology studies in Morquio chondrocytes isolated from the iliac crest of MPS IVA patients, safety pharmacology studies in rats and monkeys, pharmacokinetic/toxicokinetic studies in rats, mice, and monkeys, and repeat-dose toxicology studies of up to 26 weeks in rats and 52 weeks in monkeys. Reproductive and developmental toxicity was evaluated in rats and rabbits.

Cellular uptake of BMN 110 was demonstrated in the human Morquio fibroblast cell line (b) (4) and in human Morquio chondrocytes isolated from the iliac crest of MPS IVA patients. The uptake of BMN 110 was followed by transfer to lysosomes. The calculated K_{uptake} was approximately 2.5 nM and the intracellular $t_{1/2}$ was approximately 6 days. The internalization of BMN 110 to human Morquio chondrocytes resulted in intracellular KS clearance and restoration of GALNS activity in lysosomes. After a single or repeat-dose intravenous (IV) bolus injections (10 mg/kg) in mice, BMN 110 was detected in the lysosomal compartment of MPS IVA target tissues (growth plate, liver, and heart valves) using confocal microscopy.

BMN 110 was rapidly cleared from plasma in rats and monkeys. The $t_{1/2}$ values in rats were 2-9 minutes among all dose groups following the initial IV bolus administration of

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BMN 110 in the 26-week toxicity study. On week 26, the $t_{1/2}$ in the high-dose group was increased to 26 minutes. Following repeated IV (weekly) infusion of BMN 110 in monkeys, the $t_{1/2}$ values increased from approximately 10 minutes on week 1 to 30-90 minutes on week 52. Systemic exposure (C_{max} and AUC_{0-t}) in monkeys was increased at week 52, compared with the initial dose (e.g. 2 to 3-fold increase in the 6 and 20 mg/kg groups). The prolongation of $t_{1/2}$ and increase in systemic exposure in rats and monkeys after repeated administration was likely due to the formation of anti-drug antibodies, which included neutralizing antibodies in both species. BMN 110 was detected in fetal rabbit plasma in a dose-ranging embryo-fetal development study, and in milk in the pre-/postnatal developmental study in rats.

A 26-week IV toxicity study in rats and a 52-week IV toxicity study in juvenile monkeys (age 2-3 years) were conducted. In both studies, BMN 110 was dosed at 1, 6, and 20 mg/kg once weekly. The rat study included the pretreatment of BMN 110-treated groups with diphenhydramine (DPH) starting on week 3 to eliminate or minimize anaphylactic-type reactions. Therefore, a second control group treated with DPH alone was included in this study. Among the two chronic toxicity studies, the major findings occurred in rats, and were related to anaphylactic-type reactions (i.e. red skin on all paws or ears, labored respiration, hypoactivity, squinted eyes, and dilated pupils). These effects were likely due to the administration of an antigenic heterologous protein. Three unscheduled deaths in the 26-week rat toxicity study were attributed to adverse drug-related anaphylactic-type reactions. These reactions were generally mitigated by DPH pretreatment at a dose of 2 mg/kg (oral). BMN 110 at 6 and 20 mg/kg/week increased urine volume and pH, and decreased urine specific gravity in rats. However, no associated histopathological changes were observed in kidneys. No drug-related mortalities or adverse effects were observed in the 52-week IV (infusion) monkey toxicity study. BMN 110 had no effects on monkey bone or cartilage growth. In summary, no target organ toxicity was observed after weekly administration of up to 20 mg/kg in the repeat dose toxicology studies in rats and monkeys. However, most of the animals in both of these studies developed neutralizing antibodies to BMN 110. Thus, the utility of these studies in characterizing the toxicity of BMN 110 is very limited, and the results must be interpreted with caution. Since the potential target- or pharmacology-related toxicity may have been eliminated by the neutralizing antibodies, the interpretation of results from both chronic toxicity studies (i.e. absence of target organ toxicity or any major adverse effects aside from anaphylactic reactions) is confounded.

The effects of BMN 110 on reproduction and development were evaluated in rats at 1, 6, and 20 mg/kg/day (combined fertility and embryo-fetal developmental study, and a pre-/postnatal developmental study) and in rabbits (embryo-fetal developmental study) at 1, 3, and 10 mg/kg/day. Both studies in rats included a control group treated with the vehicle for BMN 110, and a control group treated with the vehicle and DPH (referred to as DPH alone). DPH was administered at 10 mg/kg/day via intraperitoneal injection to all rats in the BMN 110 treatment groups to prevent anaphylactic reactions. The effects of BMN 110 on all study parameters were evaluated based on comparison to the DPH alone control group.

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In the combined fertility and embryo-fetal developmental study in rats, the paternal NOAEL for general toxicity was 1 mg/kg/day IV, based on decreased body weight and body weight gain at 6 and 20 mg/kg/day. BMN 110 did not produce any significant inhibition of weight gain during pregnancy, compared to the DPH alone control group. Therefore, the maternal NOAEL during pregnancy was 20 mg/kg/day IV. Fertility and embryo-fetal developmental parameters were not adversely affected by BMN 110 treatment. Thus, the NOAEL for rat fertility and embryo-fetal development was 20 mg/kg/day IV.

In the segment 2 study in rabbits, BMN 110 was administered via intravenous infusion at doses of 1, 3 and 10 mg/kg/day during gestation days 7 to 20. The maternal NOAEL was < 1 mg/kg/day IV, based on the incidence of gross effects in liver (i.e. red, tan, mottled, raised and/or pitted areas). BMN 110 had no effects on embryo-fetal developmental parameters. Thus, the NOAEL for embryo-fetal development in rabbits was 10 mg/kg/day IV.

In the pre-/postnatal study in rats, BMN 110 at 20 mg/kg/day (+ DPH) produced signs of maternal toxicity including significant reductions in body weight, body weight gain, and food consumption. A small number of deaths occurred during pregnancy in the 1, 6, and 20 mg/kg/day + DPH groups. However, mortality was not dose dependent, and 3/7 deaths were preceded by signs of anaphylaxis or DPH toxicity. Given the small number and dubious nature of these deaths, the interpretation of the offspring observations is not substantially impacted, although the possible involvement of maternal toxicity in the adverse effects in offspring cannot be excluded. The 6 and 20 mg/kg/day BMN 110 + DPH groups had a statistically significant decrease in the percentage of liveborn fetuses and a statistically significant increase in the percentage of stillbirths. These effects were dose-dependent. The increase in percentage of stillbirths at 6 and 20 mg/kg/day exceeded the range of historical control values from the testing laboratory. The percent survival of pups during postpartum days 1-4 (viability index) was significantly reduced in the 20 mg/kg/day BMN 110 + DPH group (94.4% compared to 98.7% in the DPH alone group). The reduction in viability index was correlated with a statistically significant increase in pup mortality during postpartum days 2-4 at 20 mg/kg/day (3.8% compared to 0.6% in the DPH alone group). BMN 110 was detected in the milk of F0 dams treated with BMN 110 at doses of 6 and 20 mg/kg/day. The maternal NOAEL was 6 mg/kg/day IV based on the significant reduction in body weight gain and food consumption at 20 mg/kg/day. The NOAEL for pre- and postnatal development was 1 mg/kg/day IV, based on the increase in percentage of stillbirths at 6 and 20 mg/kg/day, and the increase in postnatal deaths at 20 mg/kg/day. BMN 110 did not have effects in tests for learning and memory in the offspring, nor were other developmental parameters affected. However, several developmental tests to evaluate behavior, locomotor activity, sensory functions, and reflex development (e.g. static righting reflex, cliff aversion, startle response) were not conducted. Since the omitted tests are routinely included in pre-/postnatal developmental studies, the evaluation of neurobehavioral development was suboptimal in this study, and this should be conveyed in the Pregnancy section of the labeling.

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1.3 Recommendations

1.3.1 Approvability

VIMIZIM™ (elosulfase alfa) is recommended for approval for treatment of patients with Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome).

1.3.2 Additional Nonclinical Recommendations

None.

1.3.3 Labeling

Established Pharmacologic Class Text Phrase

Sponsor's Proposed Version:

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

VIMIZIM is a hydrolytic lysosomal glycosaminoglycan (GAG)-specific enzyme indicated for patients with Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome).

Evaluation:

The Sponsor proposed the term "hydrolytic lysosomal glycosaminoglycan (GAG)-specific enzyme" as the EPC (established pharmacologic class) text phrase for VIMIZIM (elosulfase alfa). This is the same EPC text phrase that is designated for galsulfase, idursulfase, and laronidase. All of these drugs share a similar mechanism of action, which is the enzymatic degradation and clearance of GAGs from lysosomal storage sites. The most notable mechanistic difference among elosulfase alfa and the three approved drugs with the same EPC is related to (b) (4) The four major classes of GAGs include chondroitin sulfate/dermatan sulfate, heparan sulfate, keratan sulfate, and hyaluronic acid (Yip GW, et al, Mol Cancer Ther, 2006, 5(9): 2139-2148). Keratan sulfate and chondroitin 6-sulfate are the known substrates for elosulfase alfa, whereas dermatan sulfate and heparan sulfate are the known substrates for galsulfase, idursulfase, and laronidase. Since all of these drugs are ERTs (enzyme replacement therapies) that target GAGs in lysosomal storage sites for metabolic breakdown, the proposed EPC text phrase for elosulfase alfa is considered as scientifically valid based on its apparent mechanism of action. Furthermore, the proposed indication for elosulfase alfa is Mucopolysaccharidosis type IVA, and all of the approved ERTs with the proposed EPC text phrase are indicated for the treatment of other types of Mucopolysaccharidosis diseases. Thus, the proposed EPC text phrase is considered to be clinically meaningful, and is therefore acceptable.

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Sponsor's Proposed Version:

8.1 Pregnancy Category (b) (4)

(b) (4)

Evaluation:

The proposed pregnancy category (b) (4) is not appropriate based on results of the pre-/ postnatal developmental study in rats. This study showed that BMN 110 produced a statistically significant, dose-dependent increase in the percentage of stillbirths at 6 and 20 mg/kg/day IV. In addition, the percent survival in pups during postpartum days 1-4 was significantly reduced in the 20 mg/kg/day BMN 110 + DPH group (94.4% compared to 98.7% in the DPH alone control group). There was a significant increase in the number of pups found dead on days 2 to 4 postpartum in the 20 mg/kg/day BMN 110 + DPH group, compared to the DPH alone control group. The maternal NOAEL was 6 mg/kg/day IV based on the reduced body weight gain and reduced food consumption at 20 mg/kg/day.

AUC data is available from the combined fertility and embryo-fetal developmental study in rats, which used the same dose levels as the pre-/postnatal developmental study in rats (i.e. 1, 6, and 20 mg/kg/day BMN 110 in combination with DPH). Therefore, this labeling section should include the rat to human AUC ratio, instead of the (b) (4) (b) (4) Since AUC was not measured in the embryo-fetal developmental studies in rabbits, this section should state the rabbit to human dose ratio based on mg/m^2 comparison.

In the pre-/postnatal developmental study in rats, several tests to evaluate behavior, locomotor activity, sensory functions, and reflex development (e.g. static righting reflex, cliff aversion, startle response) in the offspring were not conducted. Since the omitted tests are routinely included in pre-/postnatal developmental studies, the evaluation of F1 generation development was suboptimal in this study (b) (4) .

The recommended changes in this section were developed in collaboration with the Maternal Health Team (Erica Radden, Jeanine Best, and Melissa Tassinari).

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Recommended Version:

8.1 Pregnancy

Pregnancy Category C

Risk Summary

There are no adequate and well-controlled studies with VIMIZIM in pregnant women. [redacted] (b) (4) No effects on embryo-fetal development were observed in rabbits given daily administration of elosulfase alfa at doses [redacted] (b) (4) A dose-dependent increase in stillbirths was observed when elosulfase alfa was administered daily in rats during organogenesis through lactation at doses 5 times [redacted] (b) (4). An increase in pup mortality was observed at doses producing maternal toxicity. VIMIZIM should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

All reproductive studies with rats included pre-treatment with diphenhydramine to prevent or minimize hypersensitivity reactions. The effects of elosulfase alfa were evaluated based on comparison to a control group treated with diphenhydramine alone. Daily intravenous administration of up to 20 mg/kg elosulfase alfa in rats (33 times the human steady-state exposure at the recommended weekly dose) during a 15-day premating period, mating, and through the period of organogenesis, produced no maternal toxicity or effects on embryo-fetal development. Daily intravenous administration of up to 10 mg/kg in rabbits [redacted] (b) (4) during the period of organogenesis had no effects on embryo-fetal development. However, maternal toxicity (gross changes in liver) was observed in rabbits given doses of 1 mg/kg/day and higher [redacted] (b) (4) Elosulfase alfa produced an increase in the percentage of stillbirths when administered daily in rats at doses of 6 mg/kg IV and higher (5 times the human steady-state exposure at the recommended weekly dose) during the period of organogenesis through lactation. Daily administration of 20 mg/kg IV (33 times the human steady-state exposure at the recommended weekly dose) produced maternal toxicity and an increase in mortality of offspring during the lactation period. This study lacked a complete evaluation of [redacted] (b) (4) however, no effects of elosulfase alfa were noted in tests for learning and memory.

Sponsor's Proposed Version:

8.3 Nursing Mothers

(b) (4)

Evaluation:

The label should state that elosulfase alfa was excreted in rat milk, and should include a cross-reference to section 8.1 because of the increased mortality in rat pups nursed by mothers receiving elosulfase alfa. The supporting findings for these recommendations are the detection of elosulfase alfa in milk from rats treated with intravenous doses of 6 and 20 mg/kg/day, and the increase in pup mortality in the 20 mg/kg/day group during lactation in the pre-/postnatal developmental study. Elosulfase alfa was detected in one of five milk samples from dams administered 6 mg/kg/day (132 ng/ml) and in four of five dams administered 20 mg/kg/day (102 - 212 ng/ml).

The recommended changes in this section were developed in collaboration with the Maternal Health Team (Erica Radden, Jeanine Best, and Melissa Tassinari).

Recommended Version:

8.3 Nursing Mothers

It is not known if VIMIZIM is present in human milk. Elosulfase alfa is present in milk from rats [see *Use in Specific Populations (8.1)*]. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VIMIZIM and any potential adverse effects on the breastfed child from the drug or from (b) (4) Exercise caution when administering VIMIZIM to a nursing mother.

Sponsor's Proposed Version:

12.1 Mechanism of Action

Mucopolysaccharidoses comprise a group of lysosomal storage disorders caused by the deficiency of specific lysosomal enzymes required for the catabolism of glycosaminoglycans (GAG). Mucopolysaccharidosis IVA (MPS IVA, Morquio A Syndrome) is characterized by the absence or marked reduction in *N*-acetylgalactosamine-6-sulfatase activity. The sulfatase activity deficiency results in the accumulation of the GAG substrates, KS and (b) (4) (C6S), in the lysosomal compartment of cells throughout the body. The accumulation leads to widespread cellular, tissue, and organ dysfunction. VIMIZIM is intended to provide the exogenous enzyme N-acetylgalactosamine-6-sulfatase that will be taken up into the lysosomes and increase the catabolism of the GAGs KS and C6S. Elosulfase alfa uptake by cells into lysosomes is (b) (4) mediated by the binding of mannose-6-

phosphate-terminated oligosaccharide chains of elosulfase alfa to mannose-6-phosphate receptors.

In the absence of an [redacted] (b) (4) pharmacological activity was [redacted] (b) (4) using human primary chondrocytes from [redacted] (b) (4) Treatment of MPS IVA chondrocytes with [redacted] (b) (4) induced clearance of KS lysosomal storage from the chondrocytes [redacted] (b) (4)

Evaluation:

In the MOA (Mechanism of Action) section, the Sponsor stated that VIMIZIM induced clearance of KS lysosomal storage from MPS IVA chondrocytes [redacted] (b) (4) These statements are supported by a pharmacology study submitted in this application. The Sponsor conducted this study to evaluate the effects of elosulfase alfa on lysosomal KS, extracellular KS, and chondrogenic gene expression (type I and II collagen, aggrecan, and SRY-related SOX 9 gene) in cultured primary human Morquio chondrocytes. Immunocytochemistry showed that elosulfase alfa produced clearance of KS from lysosomal storage sites without affecting extracellular KS in this cell culture system. It has been reported that *SRY*-related *SOX9* gene, collagen type I and type II genes, and aggrecan gene are involved in chondrogenesis (Kosher, RA, et al, Journal of Cell Biology, 1986, 120: 1151-1156; Mwale F, et al, J Orthop Res, 2006, 24(8): 1791-1798; Lu Z, et al, Tissue Engineering Part A, 2010, 16(1): 81-90). The impaired expression of these genes in cultured Morquio chondrocytes was restored to the levels observed in normal chondrocytes, following treatment with elosulfase alfa. Thus, the pharmacology study conducted by the Sponsor provided evidence to support the statements related to specific aspects of the pharmacological activity of elosulfase alfa in the proposed MOA section.

For the following sentence, we recommend that [redacted] (b) (4) be replaced with "evaluated", given that this study evaluated MPS IVA chondrocytes from only one patient.

"In the absence of an animal model correlative of the disease, VIMIZIM pharmacological activity was [redacted] (b) (4) *using human primary chondrocytes from a MPS IVA patient."*

Also, we recommend that [redacted] (b) (4) be replaced with "elosulfase alfa" in the second paragraph. All other recommended changes were developed in collaboration with the review team.

Recommended Version:

12.1 Mechanism of Action

Mucopolysaccharidoses comprise a group of lysosomal storage disorders caused by the deficiency of specific lysosomal enzymes required for the catabolism of glycosaminoglycans (GAG). Mucopolysaccharidosis IVA (MPS IVA, Morquio A Syndrome) is characterized by the absence or marked reduction in *N*-acetylgalactosamine-6-sulfatase activity. The sulfatase activity deficiency results in the accumulation of the GAG substrates, KS and C6S, in the lysosomal compartment of cells throughout the body. The accumulation leads to widespread cellular, tissue, and organ dysfunction. VIMIZIM is intended to provide the exogenous enzyme N-acetylgalactosamine-6-sulfatase that will be taken up into the lysosomes and increase the catabolism of the GAGs KS and C6S. Elosulfase alfa uptake by cells into lysosomes is mediated by the binding of mannose-6-phosphate-terminated oligosaccharide chains of elosulfase alfa to mannose-6-phosphate receptors.

In the absence of an animal disease model that recapitulates the human disease phenotype, elosulfase alfa pharmacological activity was evaluated using human primary chondrocytes from (b) (4) MPS IVA patient. Treatment of MPS IVA chondrocytes with elosulfase alfa induced clearance of KS lysosomal storage from the chondrocytes.

Sponsor's Proposed Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with (b) (4) (b) (4)

Evaluation:

In the absence of carcinogenicity studies, a statement should be added to indicate the expectation of a low potential for carcinogenicity, based on the mechanism of action (see Carcinogenicity section for details). In the first sentence, (b) (4) should be replaced with "elosulfase alfa". The rat to human dose ratio was calculated based the (b) (4) However, AUC data is available from the combined fertility and embryo-fetal developmental study in rats. Therefore, this labeling section should include the rat to human AUC ratio, instead of the (b) (4) In the recommended version below, the rat $AUC_{0-\infty}$ values from the end of the dosing period are used for calculation of rat/human AUC ratios (33,004 mcg•min/mL and 20,081 mcg•min/mL in male and female rats, respectively, at 20 mg/kg/day, compared to 600 mcg•min/mL in adult patients with MPS IVA).

Recommended Version

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Reference ID: 3396720

Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with elosulfase alfa. Based on the mechanism of action, elosulfase alfa is not expected to be tumorigenic. Daily intravenous administration of elosulfase alfa in rats at doses up to 20 mg/kg (55 times the human steady-state exposure in male rats and 33 times the human steady-state exposure in female rats at the recommended human weekly dose) had no effects on fertility or reproductive performance.

Sponsor's Proposed Version:

(b) (4)

Evaluation:

None of the information in this proposed section is necessary for safe and effective use of VIMIZIM in humans. Therefore, this section should be removed. It is noted that the (b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: none

Generic Name: elosulfase alfa

Code Name: BMN 110

Trade Name: VIMIZIM™

Chemical Name: recombinant human N-acetylgalactosamine-6-sulfatase

Molecular Formula/Molecular Weight: approximately 110 kDa (i.e. 2 monomers, approximately 55 kDa for each peptide chain)

Structure or Biochemical Description:

Reference ID: 3396720

BMN 110 is recombinant human N-acetylgalactosamine-6-sulfatase (rhGALNS), a soluble homodimeric protein expressed in Chinese Hamster Ovary (CHO) cells. The mature protein monomers contain 496 amino acids, including eight cysteine residues and two consensus N-glycosylation sites. rhGALNS is a glycosylated enzyme involved in the lysosomal degradation of the glycosaminoglycans (GAGs), keratan sulfate and chondroitin sulfate. The glycosylation sites on rhGALNS contain oligosaccharides enriched in mannose and mannose-6-phosphate.

Pharmacologic Class: enzyme replacement therapy

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 101,234 (BMN 110; BioMarin Pharmaceutical Inc.)

2.3 Drug Formulation

VIMIZIM (rhGALNS) is a sterile solution for infusion, packaged in a container closure system consisting of a Type I (b) (4) glass tubing vial, (b) (4) rubber stopper, and an aluminum seal with flip off cap. Each vial is filled to a (b) (4) VIMIZIM is formulated with a target pH of 5.4. The composition of the drug product is summarized in the Sponsor's table below.

Table 1: Composition of VIMIZIM Drug Product

Name of Ingredient	Concentration	Composition per vial	Function	Compendial Grade[1]
rhGALNS	1 mg/mL	5 mg	Active Ingredient	rhGALNS Reference Material[3]
Other Ingredients				
Sodium Acetate, Trihydrate	(b) (4)	13.6 mg	(b) (4)	
Sodium Phosphate Monobasic Monohydrate[2]		34.5 mg		
L-Arginine Hydrochloride		31.6 mg		
Sorbitol		100 mg		
Polysorbate-20		0.5 mg		
(b) (4)		(b) (4)		
(b) (4)				

2.4 Comments on Novel Excipients

N/A

2.5 Comments on Impurities/Degradants of Concern

N/A

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for patients with Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome). The recommended dose is 2 mg/kg administered once a week as an intravenous infusion over approximately 4 hours.

2.7 Regulatory Background

The Sponsor conducted a Phase1/2 dose escalation clinical study in the UK prior to submission of IND 101,234. In this study, approximately 20 MPS IVA patients aged 5-18 years received weekly infusions of BMN 110 at sequentially increasing dose levels of 0.1 mg/kg (weeks 1-12), 1 mg/kg (weeks 13-24), and 2 mg/kg (weeks 25-36). The Sponsor submitted IND 101,234 on May 20, 2011. In this IND submission, the Sponsor proposed a Phase 3, randomized, double-blind, placebo-controlled, multinational study to evaluate the efficacy and safety of BMN 110 in patients with MPS IVA.

3 Studies Submitted

3.1 Studies Reviewed

Study	Report #	Lot #	Testing Laboratory	Page
PHARMACOLOGY				
Development and qualification of the rhGALNS cellular uptake assay	GNS-TR-AC-006	-	BioMarin Pharmaceutical Inc. Novato, CA	24
Half-life of rhGALNS in cellular uptake assay	GNS-TR-AC-010	-	BioMarin Pharmaceutical Inc. Novato, CA	26
Keratan sulfate clearance in Mucopolysaccharidosis IVA chondrocytes	0110-08-042	-	BioMarin Pharmaceutical Inc. Novato, CA	27
Evaluation of respiratory functions in rats (IV)	0110-08-022	11333P71	(b) (4)	39
Evaluation of cardiovascular functions in telemetry-instrumented conscious cynomolgus monkeys (IV)	011-08-025	11333P53		40
PHARMACOKINETICS/ADME				
Biodistribution *in vivo* and *in vitro*	011-08-041	-	BioMarin Pharmaceutical Inc. Novato, CA	41
TOXICOLOGY				
Single Dose Toxicity				
Rat				
CNS safety pharmacology and a single dose toxicity study (IV)	0110-08-021	11333P71	(b) (4)	37
Repeat Dose Toxicity				
Rat				
26-week IV toxicity study with a 4-week recovery period	0110-08-020	11333P53 11333P71 11333P79 11333P90 NP400801A NP400801B AP400802	(b) (4)	49
Monkey				

Reference ID: 3396720

28-day IV toxicity study	BMN110-10-100	L401012 BSJJ03, 2010-0110-09	(b) (4)	61
52-week IV toxicity study with a 4-week recovery period	0110-08-018	11333P53 11333P71 11333P79 11333P90 NP400801A NP400801B AP400802 11428P95 11541P04 11541P73		69
Reproductive and Developmental Toxicity Studies				
A combined fertility and developmental toxicity study in rats	BMN110-10-007	11615P81		89
Intravenous dose-range developmental toxicity study in rabbits	BMN110-10-008	11615P78		113
Intravenous developmental toxicity study in rabbits	BMN110-10-061	11780P15		118
A pre- and post-natal reproduction study by intravenous injection in rats	BMN110-12-013	P4014-10009		128

-: not indicated.

3.2 Studies Not Reviewed

The following Analytic Methods and Validation Reports are not reviewed:

Reagent Qualification Report: Quantitative Measurement of Recombinant Human N-Acetylgalactosamine-6-Sulfatase (rhGALNS or BMN110) in Fetal Rabbit Plasma

Validation Report: the detection of antibodies to recombinant human N-acetylgalactosamine-6- sulfatase (rhGALNS or BMN110) in human, cynomolgus monkey, and rat serum

Assay Validation Report: Detection of recombinant human N-acetylgalactosamine 6-sulfatase (rhGALNS) in human, cynomolgus monkey and rat plasma

Assay Validation Report: Detection of antibodies to recombinant human N-acetylgalactosamine 6-sulfatase (rhGALNS) in human, cynomolgus monkey, and rat

Reference ID: 3396720

serum that inhibit uptake via cation-independent mannose-6-phosphate receptor (CIMPR)

Assay Validation Report: Detection of recombinant human N-acetylgalactosamine 6-sulfatase (rhGALNS) in human, cynomolgus monkey and rat plasma

Analysis of dose concentration of rhGALNS in formulation buffer using the BCA method

Assay Validation Report: Detection of Antibodies to Recombinant Human N-acetylgalactosamine-6-sulfatase (rhGALNS or BMN110) In Rabbit Serum

Assay Validation Report: Quantitative Measurement of Recombinant Human N-Acetylgalactosamine 6-Sulfatase (rhGALNS or BMN110) in Rabbit Plasma

Assay Validation Report: Detection of Antibodies To Recombinant Human N -acetylgalactosamine-6-sulfatase (rhGALNS or BMN110) In Rat Serum

Assay Validation Report: Quantitative Measurement of Recombinant Human N-Acetylgalactosamine 6-Sulfatase (rhGALNS or BMN110) in Rat Plasma

Assay Validation Report: Detection of Recombinant Human N-Acetylgalactosamine-6-Sulfatase (rhGALNS) in Rat Breast Milk (Amendment #2)

Assay Validation Report: Detection of Anti-BMN110 (rhGALNS) Total Antibodies in Rat Serum Report Number: BMN110-12-031 (Amendment #1)

Validation of Method BN005: LC/MS/MS Assay for Quantitation of Gal-4GleNAc(6S) and Galβ1-4GleNAc(6S) Derived from Keratan Sulfate in Rat Plasma
Validation of Method BN006: Quantitation of Gal-4GleNAc(6S) and Galβ1-4GleNAc(6S) Derived from Keratan Sulfate in Monkey Plasma

A one step enzyme immunological test for the quantification of degradation products of C-terminal telopeptides of Type I collagen in non-human primate serum.

3.3 Previous Reviews Referenced

N/A

4 Pharmacology

4.1 Primary Pharmacology

Reference ID: 3396720

Development and Qualification of the rhGALNS Cellular Uptake Assay (Technical Report #: GNS-TR-AC-006)

Methods:

K_{uptake} is analogous to the Michaelis-Menten K_m, with GALNS enzyme acting as the "substrate", the cell-surface CI-M6PR (or other target receptor) acting as the "enzyme", and the internalized GALNS considered as the "product". For this analogy to be valid, the assay system needs to be operating within the parameters appropriate to Michaelis-Menten kinetics.

To evaluate the dependence of GALNS uptake rate versus time in the GALNS-deficient human fibroblast cell line (b) (4) fibroblasts were incubated with multiple concentrations (up to 75 nM) of BMN 110 (rhGALNS) for 30 min to 6 hours at 37°. The cells were then washed and lysed. The amount of BMN 110 taken up into the cells was determined by enzyme-linked immunosorbent assays (ELISA). TMB (3,3′,5,5′-tetramethylbenzidine), a chromogen, was used as the ELISA substrate. The ELISA data was then used to calculate a K_{uptake}, defined as the concentration of BMN 110 in cell culture medium that results in half saturation of intracellular BMN 110 content.

Since the GALNS uptake assay is a cell-based method that calculates a parameter derived from a transformed titration curve, precision of this method will not be as good as that of methods based on a standard curve (e.g. enzymatic activity measurement). The intermediate precision was evaluated by designating one batch of GALNS (# AP400802) as a reference material to be used in each assay to monitor precision.

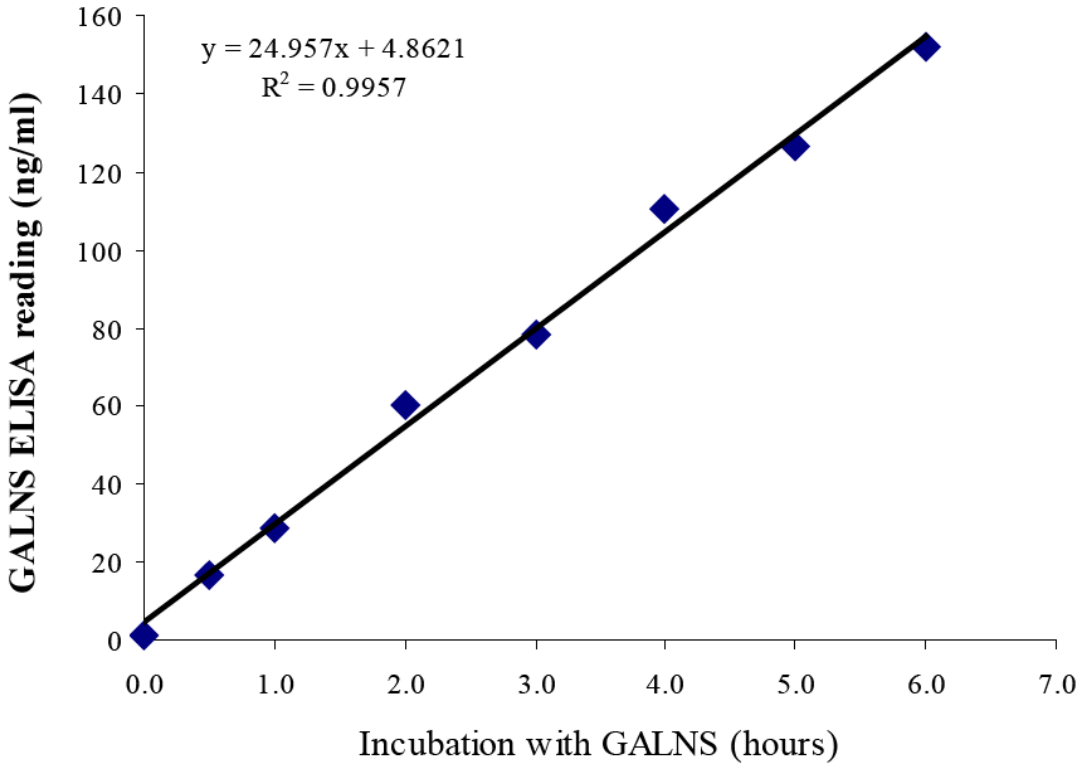
(b) (4) cells were incubated with multiple concentrations of either mannose-6-phosphate (0.4 mM to 12.5 mM) or ASB (N-acetylgalactosamine-4-sulfate sulfatase, up to 8 nM) in the presence of 2.5 nM BMN 110 to evaluate the BMN 110 uptake inhibition by these two substrates.

Results:

The Sponsor stated that the rate of uptake is well-represented by a regression line with a slope of 25 ng/ml per hour. These results show that any uptake time duration over the range tested would satisfy the necessary conditions of linear uptake rate (Figure 1). Thus, the Sponsor used a four hour incubation time for convenience and because it provided a reasonably high signal in the rhGALNS ELISA.

Reference ID: 3396720

Figure 1: Uptake of GALNS by (b) (4) Fibroblasts Increases over Time



Calculation of K_{uptake} values were based on a Hanes-Woolf plot. Data obtained from multiple assays of batch AP400802 to evaluate the intermediate precision is shown in the Sponsor's table below.

Table 2: GALNS (AP400802) Uptake in (b) (4) Fibroblasts

Date of Assay	K_{uptake} (nM)
10-02-08	3.2, 2.7
10-29-08	2.5
02-09-09	2.9
04-02-09	2.9
04-08-09	1.6
04-21-09	1.6
05-12-09	2.6
Mean K_{uptake}	2.5 ± 0.6

The Sponsor stated that the coefficient of variation was 23.8% (standard deviation divided by mean), which is within the expected range of variation for results from a cell-

based method. The mean K_{uptake} from multiple assay runs was approximately 2.5 nM in (b) (4) cells.

The BMN 110 uptake at 2.5 nM by (b) (4) fibroblasts was almost completed inhibited by 0.2 mM free mannose-6-phosphate. The lysosomal enzyme rhASB at 1 nM produced 50% inhibition of BMN 110 uptake.

The Sponsor concluded that the GALNS uptake assay had been optimized for the incubation time and selection of cell line, and that the assay exhibited adequate precision and was specific for mannose-6-phosphate receptor mediated uptake. Therefore, the assay was considered as acceptable for use in the Sponsor's quality control laboratory.

Half-life of rhGALNS in Cellular Uptake Assay (Technical report #: GNS-TR-AC-010)

Methods:

Cells from the human MPS IVA fibroblast cell line (b) (4) were incubated with 25, 50 or 100 nM rhGALNS (lot number: AP400802) for 5 hr. The medium was then changed to a normal growth (drug-free) medium and cells were incubated for periods ranging from 1 to 12 days. Individual cell cultures were then washed and lysed on days 1, 3, 4, 7 and 12. rhGALNS activity in the cell lysates was measured using an ELISA method. A synthetic substrate, 4-methylumbelliferyl-galactoside 6-sulphate (4-Mu-Gal-6-S, 1 mM) was used as a substrate for rhGALNS in the ELISA assay.

Results:

The half-life of GALNS activity obtained from multiple assays of rhGALNS is shown in the Sponsor's table below.

Table 3: GALNS Half-Life in (b) (4) Fibroblasts

GALNS Concentration (nM)	Half-life (days)
25	4.5
25	5.0
50	6.7
100	7.2
Mean Half-life	5.85 ± 1.3

The coefficient of variation was 22.3%. The Sponsor stated that this magnitude of variation is within the expected range for results from a cell-based method.

Reference ID: 3396720

Thus, the intracellular half-life of rhGALNS was approximately 6 days in the human MPS IVA fibroblast cell line (b) (4)

Keratan Sulfate Clearance in Mucopolysaccharidosis IVA Chondrocytes with BMN 110 (Report #: 0110-08-042)

Methods:

Note: This report included three studies. For clarity, certain details of the cell culture incubation conditions (e.g. treatment with BMN 110) are presented with each study in the Results section.

Primary Human Chondrocyte Cultures

Primary human chondrocytes were isolated from the iliac crest of two patients with Morquio syndrome (patients 1 and 2, MQCH cells). The majority of the experiments were performed with the cells from patient 1, whereas key experiments were repeated on cells from patient 2. Primary normal human knee chondrocytes (NHKC), isolated from normal knee cartilage, were cultured under the same conditions and used as a control.

As primary chondrocytes are known to de-differentiate into fibroblast-like cells when cultured in monolayers, the cells were grown in alginate bead suspension conditions to retain a chondrocyte phenotype, in addition to monolayer conditions.

Monolayers of MQCH and NHKC cells were cultured in chondrocyte growth medium, containing R3-IGF-1, bFGF, transferrin, insulin, FBS, gentamycin and amphotericin-B. Monolayers of MQCH and NHKC cells (b) (4) cells) were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were plated for experiments at 5000 cells/cm^2.

To induce expression of a chondrogenic phenotype, differentiation, and KS accumulation, MQCH and NHKC cells were cultured in alginate beads in the presence of IGF-1, TGFβ, transferrin, insulin and ascorbic acid for 6-15 weeks. The chondrocyte phenotype was confirmed by the presence of chondrogenic markers and extracellular matrix. The absence of GALNS activity in MQCH cells and the accumulation of keratan sulfate (KS) were confirmed by the GALNS activity assay and capillary electrophoresis (CE), respectively.

Therefore, the Sponsor studied MQCH cells in three growth phases: during their proliferation (monolayers grown to subconfluence), differentiation (6 week culture in alginate) and maturity (15 week culture in alginate) phases. The long term studies, in which MQCH cells were maintained in alginate suspension cultures for 6 -15 weeks, allowed for chondrocyte differentiation, as well as synthesis and accumulation of KS, reminiscent of the *in vivo* chondrocytes in human Morquio syndrome.

Reference ID: 3396720

Analysis of Proliferation

Slides obtained during study 2 and study 3 (see results section below) were rehydrated in PBS, permeabilized in 0.25% Triton/PBS for 5 min and mounted in ProlongGold aqueous mountant with DAPI. After curing (>24 hr at room temperature), the nuclei of cells were visualized under UV, using a fluorescence microscope (Leica DMIRE2). Nuclei of 45 cell aggregates were counted. Individual cells, distinctly separate from the cell aggregates, were excluded from counts.

Alcian Blue Staining of Cartilaginous Extracellular Matrix

Paraffin-embedded beads obtained during study 2 (see results section below) were sectioned at 5 µm, de-paraffinized, rehydrated, stained in Alcian blue, counterstained in hematoxylin, dehydrated and mounted. Bright-field photomicrographs were taken.

Immunocytochemistry

Slides obtained during studies 1, 2 and 3 (see results section below) were rehydrated in PBS, permeabilized in 0.25% Triton/PBS, blocked in 5% normal goat serum. Slides were then incubated with primary antibodies, including polyclonal anti-GALNS, monoclonal anti-KS and monoclonal or polyclonal anti-Lysosomal Associated Membrane Protein-1 (LAMP1) in blocking buffer. After a series of washes in blocking buffer and PBS, goat secondary antibodies, including anti-mouse-488, anti-rabbit-555, anti-rabbit-488 or anti-mouse-594 in blocking buffer were applied. After a series of washes, the slides were mounted in ProlongGold aqueous mountant with DAPI.

Confocal microscopy

Confocal stacks were acquired using a Zeiss LSM 510 NLO with a 40x objective, 2x zoom and 0.53 µm z increment.

qRT-PCR

RNA obtained in study 3 (see results section below) was used to generate cDNAs (High capacity RNA-to-cDNA kit; (b) (4)). Chondrocyte genes were quantified using probe-based TaqMan q-PCR kits included collagen II (COL2A1), collagen I (COL1A1), collagen X (COL10A1), aggrecan (ACAN), SOX9, and GAPDH. Results were normalized for expression of a panel of housekeeping genes (B2M, RPL13A, GAPDH and ACTB) and expressed as the fold change in MQCH cells compared to NHKC cells. Cells from patient 1 were used for these experiments.

GALNS Activity Assay

Reference ID: 3396720

GALNS in the samples were adsorbed in microtiter plate wells that were pre-coated with specific polyclonal antibodies to GALNS (5 µg/ml). Immobilized GALNS was reacted with 2 mM 4MU-galactose-6S as a synthetic substrate, which produced release of the sulfate moiety. This reaction was stopped by the addition of 25 µg/ml β-galactosidase in 300mM NaPi, pH 7.2. Released 4MU was ionized by glycine/carbonate, pH 10.6 stop buffer. Intensity of color was measured at 355 nm. GALNS concentrations in the samples were extrapolated from a standard curve with known concentrations of GALNS.

Capillary Electrophoresis (CE)

Cell lysates from studies 2 and 3 (see results section below) were digested with keratanase II, producing disaccharides from keratan sulfate with the general structure of galactose (1-4)-N-acetyl-glucosamine-6 sulfate (1-4). The disaccharide may have been sulfated or non-sulfated at the 6-galactose position. Thus, keratanase II digestion of KS present in the cell lysates generated two disaccharide peaks that are believed to be Gal6S-GlcNAc6S (peak 1) and Gal-GlcNAc6S (peak 2) in CE. The KS disaccharides in cell lysates were derivatized by reductive amination with the fluorescent dye 2-aminoacridone (AMAC) using a standardized procedure. The labeled disaccharides were measured by Laser Induced Fluorescence-Capillary Zone Electrophoresis (LIF-CZE or CE). This assay measured depletion of KS independently of its molecular size or processing state. It was assumed that all KS-like substances accumulating in the absence of GALNS activity were natural substrates of the enzyme.

Results:

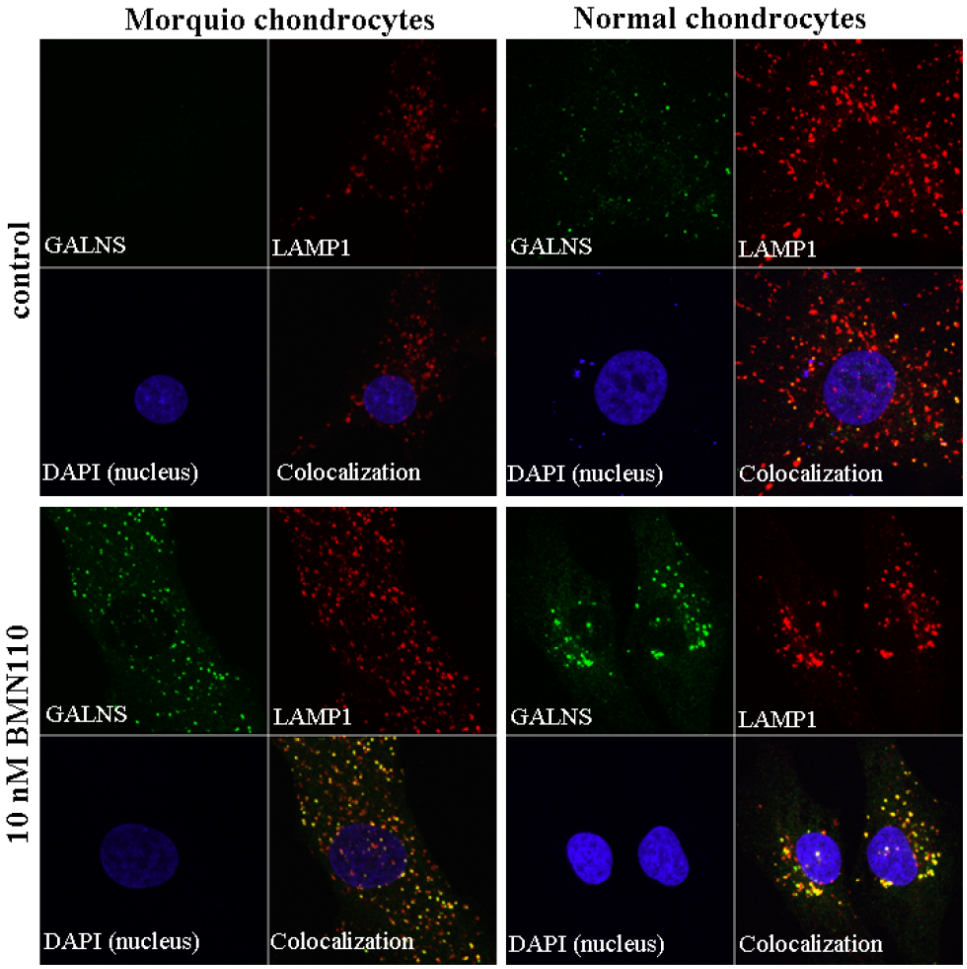
Study 1: MQCH Cells Grown in Monolayers Internalize BMN 110 into Lysosomes

MQCH cells from patient 1 and NHKC cells in monolayers were treated with either a single administration of 10 nM BMN 110 for 3 days, or two administrations of 10 nM BMN 110 (at day 0 and day 3) with incubation for a total of 6 days. The internalization of BNM 110 into lysosomes was evaluated by immunochemistry, and GALNS activity was measured by ELISA (see study 3 (2) section for GALNS activity result).

As shown in the figure below (taken from the study report), strong endogenous GALNS immunofluorescence was detected in normal chondrocytes, but not in Morquio chondrocytes. A three-day treatment with 10 nM BMN 110 resulted in a significant intracellular accumulation of BMN 110 in both Morquio and normal chondrocytes. A co-localization with LAMP-1 was observed, suggesting that BMN 110 was transported to the lysosomes following cellular uptake.

Reference ID: 3396720

Figure 2: BMN 110 Internalization into Chondrocytes Grown in Monolayers after 3-day Treatment with BMN 110



Confocal analysis of a chondrocyte monolayer after immunostaining with anti-GALNS (green), anti-LAMP-1 (red) and DAPI (blue). Colocalization of GALNS and LAMP-1 appears in orange. All images were captured with identical setting parameters. Patient 1.

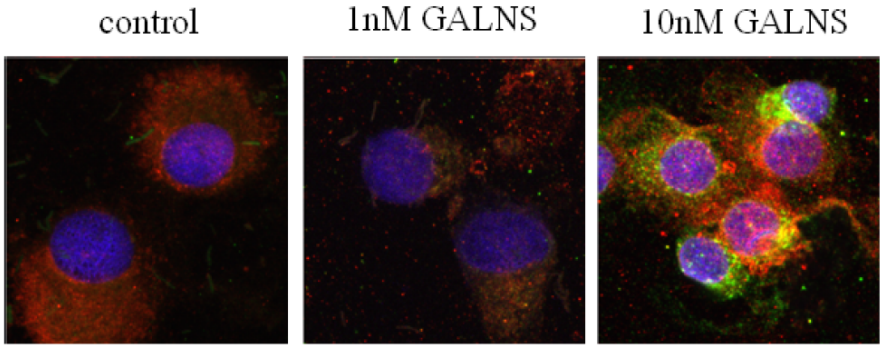
Study 2: MQCH Cells Grown in Alginate Suspension Cultures Internalize BMN 110 into Lysosomes

MQCH cells from patient 1 and patient 2, and the control NHKC cells in alginate suspension cultures were treated with 1 or 10 nM BMN 110, starting at 24 hr after initiation of the cultures. Treatment was repeated during medium change, twice a week, for 6 weeks. Bright-field photomicrographs of the cells from patient 1 were taken weekly. Weekly samples of alginate beads were taken for histology (patient 1), immunocytochemistry (patient 1 and patient 2) and estimation of proliferation (patient 1). Upon completion of the study (6 weeks of culture), total protein content was quantified using a NanoDrop spectrophotometer and protein lysates were stored at -80°C for

analysis of KS clearance by capillary electrophoresis (CE) (See Study 3 (1) B for CE results).

Treatment of MQCH cells with 1 and 10 nM BMN 110 resulted in a dose-dependent uptake of the BMN 110 into lysosomes. The Sponsor's figure below shows BMN 110 uptake after 6 weeks of treatment. The Sponsor stated that normal chondrocytes exhibited a similar dose-dependent internalization of BMN 110 when cultured under the same conditions (data not shown).

Figure 3: Dose-Dependent Internalization of BMN 110 (GALNS) in MQCH Cells Grown in Alginate Suspension Cultures for 6 Weeks



Confocal analysis of chondrocyte grown in alginate beads after immunostaining with anti-GALNS (green), anti-LAMP-1 (red) and DAPI (blue). Only colocalization (orange) of GALNS and LAMP-1 are presented. All images were captured with identical setting parameters.

Morquio chondrocytes were grown in differentiation medium for 6 weeks to allow accumulation of intracellular KS prior to BMN 110 treatment. The cells were then treated for an additional 9 weeks with 10 nM BMN 110. BMN 110 was internalized throughout the 9 weeks of treatment and transported to the lysosome as shown in the Sponsor's figure below. The results indicated that intracellular KS storage did not affect BMN 110 uptake. The cell images in the figure were obtained using cells from patient 1. The Sponsor stated that comparable observations were made in experiments with cells from patient 2, but data was not shown.

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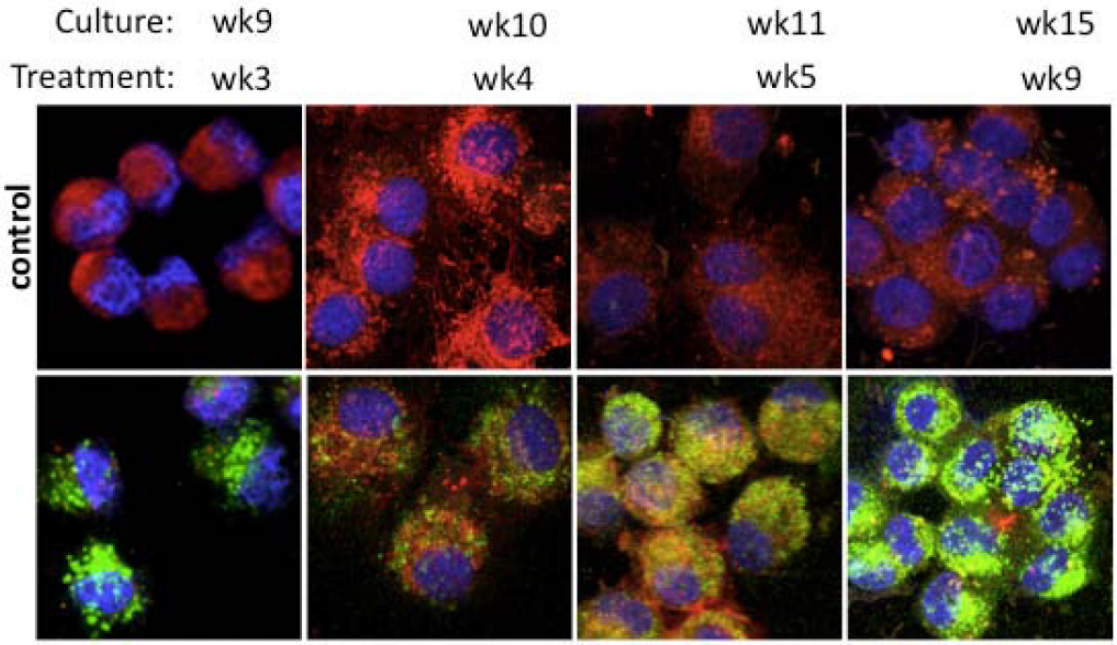
Figure 4: Uptake of BMN 110 into Lysosomes in Morquio Chondrocytes (MQCH) Cultured in Alginate Suspension Cultures for 9 Weeks



Immunostaining with anti-GALNS (green) and anti-LAMP1 (red), GALNS co-localized with LAMP1 appears in orange, nucleus is stained with DAPI (blue). Images of cells from patient 1 are shown. Comparable observations were made in experiments with cells from patient 2 (data not shown).

Study 3: Effects of BMN 110 on Differentiated MQCH Cells: Clearance of Accumulated KS, Restoration of GALNS Activity, and Normalization of Chondrogenic Gene Expression

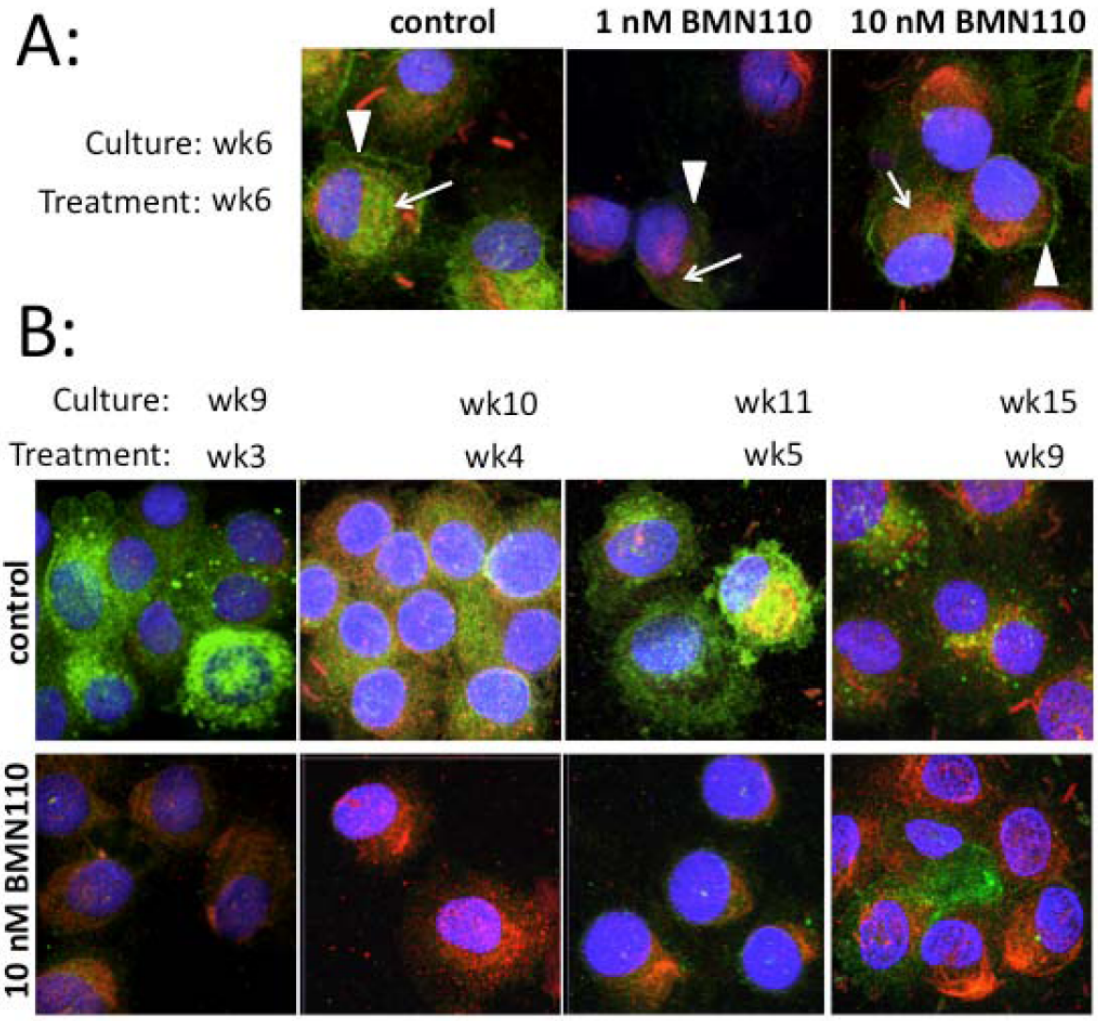
MQCH cells from patients 1 and 2, and NHKC cells were maintained in alginate suspension cultures for 6 weeks prior to treatment, in order to allow for chondrocyte differentiation and KS accumulation. BMN 110 (10 nM) was applied during medium change, twice a week, for 9 additional weeks, for a total of 15 weeks in culture. Bright-field photomicrographs of the cells from patient 1 were taken weekly throughout the culture period. At 2 and 11 weeks of culture, samples of alginate beads were harvested for RNA extraction and stored at -80°C until qRT-PCR. Throughout the treatment period, weekly samples of alginate beads were subjected to immunocytochemistry (patients 1 and 2) and estimation of proliferation (patient 1). Upon completion of the study (15 weeks of culture), total protein content was quantified using a NanoDrop spectrophotometer and protein lysates were obtained for GALNS activity assay and analysis of KS clearance by capillary electrophoresis.

Detection of KS Clearance from MQCH Cells using Immunofluorescence

After 6 weeks of treatment, BMN 110 at 1 or 10 nM reduced lysosomal KS accumulation in MQCH cells cultured in alginate beads in a dose-dependent manner. Continuous treatment with BMN 110 at 10 nM for 9 weeks reduced lysosomal KS accumulation in mature MQCH cells which had been grown in differentiation medium for 6 weeks. However, the presence of extracellular KS in the immediate vicinity of the cells was not affected by BMN 110 treatment.

Internalization of BMN 110 was observed in NHKC cells cultured in parallel with MQCH cells, throughout the 6 or 9 weeks of treatment (data was not shown in the report). However, the NHKC cells exhibited no significant change in KS immunofluorescence in response to the treatment.

Figure 5: BMN 110 Treatment Decreases Keratan Sulfate (KS) Accumulation in Morquio Chondrocytes



A: Cells were treated with 1 nM (patient 1) or 10 nM (patient 1 and 2) BMN 110 for the duration of the experiment (6 weeks). Concentration-dependent clearance of intracellular KS (arrows) was evident across the time points (representative images shown). Extracellular KS (arrowheads) was evident in all cultures. B: Cells from patient 1 and 2 were maintained for 6 weeks, then continuously treated with 10 nM BMN 110 for 9 weeks (a total of 15 weeks in culture). Untreated (control) MQCH cells exhibited significant KS staining (green, orange). Cells treated with BMN 110 showed significantly decreased KS staining. In the treated cultures, mostly lysosomal (LAMP1, red) staining was present. Nuclei were stained with DAPI (blue).

Detection of KS Clearance from MQCH Cells using Capillary Electrophoresis

Almost complete clearance of accumulated KS content in MQCH cells, based on measurement using capillary electrophoresis, was demonstrated for patient 1 following treatment of cells with BMN 110 at 1 or 10 nM for 6 weeks. Two samples were obtained from the MQCH cultures. Data presented in the Sponsor's table below shows the complete elimination of peak 1 (Gal6S-GlcNAc6S) and peak 2 (Gal-GlcNAc6S) after treatment with BMN 110 (patient 1), relative to normal chondrocytes (NHKC). Similar results were obtained with MQCH cells that accumulated KS during 6 weeks in culture with no treatment, followed by treatment 10 nM GALNS for 9 weeks (data not shown in study report).

Table 4: Keratan Sulfate Levels in Morquio Chondrocytes (MQCH) and NHKC Treated with BMN 110

BMN 110 (nM)	Morquio chondrocytes		Normal chondrocytes	
	Peak 1	Peak 2	Peak 1	Peak 2
0	1730-2165	1920-2430	140-200	340-460
1	270[a]	460[a]	300-440	910-800
10	190-240	290-350	220-330	330-550

Values are the area of the peak.
N = 2 samples (both values from the 2 samples are presented)
a: n=1 (the other sample had undetectable peaks)
Peak 1: Gal6S-GlcNAc6S
Peak 2: Gal-GlcNAc6S

BMN 110 had no effects on KS in NHKC.

Restoration of GALNS Activity by BMN 110 in Monolayer and Differentiated MQCH Cells

Treatment of MQCH cell monolayers with 10 nM BMN 110 for 3 days completely restored GALNS activity. Restoration of GALNS activity also occurred in alginate MQCH suspension cultures after 3 or 9 weeks treatment. The results are summarized in the Sponsor's table below.

Table 5: GALNS Activity in MQCH and NHKC Cells after BMN 110 Treatment

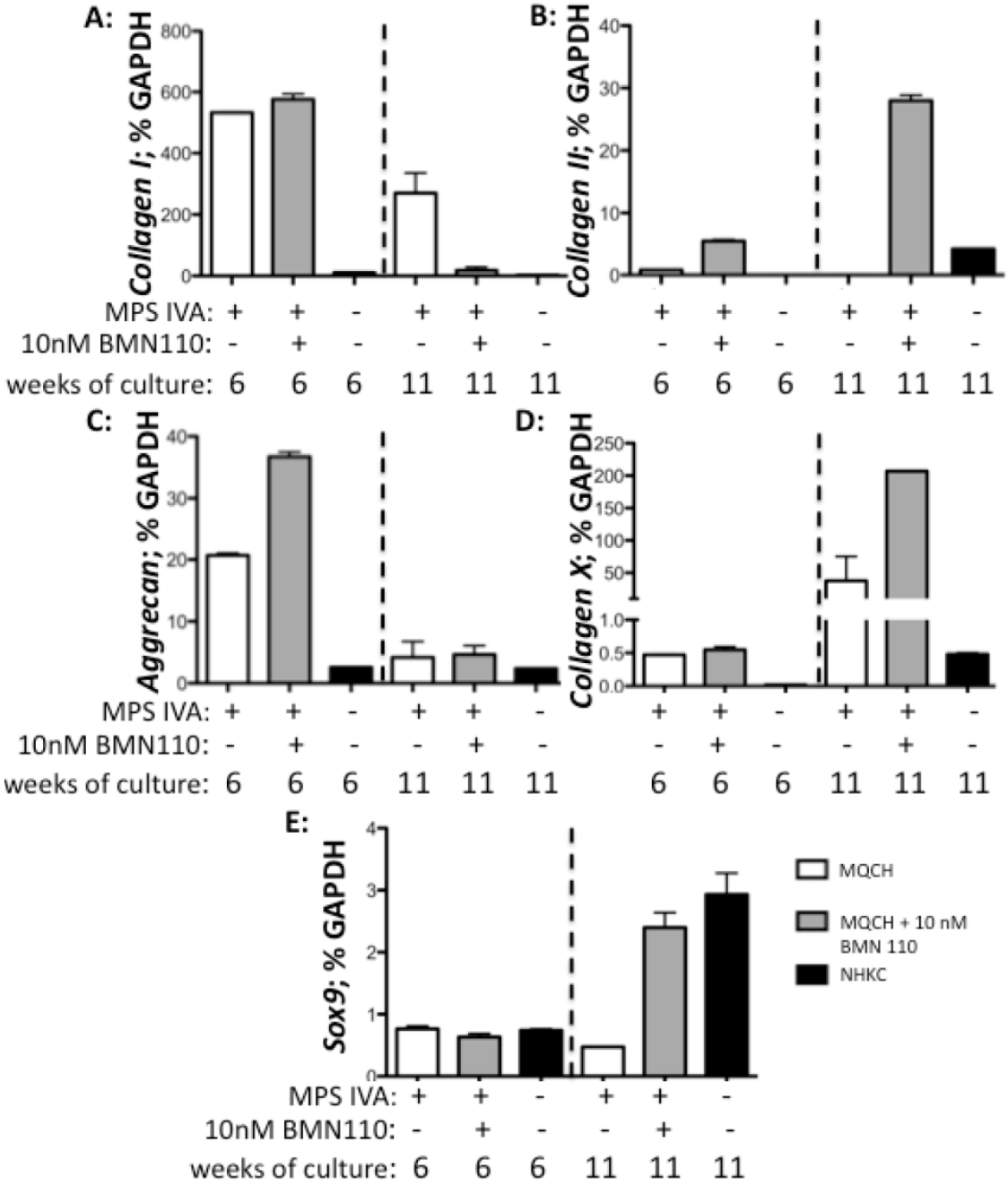
	GALNS activity (ng of active enzyme/µg protein)			
	MPS IVA chondrocytes		Unaffected chondrocytes	
nM rhGALNS:	0nM	10nM	0nM	10nM
Monolayers, 3-day culture[a]	BLOQ[c]	2.2 ± 0.18[c]	0.1 ± 0.003	2.2 ± 0.03
Alginate, 15-week culture[b]	BLOQ[c]	1.5[c]	0.3	1.6
Alginate, 3-week culture[b]	BLOQ[d]	0.8[d]	0.21	0.7

BLOQ, below the limit of detection (value <0.1); [a]n=3; [b]n=1; [c]MPS IVA patient 1; [d]MPS IVA patient 2

Normalization of Chondrogenic Gene Expression by BMN 110 in MQCH Cells

BMN 110 at 10 nM produced normalization of gene expression for collagen I and Sox 9 (sex determining region Y-box 9, a master transcription factor for the chondrogenic lineage) in differentiated MQCH cells cultured for 11 weeks. However, BMN 110 at 10 nM increased the expression of collagen II in proliferating (6-week culture) and differentiated MQCH cells, increased the expression of aggrecan in proliferating MQCH cells, and increased the expression of collagen X in differentiated MQCH cells. The effects of BMN 110 on chondrogenic gene expression profiles are summarized in the Sponsor's figure below.

Figure 6: Gene Expression in Morquio Chondrocytes after BMN 110 Treatment



In summary, primary human Morquio chondrocytes (MQCH) in alginate suspension culture expressed chondrogenic phenotype and differentiation. MQCH cells expressed chondrocyte markers including Sox 9, collagen II, collagen X and aggrecan mRNAs and elaborated extracellular matrix. MQCH cells accumulated KS and exhibited a reduced proliferation rate due to the lack of GALNS. Compared to human normal knee chondrocytes (NHKC), MQCH cells exhibited altered chondrogenic gene expression including an increase in matrix metalloprotease 13, collagen X, collagen I, aggrecan and

a decrease in Sox 9. MQCH cells internalized BMN 110 into lysosomes in a dose-dependent manner. Treatment of MQCH cells with BMN 110 at 1 and 10 nM restored intracellular GALNS activity and cleared lysosomal KS storage. BMN 110 also modulated gene expression, increasing expression of the chondrogenic genes collagen II, collagen X, aggrecan and Sox 9, and decreasing the abnormal expression level of collagen I. Thus, the Sponsor concluded that the Morquio chondrocyte model (MQCH cells) is an appropriate system for *in vitro* assessment of BMN 110 pharmacological activity.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

Effects of BMN 110 on Central Nervous System and Single Dose Toxicity Study in Rats Following Intravenous Administration (Study report #: 0110-08-021)

Methods:

This was a GLP study. Sprague-Dawley rats (6/sex/group) in the main study were treated with a single dose of BMN 110 at dose levels of 0, 1, 6, and 20 mg/kg via an intravenous bolus injection. After dosing, animals were observed for 14 days. All animals were evaluated using a modified Irwin neurological assessment during the pre-dose phase and at approximately 0.25, 2, 6, and 24 hours after dosing. The modified Irwin neurological assessment included evaluation of home cage observations, hand held observations, open field observations, and elicited behaviors.

Clinical signs and mortality were observed twice daily. Body weights were recorded before dosing on day 1 and on days 8 and 14. At study termination, blood samples were collected for hematology, coagulation, and clinical chemistry from fasted animals via jugular vein. Urine samples were collected from fasted animals for urinalysis. All animals in the main study group were subjected to necropsy at termination. The following organs were weighed at termination: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, and uterus.

The following organs or parts of organs from the main study groups were fixed in 10% buffered formalin; the organs marked with an asterisk were subjected to microscopic examination: adrenals, aorta, bone (femur), bone marrow (femur), brain*, cecum, cervix, colon, duodenum, epididymis*, esophagus, eyes with optic nerve*, harderian gland (preserved in modified Davidson's fixative), heart* (including left atrioventricular valve), ileum, injection sties*, jejunum, kidneys*, lesions*, liver*, lungs* (with large bronchi), lymph nodes (mandibular and mesenteric), mammary gland (females), ovaries, pancreas, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve,

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seminal vesicles, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen*, sternum with bone marrow, stomach, testes (preserved in modified Davidson's fixative), thymus, thyroid/parathyroid, tissue masses or tumors (including regional lymph nodes), tongue, trachea (including larynx), urinary bladder, vagina, and uterus.

In the toxicokinetics study, rats (6/sex/group) were treated with the same dose levels as in the main study. Blood samples were collected at pre-dose, 1, 5, 15, 30, 60, and 100 minutes post-dose. At 8 hr after dosing, blood samples were also collected from the TK groups to analyze keratan sulfate.

The vehicle was 20 mM sodium acetate, 1 mM sodium phosphate, 150 mM NaCl and 0.01% polysorbate 20 (pH 5.5). The dose volume was 6.6 ml/kg.

Plasma concentrations of BMN 110 were measured using a validated ELISA method. The lower limit of quantification (LOQ) was 30 ng/ml.

The basic repeating disaccharide unit within keratan sulfate is -3Galβ1-4GlcNAcβ1-, which can be sulfated at carbon position 6 (C6) on both the Gal and GlcNAc moieties, or on GlcNAc alone. Thus, Galβ1-4GlcNAc(6S) and Gal(6S)β1-4GlcNAc(6S) were measured in plasma as keratan sulfate degradation products using an LC/MS/MS method.

Results:

Two mid-dose (6 mg/kg) males in the TK group died shortly after 30 or 100 min post-dose blood sample collection. Necropsy was not performed.

There were no treatment-related changes in clinical signs, body weight, body weight gain, body temperature, clinical chemistry, hematology or urinalysis parameters.

BMN 110 had no effects on absolute and relative organ weights.

One low-dose (1 mg/kg) female (# B84469) exhibited the following changes: enlarged left kidney (pelvis), enlarged ureters, thickened urinary bladder, and moderate or slight transitional cell hyperplasia in kidney, ureter, and urinary bladder. One high-dose male (20 mg/kg) also exhibited an enlarged left kidney. Since most of these changes were observed only in one low-dose female, the changes in total were not considered as treatment-related. Thus, there were no treatment-related macroscopic or microscopic findings.

BMN 110 had no effects on body temperature or neurobehavioral parameters (open field observations, hand held observations, home cage observations, and elicited behaviors). Thus, a single IV injection of up to 20 mg/kg BMN 110 in rats had no effects on CNS function.

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The $t_{1/2}$ ranged from about 2 to 8 min as the dose increased from 1 to 20 mg/kg. The increase in AUC for BMN 110 at both dose increments was greater than dose proportional. There was no significant (>2-fold) gender-related difference in mean C_{max} or AUC_{0-t} values. The TK parameters are summarized in the Sponsor's table below.

Table 6: Toxicokinetic Parameters for BMN 110 in Rat Plasma

Dose Group	Dose Level (mg/kg)	Sex	AUC_{0-t} (ng*min/mL)	$AUC_{0-\infty}$ (ng*min/mL)	C_{max} (ng/mL)	$t_{1/2}$ (min)
6	1	M	80594	80937	12950	2.01
		F	65226	65396	14208	1.81
		Combined	72652	72906	13579	1.92
7	6	M	1652729	1652936	118908	7.16
		F	1440249	1446980	170208	6.81
		Combined	1549560	1549691	143771	9.07
8	20	M	9642416	9643451	323542	6.43
		F	11057396	11058833	404433	7.76
		Combined	10348034	10349172	354417	6.57

Note: PK parameters for the combined are derived from the mean concentration-time profile including both males and females (Figure 3).

Keratan sulfate levels in plasma of control and BMN 110-treated animals were below quantitation limits.

In summary, Sprague Dawley rats were given a single dose of BMN 110 at 1, 6, or 20 mg/kg by slow push intravenous injection. BMN 110 had no effects on clinical signs, body weight, body weight gain, clinical chemistry, hematology, or urinalysis parameters. There were no treatment-related macroscopic or histopathologic findings. BMN 110 had no effects on modified Irwin neurological assessments. The $t_{1/2}$ increased with increasing dose. The systemic exposure (AUC) increased with dose in manner that was greater than dose proportional. The no observed adverse effect level (NOAEL) was 20 mg/kg, with respect to the evaluation of CNS function.

Evaluation of Respiratory Functions Using Head-Out Plethysmography Following Intravenous Administration of BMN 110 to Rats (Study Report #: 0110-08-022)

Methods:

This was a GLP study. Male Sprague-Dawley rats (8/group) were treated with a single dose of BMN 110 at dose levels of 0, 1, 6, and 20 mg/kg via an intravenous bolus injection. The control article was 20 mM sodium acetate, 1 mM sodium phosphate, 150 mM NaCl and 0.01% Tween 20. Clinical signs and mortality were observed twice daily. Respiratory parameters (tidal volume, respiration rate, and minute volume) were

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analyzed at baseline and at approximately 0.5, 1, 2, 4, 6, and 24 hours post-dose. Baseline data were collected for at least 90 minutes on the day prior to dose administration.

Results:

BMN 110 had no effects on tidal volume, respiration rate, or minute volume. The NOAEL was 20 mg/kg.

Evaluation of Cardiovascular Functions in Telemetry-Instrumented Conscious Cynomolgus Monkeys Following Intravenous Infusion of BMN 110 (Study Report #: 011-08-025)

Methods:

This was a GLP study. Eight male research-naïve cynomolgus monkeys (*Macaca fascicularis*) were assigned to a double Latin square crossover study design. Each animal received one of the four doses (0, 1, 6, and 20 mg/kg) by intravenous infusion on days 1, 3, 5, and 8 with a dose rate of 1.67 mL/kg/hour. The doses were infused for 4 hours. The study design is summarized in the table below (taken from the study report).

Table 7: Study Design for Cardiovascular Function Evaluation in Monkeys

Animal ID	Dose Level Designation on Specified Dosing Days (mg/kg)[a, b,]			
Male	Day 1	Day 3	Day 5	Day 8
1	1	0	20	6
2	6	20	0	1
3	20	1	6	0
4	0	6	1	20
5	0	20	6	1
6	20	1	0	6
7	6	0	1	20
8	1	6	20	0

[a] BMN 110 lot 11333P53 Vehicle lot 11410P93 (20 mM sodium acetate, 1 mM sodium phosphate, 150 mM NaCl in 0.01% Polysorbate 20 pH 5.5).

[b] BMN 110 or BMN 110 vehicle dosed at a volume of 6.68 mL/kg/day and an infusion rate of 1.67mL/kg/hr.

Clinical signs and mortality were observed twice daily. Body weight was recorded weekly prior to dose, on day 1 (dosing day), and on day 7 of the dosing phase. The final body weight was recorded after the end of the telemetry collection. Qualitative food consumption was recorded once daily.

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At least 1 week before initiation of treatment, animals were anesthetized, and a catheter was surgically implanted into a femoral vein. Two weeks prior to study initiation, an electrocardiogram (ECG) and pressure transmitter (Model No. TL11 M2-D70-PCT) was implanted into the abdomen and sutured to the abdominal wall. The pressure catheter was placed in an artery and advanced into the abdominal aorta. (b) (4) telemetry equipment was used to generate and acquire the data input.

On each day of dosing, ECG, abdominal body temperature, and blood pressure measurements were recorded for approximately 90 minutes prior to dosing (baseline) and continuously for approximately 7 hours from the start of infusion. After the end of the continuous data collection, telemetry data were recorded during at least the final 15 minutes of each hour through at least 24 hours after the end of infusion. The information collected included heart rate, systolic pressure, diastolic pressure, mean arterial pressure, arterial pulse pressure, respiration rate, ECG and intra-abdominal body temperature.

Blood was collected one day before each scheduled dosing day and once following the final telemetry collection on day 9 of the dosing phase for determination of anti-GALNS (anti-BMN 110) antibody levels using an ELISA method.

Results:

There were no treatment-related changes in clinical signs, body weight, food consumption, or body temperature. No deaths occurred.

BMN 110 had no effects on ECG parameters (heart rate, PR, QRS, QT, or QT_c (Bazett correction) intervals), hemodynamic parameters (systolic, diastolic, mean arterial, and arterial pulse pressure), or respiratory rates at any time point.

Total anti-BMN 110 antibody titers were below the limit of detection (30 ng/ml) on day 1 and at the end of study on day 9.

In summary, male cynomolgus monkeys were administered a single dose of 0, 1, 6 or 20 mg/kg BMN 110 by intravenous infusion. There were no treatment-related changes in clinical signs, body weight, food consumption, abdominal temperature, or cardiovascular (hemodynamic and ECG) parameters. Anti-BMN 110 antibodies were not detected in monkey plasma during the study period. The NOAEL was 20 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Evaluation of the Biodistribution of Human Recombinant N-acetylgalactosamine-6-sulfatase (rhGALNS) *In Vivo* and *In Vitro* (Study report #: 011-08-041)

Reference ID: 3396720

Methods:

The test articles used in this study included rhGALNS, rhGALNS-A488 (rhGALNS conjugated to Alexa Fluor 488 fluorophore, and rhGALNS-A555 (rhGALNS conjugated to Alexa Fluor 555 fluorophore). rhGALNS-A488 and rhGALNS-A555 were covalently modified at Cys165 of rhGALNS, through maleimide chemistry. Intracellular distribution was determined after incubation of rabbit synoviocytes with the test articles. Tissue distribution was determined after administration of the test articles in wild-type (wt) mice. PBS, ASB and Alexa 488 fluorophore (PBS/cys-A488) were used as controls.

Methods for *in vitro* study: rabbit synoviocytes and CHO cells

Normal synoviocytes, isolated from a female rabbit, were cultured in Ham's F12 medium, supplemented with 10% fetal bovine serum and antibiotics (penicillin and streptomycin). Normal CHO G71S cells and CHO G71S cells over-expressing rhGALNS were cultured in UltraCHO medium, supplemented with 5% fetal bovine serum, antibiotics (penicillin and streptomycin) and L-glutamine.

Normal CHO G71S cells, CHO G71S cells over-expressing rhGALNS, and rabbit synoviocytes were grown in 12-well plates or chamber slides to 80-90% confluence. They were treated with 100, 50, 25, 12.5, 6.25 or 3.125 nM rhGALNS, rhGALNS-488 or rhGALNS-555 for 4 hours, followed by iodo-deoxyuridine (IDU) for 2 hours (the Sponsor did not provide the concentration for IDU).

Cells in the 12-well plates were lysed in 0.1% triton X-100 in PBS. The released rhGALNS molecules were immobilized by specific polyclonal antibodies to rhGALNS, and were measured by an ELISA method. The enzyme activity from bound rhGALNS was determined by a chromogenic reaction using hydrogen peroxide/tetramethyl-benzidine. rhGALNS concentrations in the samples were extrapolated from a standard curve with known concentrations of rhGALNS.

Cells grown in chamber slides were fixed in 4% paraformaldehyde (PFA) and permeabilized in 0.25% Triton X-100. Polyclonal rabbit primary antibodies raised against human GALNS and covalently conjugated with Alexa-488 and Alexa-555 fluorophores were applied. After brief fixation (4% PFA), sides were washed in PBS and mounted in ProLong Gold mountant with DAPI. The uptake of GALNS was identified by immunocytochemistry.

Confocal microscopy was used to confirm that subcellular compartmentalization of rhGALNS-488 colocalized with that of the rhGALNS.

Methods for *in vivo* studies

1. Single Dose Pilot Study

Two Balb/c female mice (4-5 weeks old) per time point were administered a single IV bolus injection (10 mg/kg) of rhGALNS-A488 (GALNS-488), rhGALNS (GALNS), vehicle

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(PBS/Cys-488) or ASB (N-acetylgalactosamine-4-sulfate sulfatase). Heart, liver, femurs and tibias were harvested at 2 and 24 hours after the injection. These tissues were dissected and fixed in 4% paraformaldehyde, and sectioned. The presence of rhGALNS-A488 in the growth plate, heart valves and liver was analyzed by confocal microscopy. The study design is summarized in the Sponsor's table below.

Table 8: Single Dose Pilot Study Design

	Numbers of mice	
GROUP	**2 hr***	**24 hr***
PBS	2	2
GALNS-488	2	2
GALNS	2	2
ASB	1	0

*Duration of treatment prior to euthanasia.

2. Repeat Dose Study

One to three Balb/c female mice (4-5 weeks old) per time point were administered an IV bolus injection (10 mg/kg) of rhGALNS-A488 (GALNS-488) every other day for a total duration of 8 days (5 injections). Female mice treated with IV bolus injections of PBS, PBS/cys-A488 or ASB were used as control groups. Heart, liver, femurs and tibias were harvested at 2, 4 and 8 hours after the injection. The study design is summarized in the Sponsor's table below.

The tissues were dissected and fixed in 4% paraformaldehyde, and sectioned. All tissue sections were incubated with polyclonal rhGALNS antibodies. To demonstrate the lysosomal localization of rhGALNS-A488 in heart valves, the sections of heart valves were also incubated with monoclonal LAMP-1 (lysosomal-associated membrane protein-1) antibody.

After the PBS washes, secondary donkey anti-mouse or anti-rabbit antibodies, conjugated to Alexa-555 were applied. Sections were mounted in ProLong Gold with 4',6-diamidino-2-phenylindole (DAPI). To quantify the cellular uptake of rhGALNS-A488 in the tissues, confocal microscopy captured a "confocal stack", a series of images taken at different horizontal planes within a single sample. Through a process called "volumetric analysis", the volume of intracellular space occupied by internalized rhGALNS-A488 was determined using the confocal stacks.

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Table 9: Study Design for Repeat Dose Study

	Numbers of mice		
GROUP*	**2 hr****	**4 hr****	**8 hr****
PBS	1	0	1
PBS/Cys-448	2	0	2
GALNS-488	3	3	3
GALNS	3	0	3
ASB	2	0	1

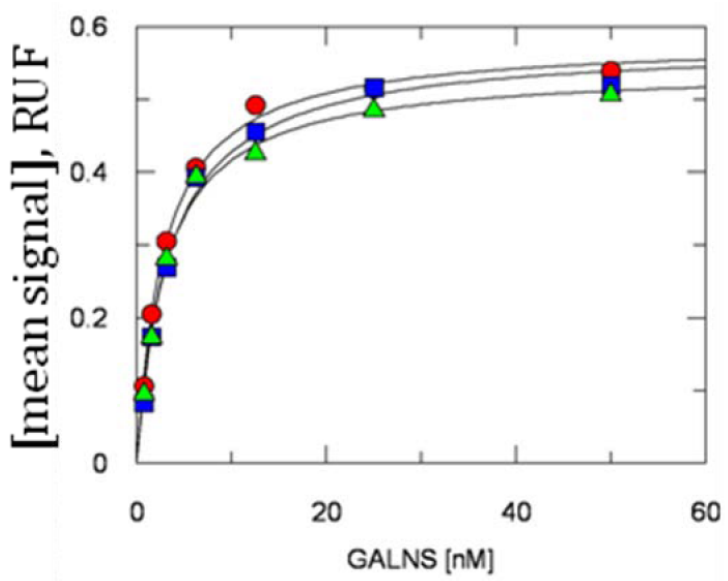
*Five doses were administered every other day. **Duration of treatment prior to euthanasia.

Results:

***In vitro* studies**

As shown in the Sponsor's figure, the levels of the intracellular rhGALNS-A488 (K_d = 3 nM) and rhGALNS-A555 (K_d = 4.5 nM) in synoviocytes were comparable to levels of rhGALNS (K_d = 5.8 nM) control. Thus, the cellular uptake of the enzyme (rhGALNS) was not substantially affected by fluorophore conjugation.

Figure 7: Uptake of GALNS, GALNS-488 and GALNS-555 by Rabbit Synoviocytes

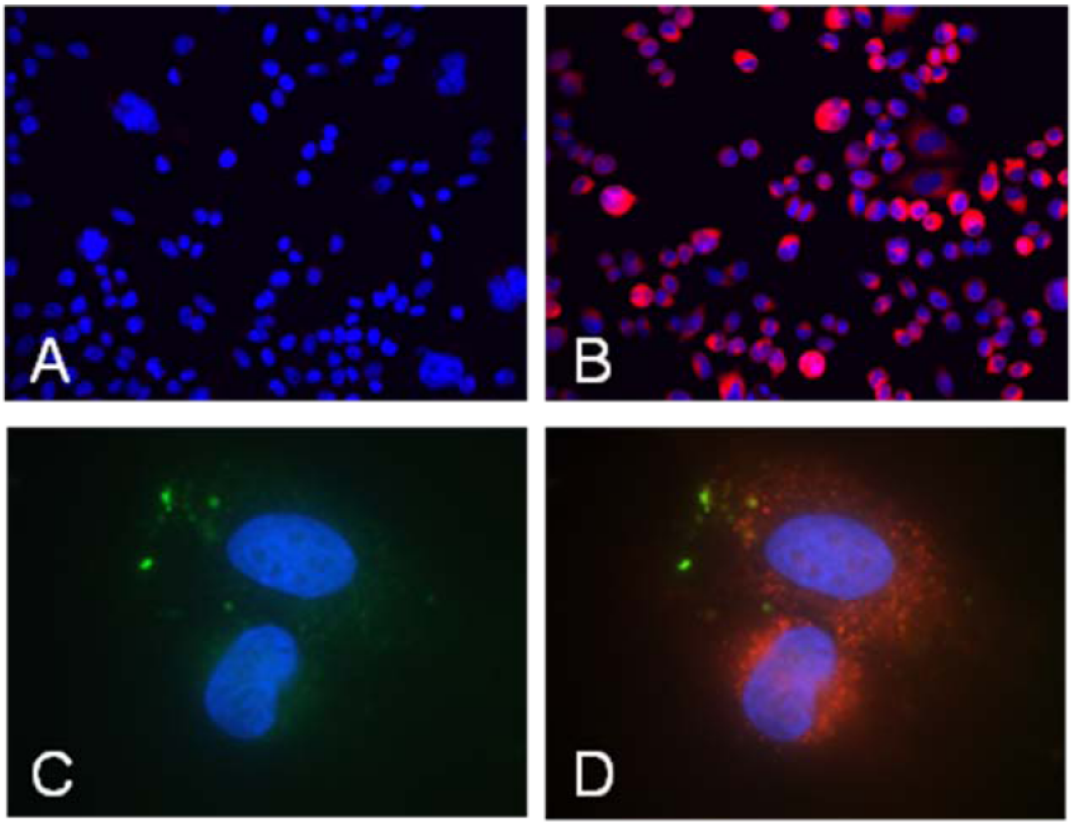


Synoviocytes exhibit comparable uptake of GALNS (red circle; K_d = 5.8 nM), GALNS-488 (blue square; K_d = 3.0 nM) and GALNS-555 (green triangle; K_d = 4.5 nM), measured by GALNS ELISA).

CHO G71S over-expressing rhGALNS exhibited strong immunoreactivity with rabbit anti-human GALNS antibodies, in comparison to the native CHO G71S cells that served

as a negative control (see panels A and B in figure 11 below from the study report). The location of rhGALNS-A488 following uptake in synoviocytes was directly visualized (green fluorescence) in panel C and D, and indirectly visualized (red fluorescence) in panel D. The strong overlap in direct and indirect rhGALNS-A488 signals indicates that the Alexa 488 fluorophore remained stably associated with the conjugated rhGALNS protein in synoviocytes.

Figure 8: rhGALNS Immunoreactivity and rhGALNS-A488 Fluorescence in CHO71S Cells and Rabbit Synoviocytes



A: CHO 71S cells show no GALNS staining (red). **B:** CHO 71S cells over-expressing rhGALNS show strong immunoreactivity (red) with polyclonal rhGALNS antibodies and secondary antibodies conjugated to Alexa-555. **C:** Rabbit synoviocytes incubated with rhGALNS-A488 show fluorescence (green). **D:** Immunoreactivity (red) in synoviocytes incubated with polyclonal rhGALNS antibodies and secondary antibodies conjugated to Alexa-555 shows a correlation with fluorescence localization in panel C. Nuclei are stained with DAPI (blue).

Therefore, the *in vitro* studies indicate that native and fluorescent GALNS enzymes are both taken up by synoviocytes through a common pathway, and the internalization of the enzyme is not affected by its conjugation to a fluorophore. The Sponsor concluded that rhGALNS-A488 was a suitable tool for studying GALNS tissue distribution *in vivo*.

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***In vivo* studies**

Growth plate cartilage and resting cartilage are major sites of keratan sulfate accumulation that lead to skeletal dysplasia in MPS IVA patients. Keratan sulfate also accumulates in the heart valve, and this accumulation represents a major cause of morbidity in MPS IVA. Some patients also experience morbidity from liver dysfunction, due to keratan sulfate accumulation in Kupffer cells and the resulting hepatomegaly. Thus, the Sponsor evaluated tissue distribution of rhGALNS, primarily focusing on the growth plate, heart valve and liver.

1. Single dose study (pilot study)

The Sponsor stated that IV bolus injection of 10 mg/kg rhGALNS-488 in mice was sufficient to result in significant delivery of the test article to growth plate chondrocytes, heart valve and liver. The data was not shown.

2. Repeat dose study

Distribution of rhGALNS-A488 in growth plate, articular cartilage and bone marrow

After five IV bolus injections of rhGALNS-A488 in mice during the eight-day dosing phase, significant fluorescence was observed in the resting cartilage and proliferating chondrocytes at the edges of the growth plate, which are closest to the highly vascularized bone. Less fluorescence was observed in the maturing/hypertrophic zones at the center of the growth plate. Mineralized cartilage, bone and bone marrow exhibited strong fluorescence (see figure below from study report).

Volumetric measurements of rhGALNS-A488 fluorescence in the growth plate ranged from 0 to 756 μm^3. The average volumes for the 2, 4, and 8 hr time-points were 53.9, 329.8, and 60.6 μm^3, respectively. There was no consistent relationship between time of tissue collection and content of rhGALNS-A488 in chondrocytes and bone marrow, and significant variation was observed within a single time-point. rhGALNS-A488 was present in the zone of resting cartilage in each animal, with a range of 31-803 μm^3.

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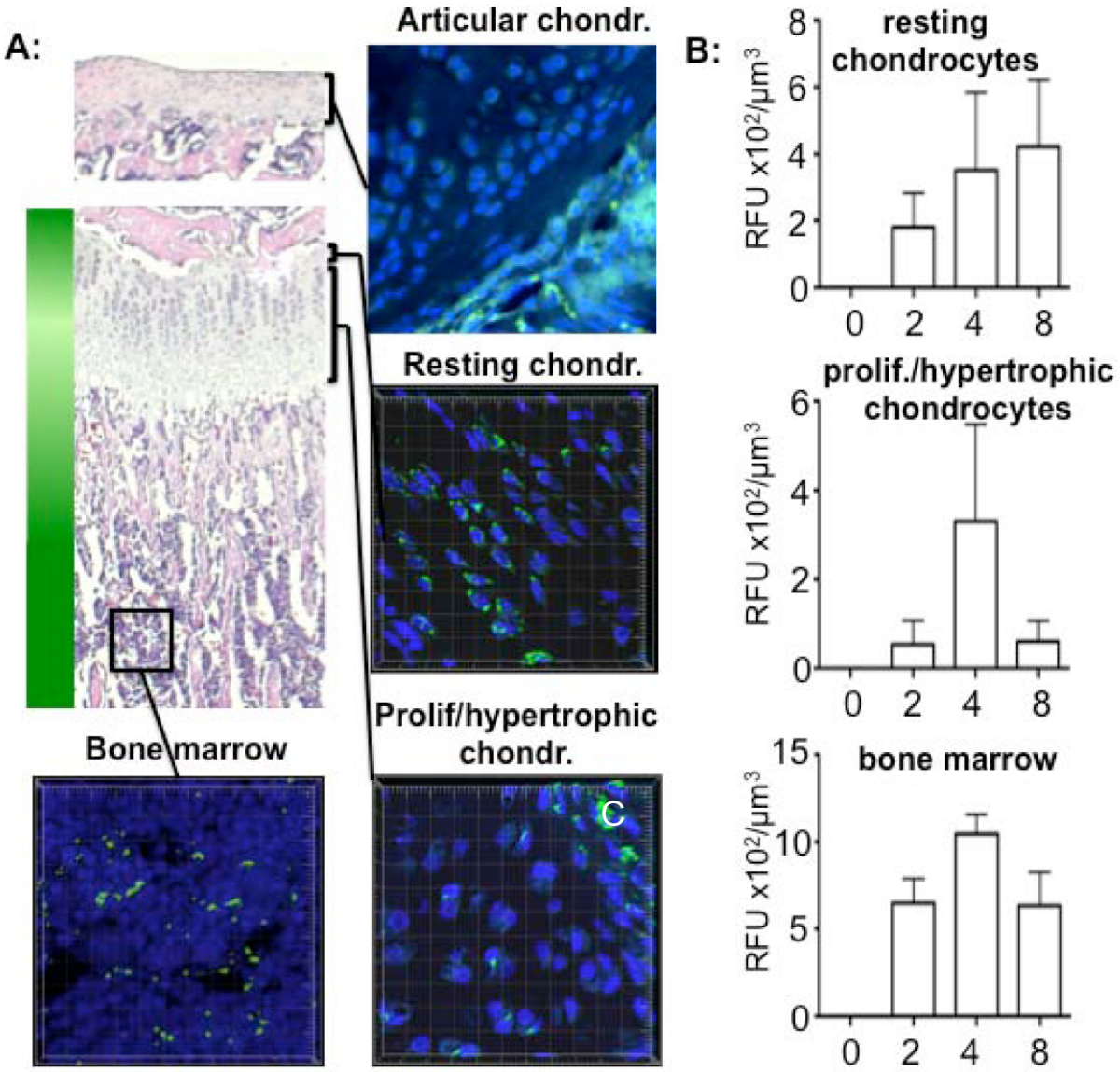
Figure 9: rhGALNS-A488 Uptake in Growth Plate



A: Confocal microscopy of direct rhGALNS-A488 fluorescence (green) in growth plate cartilage (resting, proliferating and hypertrophic chondrocytes), articular cartilage and bone marrow cells. Blue = DAPI nuclear staining. Original magnification 80x. **B:** Confocal stacks are analyzed for fluorescence intensity and data presented as average signal (RFU)/μm^3. Measurements of sections from mice that were either injected with A-488/PBS (0), or rhGALNS-A488 and sacrificed at 2 hr (2), 4 hr (4), or 8 hr (8) after injection (n=3). Means ± SEM are shown.

Distribution of rhGALNS-A488 in heart

After five IV bolus injections of rhGALNS-A488 in mice during the eight-day dosing phase, rhGALNS-A488 was present in the septum, atrium, and heart valves (mitral valves). The volumetric measurements of rhGALNS-A488 in the heart valves ranged widely from 444 to 4832 μm^3. Both the minimum and the maximum values were obtained from animals sacrificed at the 2 hr time-point. The average volumes for the 2, 4, and 8 hr time-points were 2698 μm^3, 1514 μm^3, and 1659 μm^3, respectively. The Sponsor did not submit the data for volumetric measurements of rhGALNS-A488 in the septum, and atrium.

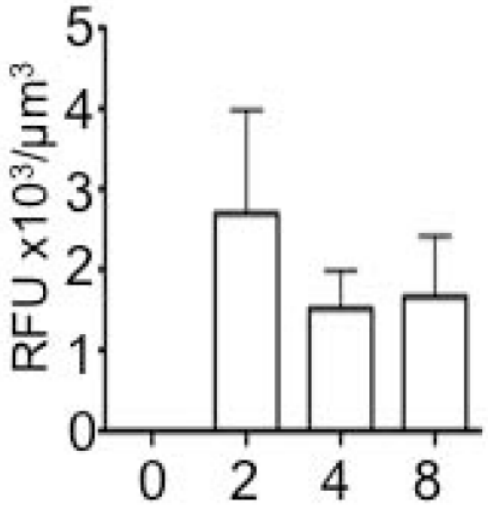
Animals injected with PBS or PBS/cys-488 did not have any detectable fluorescence signal in the heart.

rhGALNS-A488 was co-localized with LAMP-1, indicating that rhGALNS-A488 was trafficked to the lysosomes.

Distribution of rhGALNS-A488 in liver

Following repeated administration of rhGALNS-A488 in mice, the test article was present in the sinusoidal and Kupffer cells of the liver. Hepatocytes did not exhibit overt fluorescence. The volumetric measurements for rhGALNS-A488 in liver ranged from 3,188 to 10,463 μm^3, with the minimum and maximum values obtained at the 8 hr time-point. The average volumes for the 2, 4, and 8 hr time-points are shown in the Sponsor's figure below.

Figure 10: rhGALNS-A488 uptake in liver



rhGALNS-A488 volumetric measurements in cubic micrometers, from the liver of mice that were either injected with fluorophore alone (0), or rhGALNS-A488 and sacrificed at 2 hr (2), 4 hr (4), or 8 hr (8) time points after the last injection. Means ±SEM are shown (n=3).

In summary, the *in vitro* studies indicate that GALNS and fluorescent-labeled GALNS enzymes are both taken up by synoviocytes through a common pathway, and the internalization of the enzyme is not affected by its conjugation to the fluorophore. Female mice were treated with IV bolus injections of 10 mg/kg rhGALNS or rhGALNS-A488 every other day for a total duration of 8 days (5 injections). Tissues were harvested at 2, 4, and 8 hours after the final injection. rhGALNS-A488 was detected in the heart (mitral valves, atrium and septum), growth plate, articular cartilage, bone, bone marrow, and sinusoidal and Kupffer cells of the liver. rhGALNS-A488 was localized in lysosomes.

5.2 Toxicokinetics

(see toxicology studies)

6 General Toxicology

6.1 Single-Dose Toxicity

See Safety Pharmacology Study Section.

6.2 Repeat-Dose Toxicity

Study title: 26-Week Once Weekly Intravenous Toxicity and Toxicokinetic Study in Sprague-Dawley Rats Using BMN 110 with a 4-week Recovery Phase

Study no.:	0110-08-020
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 10, 2008
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

SD rats were treated once weekly with BMN 110 at doses of 0, 1, 6, or 20 mg/kg via IV bolus injection for 26-weeks. There were four unscheduled deaths. One female in the 1 mg/kg dose group died of complications related to the blood collection procedure. The deaths of two males and one female in the 6 mg/kg dose group were attributed to adverse test article-related anaphylactic-type reactions. All drug-related clinical findings (i.e. red skin on paws and ears, labored respiration, hypoactivity, squinted eyes, and dilated pupils) were considered to be anaphylactic type reactions and were observed across all treatment groups immediately following dose administration during week 3. These reactions were prevented with DPH (diphenhydramine) pre-treatment, which was

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initiated on week 3. BMN 110 at 6 and 20 mg/kg increased urine volume, and pH, and decreased urine specific gravity. Histopathologic changes were observed at the injection site (subacute inflammation and hemorrhage). All BMN 110-treated animals, with one exception, were positive for anti-BMN 110 antibodies during the dosing phase and the recovery period. Anti-BMN 110 antibodies were detected as early as day 22, the first day of blood sampling. Neutralizing anti-BMN 110 antibodies were detected in most BMN 110-treated animals during the dosing phase and recovery period (detection was based on neutralization of BMN 110 binding to M6PR). No target organ of toxicity was identified. The tolerated dose was 20 mg/kg/week IV in rats receiving an oral DPH pre-treatment of 2 mg/kg/week, based on the changes in urinalysis parameters. However, given the high incidence of neutralizing antibodies, it is likely that the pharmacological activity of BMN 110 was inhibited in most of the animals. Therefore, the results of this study must be interpreted with caution.

Methods

Doses:	1, 6, 20 mg/kg
Frequency of dosing:	once weekly
Route of administration:	slow push (~2 minutes) IV bolus injection
Dose volume:	6.6 – 6.9 ml/kg/dose
Formulation/Vehicle:	The vehicle was 20 mM sodium acetate, 1 mM sodium phosphate, and 150 mM NaCl in 0.01% Tween 20 solution. BMN110 was diluted in the vehicle at concentrations of 0.15, 0.9 and 3 mg/ml.
Species/Strain:	Sprague Dawley (Crl:CD(SD)) rats
Number/Sex/Group:	10/sex/group for main study
Age:	48 - 54 days old
Weight:	246 - 359 g for males and 156 - 238 g for females
Satellite groups:	Recovery groups: 6/sex/group Toxicokinetic study: 6/sex/group
Unique study design:	A group of rats (16/sex) was treated with oral diphenhydramine (DPH) at a dose of 2 mg/kg as a control group.
Deviation from study protocol:	Minor deviations had no impact on data integrity or interpretation.

During administration of the third weekly dose, several animals across all BMN 110-treated groups exhibited clinical signs consistent with anaphylactic type reactions. These anaphylactic type reactions were resolved by SC or IV administration of 4 mg/kg diphenhydramine (DPH), except for two males in the 6 mg/kg BMN 110 group that died. The anaphylactic reactions and deaths were expected, given that BMN 110 is an exogenous protein in rats. Anaphylactic type reactions were prevented by oral pretreatment with 2 mg/kg DPH, for all subsequent BMN 110 administrations. The study design is summarized in the Sponsor's table below.

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Table 10: Study Design for a 26-Week Toxicity and Toxicokinetic Study in Rats

Groups	BMN 110[a,b] Level (mg/kg)	Dosing Frequency	Number of Animals			
			Male		Female	
			Main	Recovery	Main	Recovery
Toxicity						
1	0	Once Weekly	10	6	10	6
2[c]	0	Once Weekly	10	6	10	6
3[d]	1	Once Weekly	10	6	10	6
4[d]	6	Once Weekly	10	6	10	6
5[d]	20	Once Weekly	10	6	10	6
Toxicokinetic						
6	0	Once Weekly	3	NA	3	NA
7[d]	1	Once Weekly	6	NA	6	NA
8[d]	6	Once Weekly	6	NA	6	NA
9[d]	20	Once Weekly	6	NA	6	NA

[a] BMN 110 lots 11333P53, 11333P71, 11333P79, 11333P90, NP400801A, NP400801B, AP400802; Vehicle lots 11410P93 and AB4022-08004T (20 mM sodium acetate, 1 mM sodium phosphate, 150 mM NaCl in 0.01% Polysorbate 20 pH 5.5).

[b] Depending on BMN 110 lot concentration, the dose volume ranged from 6.6 to 6.9 mL/kg.

[c] Vehicle treated animals that received DPH to control for the administration of DPH to BMN 110 treated animals. The DPH control animals received an oral pretreatment of DPH (2 mg/kg) at least 30 minutes prior to dosing on Week 4 and at least 1 to 2 hours prior to dosing on Week 5 through the end of the dosing phase. The animals were euthanized during Week 28 and thereby received an extra dose of vehicle/DPH. This was done to match the number of DPH pretreatment doses that BMN 110-treated animals received.

[d] DPH was administered IV or subcutaneously (SC; 4 mg/kg) following Week 3 dose administration to animals given 1, 6 and 20 mg/kg that exhibited anaphylactoid-type reactions. The animals (both male and female toxicity and TK animals) received an oral (per os; PO) pretreatment of DPH (2 mg/kg) at least 30 minutes prior to dosing on Week 4 and at least 1 to 2 hours prior to dosing on Week 5 through the end of the dosing phase.

The table below provides information about the multiple lots of BMN 110 used in this study.

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Table 11: Lot Number, Purity and Specific Activity of BMN 110 Used in a 26-week IV Toxicity Study in Rats

Lot number	Purity	Specific Activity and Cellular Uptake Measurements for BMN 110			
		Specific Activity with 4MU-Gal-6S substrate (U/mg protein)	Uptake into Synoviocytes (K_{uptake}, nM)	Uptake into (b) (4) Fibroblasts (K_{uptake}, nM)	Calculated Specific Activity (U/mg protein)*
11333P53	96.8%	3.8	17.1	-	-
11333P71	98%	5.1	20.7	-	-
11333P79	97.3%	6.5	15.7	-	-
11333P90	97.4%	5.1	-	1.5	-
NP400801A	95.1%	4.3	-	3.6	-
NP400801B	95.1%	5.4	-	5.6	-
AP400802	97%	-	-	3	4

* Method was not specified.
- This method was not used to measure activity.

Observations and Results

Mortality

Mortality was observed twice daily.

There were four unscheduled deaths. One TK female in the 1 mg/kg dose group died of the complications related to the blood collection procedure on day 50. The deaths of two males and one female in the 6 mg/kg dose group were attributed to treatment-related anaphylactic reactions. Discolored GI track (stomach, duodenum, ileum, and jejunum) was observed in the two males, but not in the female. A female given 20 mg/kg was euthanized on day 81 due to a perforated esophagus caused by the animal swallowing a gavage needle during the DPH administration.

Clinical Signs

Clinical signs of abnormalities, signs of pain or distress were observed twice daily. Detailed clinical observations were conducted once during the pre-dose phase and once weekly thereafter.

Drug-related, anaphylactic-type reactions were observed in all animals treated with BMN 110 on day 15 (week 3) of the dosing phase. Clinical signs included one or more of the following: red skin (all paws or ears), labored respiration, hypoactivity, squinted eyes, and/or dilated pupils. Pretreatment with 2 mg/kg DPH, given orally, was instituted for all DPH controls (group 2) and all animals given 1, 6, or 20 mg/kg starting on week 4 of the dosing phase. The DPH pretreatment prevented the anaphylactic-type reactions.

Body Weights

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Animals were weighed on day 1 and weekly thereafter up to week 15, and on weeks 19 and 23. In addition, all main study animals (groups 1 to 5) were weighed during week 27 of the dosing phase and group 2 animals were weighed on week 28 of the dosing phase. Body weights were recorded for all recovery animals during the week 4 of the recovery phase.

There were no treatment-related changes in body weights during the treatment period and the recovery period. The body weights on days 1 and 183 are summarized in the table below.

Table 12: Body Weights in a 26-Week Rat Toxicity Study

Day	Dose Groups				
	1 **Control**	**2** **Control with 2 mg/kg DPH**	**3** **1 mg/kg BMN 110**	**4** **6 mg/kg BMN 110**	**5** **20 mg/kg BMN 110**
	Body Weight in Males (g)				
1	296	291	293	292	294
183	674	680	665	671	673
	Body Weight in Females (g)				
1	195	197	197	198	195
183	353	372	364	373	353

A significant decrease in body weight gain was observed in males in the 1 mg/kg dose group (↓24%, $p \leq 0.05$) on days 15-22 when compared to group 1 control males and group 2 DPH control males. It is likely that the decreased body weight gain was due to test article related anaphylactic reactions. A significant increase in body weight gain was observed in all males treated with BMN 110 on days 85-92 when compared to group 1 control males and group 2 DPH control males. Significant increases in body weight gain were also observed in males in the 1 and 6 mg/kg dose groups compared to the group 1 control males (↑37% and ↑30%, respectively) and group 2 DPH control males (↑32% and ↑25%, respectively) on days 99-127. There were no significant changes in body weight gain in females treated with BNM 110. The changes in body weight gain in treated males are not treatment-related because the changes were not dose-dependent. The changes in body weight gain are summarized in the table below. There were no treatment-related changes in body weight gain in during the recovery period.

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Table 13: Changes in Male Body Weight Gain in a 26-Week Rat Toxicity Study

	Dose Groups				
Days	1 Control	2 Control with 2 mg/kg DPH	3 1 mg/kg BMN 110	4 6 mg/kg BMN 110	5 20 mg/kg BMN 110
	Body Weight Gain in Males (g)				
15-22	38 ± 10.7	38 ± 7.1	29 ± 8.4*#	32 ± 6.7	37 ± 7
85-92	0 ± 4.6	- 3 ± 5	5 ± 4.5*#	1 ± 5.7#	5 ± 5.3*#
99-127	27 ± 6.4	28 ± 8.8	37 ± 12.7*#	35 ± 8.7*	33 ± 12

*: p ≤ 0.05 compared to group 1 (vehicle control)
#: p ≤ 0.05 compared to group 2 (DPH control)

Feed Consumption

Food consumption was assessed weekly in main study animals (groups 1-5) on weeks 1 through 14 and weeks 18, 22, and 26 (week 27 for group 2) of the dosing phase and on week 3 to 4 of the recovery phase. BNM 110 had no effects on food consumption.

Ophthalmoscopy

Ophthalmology examinations were performed on all main study animals during the pre-dose phase, at week 26 of the dosing phase and at week 4 of the recovery phase. No drug-related observations were noted in the ophthalmic examinations.

ECG

Not performed.

Hematology

Blood samples for hematology and coagulation parameters were collected from fasted animals (groups 1-5) on days 23, 86, and 184 of the dosing phase and day 29 of the recovery phase.

Animals receiving BMN 110 treatment had increased WBC (up to 42%, p ≤ 0.05) and neutrophils (up to 2.7-fold, p ≤ 0.05) on day 23 compared to the vehicle controls. On days 86, and 184, the WBC and neutrophil counts were comparable to the vehicle control and DPH control values. Most of the animals that had increased WBC and neutrophils in the BMN 110 treatment groups had clinical signs of anaphylactic-type reactions prior to the first administration of DPH (week 3, day 15). Thus, the increased WBC and neutrophils on day 23 were most likely related to anaphylactic-type reactions.

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Clinical Chemistry

Blood samples for clinical chemistry were collected from fasted animals (groups 1-5) on days 23, 86, and 184 of the dosing phase and day 29 of the recovery phase. There were no treatment-related changes.

Urinalysis

Urine was collected from fasted animals (groups 1-5) on days 23, 86, and 184 of the dosing phase and day 29 of the recovery phase.

DPH treatment had no effects on urine pH, specific gravity, or volume. BMN 110 produced dose-dependent changes in urine volume, pH, and specific gravity. Urinalysis demonstrated increases in male urine volume (up to 2-fold) and pH (up to 7.4 in males of the 6 mg/kg group vs. 6.5 in the DPH control males), and a decrease in specific gravity (1.022 in the 20 mg/kg group males vs. 1.032 in the DPH control males). The changes in urine volume, pH and specific gravity were also observed in females treated with 6 and 20 mg/kg/week BMN 110, but the effects were smaller in magnitude. The changes in urine volume, pH and specific gravity were reversed at the end of the recovery period. Although there were no histopathology changes in the kidney, it is likely that the changes in urine volume, pH and specific gravity were related to BMN 110 treatment.

Gross Pathology

No treatment-related macroscopic changes were observed.

Organ Weights

The following organs were weighed: adrenal glands, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary gland, prostate, salivary gland, seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, and uterus. There were no treatment-related changes in absolute or relative organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: No

Preserved tissues were examined microscopically from each animal in the control and high dose groups, as well as each animal that died or was euthanized at an unscheduled interval. The following tissues from each animal were preserved in 10% neutral buffered formalin, unless otherwise indicated below (a: Preserved in modified Davidson's fixative): adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis[a],

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esophagus, eyes[a], femur with bone marrow (articular surface of the distal end), heart, ileum, injection site(s), jejunum, kidney, lesions, liver, lung with large bronchi, lymph node (mandibular and mesenteric), mammary gland (females), optic nerve[a], ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis[a], thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina.

Bone marrow smears were made from the femur of each animal at scheduled euthanasia, and slides were prepared and stored for possible future evaluation.

Histological Findings:

Subacute inflammation and hemorrhage at the injection site was observed with greater incidence in males given 20 mg/kg compared with control groups 1 or 2. However, in females given 20 mg/kg, the incidence of hemorrhage and subacute inflammation was comparable with vehicle control group 1. None of these changes were observed at the end of the recovery period. Therefore, it is uncertain whether the changes at the injections site were test article-related or dosing procedure-related. The incidence of injection site changes is summarized in the table below.

No target organ of toxicity was identified. The tolerated dose was 20 mg/kg/week (IV) in rats receiving an oral DPH pre-treatment of 2 mg/kg/week.

Table 14: Incidence of Histopathological Changes at the Injection Site in a 26-Week Rat Toxicity Study

Tissues		Groups		
		1 (control)	2 (control + DPH)	5 (20 mg/kg + DPH)
		Males		
Intravenous site	Inflammation, granulomatous	1/10	0	0
	Hemorrhage	1/10	0	5/10
	Inflammation, subacute	0	0	3/10
	Vasculitis	0	0	1/10
		Females		
Intravenous site	Inflammation, granulomatous	0	0	0
	Hemorrhage	5/10	0	5/9
	Inflammation, subacute	6/10	0	4/9
	Vasculitis	0	0	0

Special Evaluation

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Detection of total anti-BMN 110 antibodies and neutralizing anti-BMN 110 antibodies in serum

Serum was collected from all animals for antibody analysis during the pre-dose phase and from main study animals prior to dosing, on days 22, 50, 106, and 176 of the dosing phase and days 1, 22 and 29 of the recovery phase.

A total of 1046 samples were analyzed for total anti-rhGALNS (anti-BMN 110) antibodies using a bridging electrochemiluminescence (ECLA) assay. The limit of detection in rat was 5 ng/mL. To test for neutralizing antibodies, plasma samples that were positive for anti-rhGALNS antibodies (n = 551) were analyzed for antibodies that inhibit the binding of rhGALNS to its receptor (M6PR), using an immunoassay.

During the dosing and recovery phase, a total of 18 control animals (12 of 24 males and 6 of 26 females) had anti-rhGALNS antibody titers ranging from 30 to 196,830. Among the 12 control males, nine had anti-rhGALNS antibody titers that ranged from 90 to 196,830. Six control females had titers ranging from 30 to 90. The Sponsor stated that animals with titers that never exceeded a dilution factor of 810 were false positive responses. Therefore, six males were considered to be positive for anti-rhGALNS antibodies, with antibody titers ranging from 2430 to 196,380. No explanation was given for the positive response in the control males. Three of 15 control males and 1 of 10 control females were positive for neutralizing anti-BMN 110 antibodies during the dosing phase (day 106).

The presence of anti-BMN 110 antibodies was detected in almost all BMN 110-treated animals starting on day 22. At dosing day 106, positive titer values were observed in all groups, with a higher incidence in animals given 1 mg/kg BMN 110. All BMN 110-treated animals, with one exception, were positive for anti-BMN110 antibodies during the dosing phase and the recovery period. One male given 20 mg/kg (animal # B82122) was sero-negative at each collection interval. There were no gender-related differences. There was no clear pattern of change in the titer values or in the proportion of animals with anti-drug antibodies during the recovery phase. Neutralizing antibodies were detected in most BMN 110-treated animals during the dosing phase (1 mg/kg group: 12 males and 16 females; 6 mg/kg group: 14 males and 12 females; 20 mg/kg group: 15 males and 16 females). Neutralizing antibodies were still present during the recovery period (1 mg/kg group: 1 male and 6 females; 6 mg/kg group: 5 males and 5 females; 20 mg/kg group: 6 males and 4 females).

Toxicokinetics

Plasma samples for TK analysis were collected from all TK animals (groups 6-9) under non-fasted conditions on weeks 1, 4, 8, 13 and 26, at pre-dose and 30 minutes post-dose in the vehicle-treated animals. For BMN 110-treated animals, TK samples were collected at pre-dose and approximately 5, 30, and 100 minutes post-dose for one set of three animals/sex/group and at approximately 1, 15, and 60 minutes post-dose for the second set of animals.

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Overall, mean $t_{1/2}$ increased with repeated doses and with increasing dose levels. At dose 26, the mean $t_{1/2}$ increased from about 11 minutes at 6 mg/kg to 26 minutes at 20 mg/kg BMN 110. Clearance appeared to decrease with increased duration of dosing and with dose levels, which could be due to saturation of the clearance mechanism such as protease-mediated degradation or receptor-mediated uptake. Exposure to BMN 110 (C_{max} and AUC_{0-t}) increased with increasing dose. The increase in exposure in males was greater than dose proportional between 6 and 20 mg/kg. Females did not show a consistent relationship for proportionality between exposure and dose. The TK parameters are summarized in the Sponsor's tables below.

Table 15: Toxicokinetic Parameters for BMN 110 in Rat Plasma

Dose Group [a]	Dose Level (mg/kg)	Week	Sex	AUC_{0-t} (ng*min/mL)	$AUC_{0-\infty}$ (ng*min/mL)	C_{max} (ng/mL)	$t_{1/2}$ (min)
7	1	1	M	97339	97678	20477	1.91
			F	97132	97257	19763	1.62
			Combined[b]	97226	97453	20120	1.78
		26	M	--[c]	--[c]	--[c]	--[c]
			F	--[c]	--[c]	--[c]	--[c]
			Combined[b]	--[c]	--[c]	--[c]	--[c]
8	6	1	M	1320300	1320552	93833	4.77
			F	1632009	1638976	164750	4.23
			Combined[b]	1480777	1480886	118646	4.12
		26	M	420462	422523	25000	14.29
			F	1514493	1518598	124583	10.46
			Combined[b]	967290	970274	74792	11.12
9	20	1	M	7665983	7671032	545208	11.23
			F	6537385	6541871	413333	9.72
			Combined[b]	7099417	7104155	462083	10.4
		26	M	28349426	30834702	658333	29.26
			F	19606499	20263691	683333	21.07
			Combined[b]	23971940	25498895	670833	26.30

AUC_{0-t}, area under the plasma concentration-time curve from time 0 to the last measurable concentration, estimated by linear trapezoidal rule; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 extrapolated to infinity; C_{max}, maximum concentration in plasma; $t_{1/2}$, terminal half-life.

[a] Dose Group 6 was the vehicle control group; therefore, no TK analysis was performed.

[b.] TK parameters for the combined are derived from the mean concentration-time profile including both males and females (Figure 2.6.6.3.1.1).

[c.] TK parameters for animals treated with 1 mg/kg at Dose 4, 8, 13 and 26 could not be calculated because the plasma BMN 110 concentrations were below the lower limit of quantification (30 ng/ml) in the majority of the samples.

Table 16: Changes in Plasma Exposure with Repeat Dosing of BMN 110

Ratio	Dose Group	Dose Level (mg/kg)	Sex	C_{max} Exposure Ratio [a]	AUC_{0-t} Exposure Ratio [b]
Dose 4 / Dose 1	7	1	M	--[c]	--[c]
			F	--[c]	--[c]
	8	6	M	0.81	0.89
			F	1.02	1.18
	9	20	M	0.77	2.13
			F	0.99	1.29
Dose 8 / Dose 1	7	1	M	--[c]	--[c]
			F	--[c]	--[c]
	8	6	M	0.74	0.93
			F	0.76	0.95
	9	20	M	1.01	3.16
			F	1.29	2.04
Dose 13 / Dose 1	7	1	M	--[c]	--[c]
			F	--[c]	--[c]
	8	6	M	0.37	0.37
			F	0.77	0.89
	9	20	M	1.01	2.94
			F	0.86	2.10
Dose 26 / Dose 1	7	1	M	--[c]	--[c]
			F	--[c]	--[c]
	8	6	M	0.27	0.32
			F	0.76	0.93
	9	20	M	1.21	3.70
			F	1.65	3.00

AUC_{0-t}, area under the plasma concentration-time curve from time 0 to the last measurable concentration, estimated by linear trapezoidal rule; C_{max}, maximum concentration in plasma.

[a] C_{max} exposure ratio is C_{max} at Dose 4, Dose 8, Dose 13 or Dose 26 over Dose 1.

[b] AUC_{0-t} exposure ratio is AUC_{0-t} at Dose 4, Dose 8, Dose 13 or Dose 26 over Dose 1.

[c] TK parameters for animals treated with 1 mg/kg at Doses 8, 13 and 26 could not be calculated because the plasma BMN 110 concentrations were below the lower limit of quantification (30 ng/ml) in the majority of samples.

Dosing Solution Analysis

Dose formulations were prepared on each day of dose administration, and all dose formulations, including preparations for weeks 1 and 2, were stored at room temperature until administration within 12 hours from the preparation start time.

Duplicate samples (1 ml each) were collected from the top, middle, and bottom of the 0.15 and 3 mg/mL dose preparations formulated for administration on days 1 and 71 of

the dosing phase to determine homogeneity. Duplicate samples (1 ml each) of the 0.15 and 3 mg/ml dose formulations from days 1, 71, 85 and 183 of the dosing phase were collected upon completion of dose preparation to determine stability. All samples for homogeneity and stability analysis were stored in -60 to -80°C until analyzed.
The samples for stability analysis were stored under different conditions as shown in the Sponsor's table below.

Table 17: Storage Conditions for Samples in Stability Analysis

Conc. mg/mL	Room Temp	Refrigerated 2°C to 8°C	Frozen -60°C to -80°C	Freeze/Thaw
0.15	**6 hrs**	**1.5 months**	**>60 hrs; <1.5 months**	N/A
3	**6 hrs**	**6.5 months**	**>60 hrs; <1.5 months**	**4 cycles**

The concentrations of top, middle and bottom samples from of dose formulations on day 1 ranged from 101% to 108% of the nominal concentration of 0.15 mg/ml, and from 99% to 104% of the nominal concentration of 3 mg/ml. The concentrations of top, middle and bottom samples from dose formulations on day 71 ranged from 105% to 111% of the nominal concentration of 0.15 mg/ml, and were 106% of the nominal concentration of 3 mg/ml. Thus, the dose formulation was homogenous.

As shown in the Sponsor's table below, the Sponsor only provided the stability data for samples stored at room temperature for 12 and 24 hr. The low (0.15 mg/ml) and high dose (3 mg/ml) formulations were stable for 24 hours at room temperature (94% and 100% of the nominal concentrations of 0.15 or 3 mg/ml, respectively). The Stability Memo provided by (b) (4) stated the following:

"**Formulation stability determined by the sponsor was not performed under GLP guidelines and does not cover all intervals and storage conditions for the formulations used in this study. Specifically, dose formulations from Days 1, 71, and 85 were stored at -60 to -80 ºC for a period of time that was beyond the established stability prior to being analyzed for concentration verification and homogeneity. The impact is considered to be minor since CV and homogeneity results were consistently within specifications for the Day 1, Day 71, and Day 85 timepoints and were consistent with results from samples analyzed from Day 183 which were analyzed within the stability timeframe. While not able to be confirmed, the results suggest the samples were stable for this period of time prior to analysis.**"

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Table 18: Stability Analysis of Two Dose Formulations Differing in Sodium Phosphate Content*

Expected Conc. mg/mL	Formulation	Conc. (mg/mL) at 0 Hrs and RT	Average Conc. (mg/mL) at 12 Hrs and RT	% of Expected Conc.	Average Conc. (mg/mL) at 24 Hrs and RT	% of Expected Conc.
0.15	1 mM NaPO4	0.16	0.15	94	0.15	94
3	1 mM NaPO4	2.9	2.9	100	2.9	100
0.15	100 mM NaPO4	0.16	0.16	100	0.16	100
3	100 mM NaPO4	3.0	3.0	100	3.0	100

* Vehicle used in this study was 20 mM sodium acetate, 1 mM sodium phosphate, and 150 mM NaCl in 0.01% Tween 20 solution.

In summary, SD rats were treated once weekly with BMN 110 at doses of 0, 1, 6, or 20 mg/kg via IV bolus injection for 26-weeks. There were four unscheduled deaths. One female in the 1 mg/kg dose group died of the complications related to the blood collection procedure. The deaths of two males and one female in the 6 mg/kg dose group were attributed to adverse BMN 110-related anaphylactic reactions. All BMN 110-related clinical findings were associated with anaphylactic reactions and were observed across all treatment groups following dose administration during week 3. These reactions were prevented with DPH pre-treatment. BMN 110 at 6 and 20 mg/kg increased urine volume and pH, and decreased urine specific gravity. All BMN 110-treated animals, with one exception, were positive for anti-BMN 110 antibodies during the dosing phase and the recovery period. Anti-BMN 110 antibodies were detected as early as day 22, the first day of blood sampling. Neutralizing antibodies were detected in most BMN 110-treated animals during the dosing phase and the recovery period. No target organ of toxicity was identified. The tolerated dose was 20 mg/kg/week IV in rats receiving an oral DPH pre-treatment of 2 mg/kg/week, based on the changes in urinalysis parameters. However, given the high incidence of neutralizing antibodies, it is likely that the pharmacological activity of BMN 110 was inhibited in most of the animals. Therefore, the results of this study must be interpreted with caution.

Study Title: 28-Day Once Weekly Infusion Toxicity and Toxicokinetic Study in Cynomolgus Monkeys Assessing Different Lots of BMN 110 from Differing Production Processes

Study no.: BMN110-10-100
Study report location: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: October 21, 2010
GLP compliance: Yes
QA statement: Yes

Key Study Findings

The objective of this study was to evaluate the toxicity and toxicokinetics of three different lots of BMN 110 (one lot from the Phase 1/2 production process and two lots from the Phase 3 production process). Cynomolgus monkeys were intravenously administered three different lots of BMN 110 at 20 mg/kg, given once weekly for four weeks. For all three of the tested lots, there were no effects on body weight gain, food consumption, hematology, clinical chemistry, urinalysis, ECG, or organ weights. No target organs of toxicity were identified. A slight increase in plasma exposure to BMN 110 was observed with the two Phase 3 lots compared to the Phase 1/2 lot. Anti-BMN 110 antibodies were detected in all treated animals. The no observed adverse effect level (NOAEL) was 20 mg/kg/week for each of the tested lots.

Methods

Doses:	1, 6, 20 mg/kg
Frequency of dosing:	once weekly
Route of administration:	IV infusion for approximately 4 hr via a jugular venous catheter
Dose rate:	5 ml/kg/hour
Formulation/Vehicle:	Refer to study design table below for details about formulations. BMN 110 was diluted in vehicle at nominal concentrations of 1 mg/ml.
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	4/sex/group
Age:	2-3 years old
Weight:	2.1 to 2.9 kg for males and 2.3 to 2.6 kg for females
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	Minor deviations had no impact on data integrity or interpretation.

The study objective was to evaluate the toxicity and determine the TK parameters of three lots of BMN 110 produced using different processes (one lot of Phase 1/2 material and two lots of Phase 3 material). The study design is summarized in the Sponsor's table below.

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Table 19: Study Design for a Repeat Dose Toxicity and Toxicokinetic Study in Cynomolgus Monkeys with Three Different Lots

Group	Compound	Lot	Dose (mg/kg)	Dosing Frequency[e]	Dosing Phase No. and Gender per Group
1	Vehicle[a]		0	Once Weekly	4M, 4F
2	BMN 110	Phase 1/2 (L401012) [b]	20	Once Weekly	4M, 4F
3	BMN 110	Phase 3 (BSJJ03) [c]	20	Once Weekly	4M, 4F
4	BMN 110	Phase 3 (2010-0110-09) [d]	20	Once Weekly	4M, 4F

[a] Group 1 received vehicle only (20 mM sodium acetate, 50 mM sodium phosphate, 30 mM L-arginine hydrochloride, 2 % sorbitol, 0,01% polysorbate 20, pH 5.4)

[b] Lot No. L401012 (Phase 1/2 process) formulated in 20 mM sodium acetate, 100 mM sodium phosphate, 150 mM sodium chloride, 0.01% polysorbate 20, pH 5.5

[c] Lot No. BSJJ03 (Phase 3 process) formulated in 20 mM sodium acetate, 50 mM sodium phosphate, 30 mM L-arginine hydrochloride, 2 % sorbitol, 0,01% polysorbate 20, pH 5.4

[d] Lot No. 2010-0110-09 formulated in 20 mM sodium acetate, 50 mM sodium phosphate, 30 mM L arginine hydrochloride, 2 % sorbitol, 0,01% polysorbate 20, pH 5.4

[e] Four doses total were delivered

All BMN 110 lots were provided at a nominal concentration of 1 mg/ml and were administered at a rate of 5 mL/kg/hr. Vehicle was administered at a rate of 5 ml/kg/hr.

The table below provides information about the multiple lots of BMN 110 used in this study.

Table 20: Lot Number, Purity and Specific Activity of BMN110 in a 28-day IV Toxicity Study in Monkeys

Lot numbers	Purity	Specific activity	
		QCU-13-007 (U/mg protein)	Calculation (U/mg protein)*
L401012	96%	-	12
BSJJ03	98%	-	3
2010-0110-09	95%	5	-

* Method was not specified.
- Specific activity was not measured by this method.

Observations and Results

Mortality

Animals were observed twice daily for mortality. All animals survived until the scheduled termination.

Clinical Signs

Detailed observations were performed five times during the pre-dose phase, prior to dosing on day 1, and weekly throughout the dosing phase. There were no treatment-related changes in clinical signs.

Body Weights

Body weight was recorded during the pre-dose phase, on day -1 of the dosing phase, and weekly thereafter. There were no drug-related changes in body weight or body weight gain.

Feed Consumption

Qualitative food consumption was assessed once daily during the pre-dose and dosing phases. There was no drug-related change in food consumption.

Ophthalmoscopy

Ophthalmoscopy was performed once during the pre-dose phase and on day 23 of the dosing phase. No drug-related changes in ophthalmic evaluations were observed.

ECG

ECG was recorded once during the pre-dose phase and on day 23 of the dosing phase. BMN 110 had no effects on PR interval, QRS duration, QT interval, QT_c interval (Bazett method), RR interval, or heart rate.

Hematology

Blood samples were collected for hematology and coagulation from fasted animals via the femoral vein during the pre-dose phase and on day 29 of the dosing phase. No drug-related changes in hematology or coagulation parameters were observed.

Clinical Chemistry

Blood samples were collected for clinical chemistry from fasted animals via the femoral vein during the pre-dose phase and on day 29 of the dosing phase. No drug-related changes were observed.

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Urinalysis

Urine samples were collected overnight once during the pre-dose phase and on day 29. No drug-related changes in the urine volume, specific gravity, or urine pH were observed.

Gross Pathology

All animals were subjected to necropsy at termination (day 29). There were no treatment-related macroscopic findings.

Organ Weights

At scheduled time points, the following organs were weighed: adrenal, brain, epididymis, heart, kidney, lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, uterus, and liver with gallbladder.

A significantly higher mean absolute testis weight (1.09 ± 0.19 g vs. 0.64 ± 0.16 g in the control group; $p \leq 0.05$) and mean testis-to-body weight percentage (0.047 ± 0.007 % vs. 0.029 ± 0.01% in the control group; $p \leq 0.05$) were observed in group 3 males (Lot BSJJ03). A significantly lower mean absolute brain weight was observed in group 4 females (Lot 2010-0110-09) (57.8 ± 1.8 g vs. 66.8 ± 1.7 g in the control group; $p \leq 0.05$), but no corresponding statistically significant change occurred in mean brain-to-body weight percentage (2.5 ± 0.22 % vs. 2.87 ± 0.12% in the control group).

Histopathology

Adequate Battery: Yes

Peer Review: No

The following tissues were collected from animals at scheduled sacrifice, and from animals euthanized at unscheduled intervals:

adrenal, aorta, brain, catheterization site, cecum, cervix, colon, duodenum, epididymis, esophagus, eye, femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, infusion site, jejunum, kidney, lacrimal gland, lesions, liver, lung with large bronchi, lymph node (mandibular, mesenteric), mammary gland (females), optic nerve, ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

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The tissues were preserved in 10% neutral-buffered formalin, except for the eyes, optic nerves, and testes, which were preserved in modified Davidson's fixative. All tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Histological Findings:

There were no treatment-related histopathological changes in tissues or organs in the groups treated with different lots, with the exception of increased incidence of thrombi in Lot BSJJ03 and Lot 2010-0110-09 formulations.

Thrombi were observed at the different catheter sites (A, B and C) across all groups. A higher incidence was observed at site A (1 cm proximal to catheter tip) in the BMN 110-treated animals, especially for groups 3 and 4. Although the presence of thrombi was most likely related to the presence of the catheter, as this is a common finding in animals chronically implanted with indwelling catheters, the increased incidence in groups 3 and 4 suggests that Lot BSJJ03 and Lot 2010-0110-09 formulations may accelerate the formation of thrombi in the catheter. The incidence of thrombi in the catheter sites are summarized in the Sponsor's table below.

Table 21: Incidence of Thrombus Formation at the Different Catheter Sites in Monkeys Treated with Three Different Lots of BMN 110

	Males				Females			
	Group 1 (Vehicle)	Group 2 (Lot L401012)	Group 3 (Lot BSJJ03)	Group 4 (Lot 2010-0110-09)	Group 1 (Vehicle)	Group 2 (Lot L401012)	Group 3 (Lot BSJJ03)	Group 4 (Lot 2010-0110-09)
Catheter site A[a]	1/4	2/4	4/4	4/4	1/4	0/4	3/4	3/4
Catheter site B[b]	1/4	2/4	1/4	1/4	1/4	0/4	1/4	0/4
Catheter site C[c]	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4

[a] 1 cm proximal to the catheter tip

[b] At the catheter tip

[c] 1 cm distal to the catheter tip

Special Evaluation

Antibody Analyses

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Blood samples (approximately 1 ml) were collected from non-fasted animals via the femoral vein on day 1 (pre-dose) and from fasted animals on day 29 of the dosing phase. Antibody analysis was performed using a validated ELISA method.

No anti-BMN 110 antibodies were detected in any group 1 vehicle animals prior to dosing on day 1 or on day 29. Anti-BMN110 antibodies were detected prior to dosing on day 1 in one group 2 male animal, and in two group 3 animals (one male and one female). All BMN 110-treated animals had detectable anti-drug antibodies by the end of the study (day 29). Titers on day 29 ranged from 90 to 21,900 for animals in groups 2 and 4, and from 2430 to 21,900 for animals in group 3.

Toxicokinetics

Blood samples (approximately 1 ml) were collected from non-fasted animals via the femoral vein at 0 min (pre-dose), 30 min after the start of infusion, 2 min prior to the end of infusion, and 5, 15, 30, 60, 100, and 180 min after the end of infusion on days 1, 8, and 22. On day 15, blood was collected only at pre-dose and 2 minutes prior to the end of infusion.

The TK parameters in monkeys after IV infusion of BMN 110 weekly for 28-days are summarized in the Sponsor's table below. No gender-related differences were observed. Overall, there was a modest increase in exposure (AUC_{0-t}) to BMN 110 for both Phase 3 lots (approximately 1.5-fold) in comparison to the Phase 1/2 lot as measured over the course of 28 days. No toxicological profile differences were observed over the range of exposure.

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Table 22: Mean Toxicokinetic Parameters for Monkeys Administered Three Different Lots of BMN 110 at 20 mg/kg Weekly for 28-Days

Day	Group	Process (lot #)		AUC_{0-t} (min*ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (min)
1	2	Phase 1/2 (L401012)	Mean	8557274	46025	8.33
			SD	1644172	12723	2.44
	3	Phase 3 (BSJJ03)	Mean	10218773	71200	9.80
			SD	2588853	40458	4.69
	4	Phase 3 (2010-0110-09)	Mean	17079449	116375	14.40
			SD	4777935	29403	4.24
8	2	Phase 1/2 (L401012)	Mean	8447041	44988	7.15
			SD	3511830	21146	2.25
	3	Phase 3 (BSJJ03)	Mean	10661453	55350	7.24
			SD	4107450	26508	1.94
	4	Phase 3 (2010-0110-09)	Mean	13493127	69288	10.70
			SD	6982034	34341	2.51
22	2	Phase 1/2 (L401012)	Mean	7813560	41863	13.72
			SD	3134709	19340	12.33
	3	Phase 3 (BSJJ03)	Mean	9891332	58613	8.24
			SD	4320818	38889	2.26
	4	Phase 3 (2010-0110-09)	Mean	11472389	63800	15.98
			SD	4094411	25373	10.66

All BMN 110-treated animals had anti-drug antibodies on day 29 with comparable titers (90-21,900 for lot # L401012; 2430-21,900 for lot # BSJJ03 and 90-21,900 for lot # 2010-0110-09). There were no gender-related differences. The presence of anti-drug antibodies most likely did not reduce BMN 110 exposure, as AUC_{0-t} and C_{max} values on day 22 were similar to AUC_{0-t} and C_{max} values on day 1 or day 8 (see table 22 above).

Dosing Solution Analysis

Dose formulations were prepared once weekly. Samples (1 ml) were taken from all dose preparations for weeks 1 and 4. All samples were stored in a refrigerator at 2 to 8°C, until the time of analysis.

Concentrations of the prepared dose formulations ranged from 97.5 to 100% for lot #. L401012 (Phase 1/2 production process), 101.5 to 104% for lot # BSJJ03 (Phase 3 production process), and 105 to 108% for lot # 2010-0110-09 (Phase 3 production process) of the target concentrations. Thus, the dose formulations were stable under the storage conditions.

In summary, cynomolgus monkeys were intravenously administered three different lots of BMN 110 at 20 mg/kg, given once weekly for four weeks. Body weight gain, food consumption, hematology, clinical chemistry, urinalysis, ECG, and organ weights were not adversely affected by the three lots. No target organs of toxicity were identified. A slight increase in plasma exposure to BMN 110 was observed in the two Phase 3 lots compared to the Phase 1/2 lot. Anti-BMN 110 antibodies were detected in all treated animals. The no observed adverse effect level (NOAEL) was 20 mg/kg/week for each of the tested lots.

Study title: 52-Week Once Weekly Infusion Toxicity and Toxicokinetic Study in Cynomolgus Monkeys Using BMN 110 with a 4-Week Recovery Period

Study no.:	0110-08-018
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
	(b) (4)
	(b) (4)
Date of study initiation:	May 13, 2008
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Cynomolgus monkeys were treated once weekly with BMN 110 at doses of 0, 1, 6, or 20 mg/kg via IV infusion for 52-weeks. This study was originally designed as a 39-week repeated dose toxicity and TK study. Due to the observation of dislodged needles in some animals at the end of infusion, this study was extended to 52 weeks to ensure that all BMN 110-treated animals received at least 39 doses. There were no test article-related deaths or clinical signs. BMN 110 had no effects on body weight, body weight gain, food consumption, ophthalmic examination, ECG, hematology, clinical chemistry, or urinalysis. BMN 110 had no effects on long bone growth or circulating keratan sulfate levels. The bone resorption marker, serum crosslinked C-telopeptide of type 1 collagen, was not affected by administration of BMN 110 at 20 mg/kg/week. There were no treatment-related macroscopic or histopathologic changes. Most animals treated with BMN 110 were positive for anti-drug antibodies and neutralizing anti-drug antibodies. The antibody titers were similar at all dose levels. No target organs of toxicity were identified. Systemic exposure was dose proportional between dose levels

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of 1 and 6 mg/kg, and greater than dose proportional at dose levels of 6 and 20 mg/kg. The NOAEL was 20 mg/kg/week. However, given the high incidence of neutralizing antibodies, it is likely that the pharmacological activity of BMN 110 was inhibited in most of the animals. Therefore, the results of this study must be interpreted with caution.

Methods

Doses:	1, 6, 20 mg/kg
Frequency of dosing:	Weekly
Route of administration:	IV infusion for approximately 4 hr via a femoral or jugular venous catheter or venous access port. Animals were chair dosed due to catheter issues or animal behavior issues.
Dose volume:	Dose volumes ranged from 6.4 to 6.87 ml /kg, based on the concentration of test article lot used.
Formulation/Vehicle:	A total of 10 lots of BMN 110 were used in this study. The actual concentrations based on a 6-month stability study ranged from 2.9 to 4 mg/ml. BMN 110 was formulated in two different vehicle solutions as follows: On weeks 1 to 31, BMN 110 was formulated in a vehicle solution containing 20 mM sodium acetate, 1 mM sodium phosphate, 150 mM NaCl in 0.01% Tween 20. On weeks 32 to 52, BMN 110 was formulated in a vehicle solution containing 20 mM sodium acetate, 100 mM sodium phosphate, 150 mM NaCl in 0.01% Tween 20. Dosing formulations were prepared on each day of dose administration at nominal concentrations of 0.15, 0.9, and 3 mg/ml.
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	4/sex/group
Age:	2 – 3 years old
Weight:	2.4 to 3.3 kg for males and 2.2 to 3.4 kg for females
Satellite groups:	Recovery groups: 3/sex in the control and high-dose (20 mg/kg/week) groups

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Unique study design:	Animals were catheterized for intravenous infusion via a femoral vein. At least 1 week before initiation of treatment, animals were fasted overnight and anesthetized, and a catheter equipped with a subcutaneous vascular access port was surgically implanted into a femoral vein. When the catheter failed to work in the right and/or left femoral veins, animals were chair dosed until re-catheterized via a jugular vein or chair dosed until completion of the dosing phase. This study was originally designed as a 39-week repeat dose toxicity and TK study. Due to the observation of dislodged needles in some animals at the end of infusion, this study was extended to 52 weeks to ensure that all BMN 110-treated animals received at least 39 doses. The Sponsor summarized the number of doses received by animals during the treatment period in the table below.
Deviation from study protocol:	Deviations had no impact on the integrity or interpretation of the study.

Animals were dosed by IV infusion using an infusion jacket, tether system and a subcutaneous Smiths Medical access port to provide vascular access via the right femoral vein. Vehicle or BMN 110 was administered by continuous IV infusion over 4 hours. Animals were infused with saline (0.9% NaCl) during the intervals between administration of BMN 110 or vehicle.

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Table 23: Incidence of Needle Dislodgement During the 52-week Treatment Period

Dose (mg/kg)		Total No. of Infusions over 52 Weeks	Total No of Noted Dislodgements	Total No of Dislodgements with Expected TK Profile[a]	No of Dislodgement with Potential Impaired Exposure[b]	No of Dose Holidays	No of Uncompromised Doses (average number of doses per animal)
0	M	322	97	n/a	97	10	215 (35)
	F	364	71	n/a	71	0	293 (42)[c]
	combined	686	168	n/a	168	10	508 (39)
1	M	208	83	54	29	0	179 (45)
	F	208	12	8	4	0	204 (51)
	combined	416	95	62	33	0	383 (48)
6	M	208	62	23	39	0	169 (42)
	F	208	43	20	23	0	185 (46)
	combined	416	105	43	62	0	354 (44)
20	M	343	53	31	22	0	321 (49)[d]
	F	364	30	15	15	0	349 (50)
	combined	707	83	46	37	0	670 (49)

[a] Determined from C_{eof} and TK profiles.

[b] TK profile consistent with a subcutaneous administration.

[c] Animal I06593 was euthanized on Day 67 prior to Dose 11 and Animal I06594 was on dose holiday for dose 10.

[d] Animal I06606 (male given 20mg/kg) was euthanized on Day 272, five days after receiving dose 39 and animal I06609 (male given 20mg/kg) was euthanized on Day 308 prior to dose 45.

The table below provides information about the multiple lots of BMN 110 used in this study.

Table 24: Lot Number, Purity, Specific Activity, and K_{uptake} of BMN 110 in a 52-week IV Toxicity Study in Monkeys

Lot number	Purity	Specific Activity and Cellular Uptake Measurements for BMN 110			
		Specific Activity with 4MU-Gal-6S substrate (U/mg protein)	Calculated Specific Activity (U/mg protein)*	Uptake into (b) (4) fibroblasts (K_{uptake}, nM)	Uptake into Synoviocytes (K_{uptake}, nM)
11333P53	96.8%	3.8	-	-	17.1
11333P71	98%	5.1	-	-	20.7
11333P79	97.3%	6.5	-	-	15,7
11333P90	97.4%	5.1	-	1.5	-
NP400801A	95.1%	4.3	-	3.6	-
NP400801B	95.1%	5.4	-	5.6	-
AP400802	97%	-	4	3	-
11428P95	97%	-	5	-	-
11541P04	96.4%	-	8	2	-
11541P73	100%	-	22	2	-

* Method was not specified.

\- This method was not used to measure specific activity or uptake.

Observations and Results

Mortality

Animals were observed twice daily for mortality. There were no treatment-related deaths. Three animals were euthanized on day 67 (one control male due to sepsis), day 272 (one 20 mg/kg male (# I06606) due to pulmonary inflammation caused by campylobacter infection, attributed to secondary effects of chronic catheterization), and day 308 (one 20 mg/kg male (# I06609) due to dislocation of the left shoulder and broken left humerus).

Clinical Signs

Clinical signs were observed twice daily. Detailed observations were performed once during the pre-dose phase, on the day prior to dosing, on the day of scheduled euthanasia, and weekly during the recovery phase. There were no treatment-related clinical signs. Two males in the 20 mg/kg group had discolored feces (black, tan, or red) on day 18 pre-dose and during the dosing phase. These animals were treated with antibiotics (erythromycin, trimethoprim sulfa, difloxacin, and pepto-bismol/doxycycline). The fecal test for one of the males was positive for campylobacter. One male animal in the 20 mg/kg group had a positive fecal test for campylobacter on day 232. This animal was euthanized due to pulmonary inflammation caused by campylobacter infection.

Clinical signs related to the dosing apparatus and catheter included infection of the femoral catheter incision site, exteriorization of the original catheter tip, swelling at the port area or along the catheter tract, and in the inguinal region. The control, 6, and 20 mg/kg/week groups had similar incidence of clinical signs related to the dosing apparatus and catheter (7/14, 4/8, and 6/14 animals, respectively). One male in the 1 mg/kg/week group showed swelling at the port area, and the dosing apparatus and catheter were replaced. One male and one female in the 1 mg/kg/week group had generalized or local superficial dermatitis and were treated with topical antibiotics (Betagen). Most animals with clinical signs related to the dosing apparatus and catheter were treated with either topical, oral, or systemic antibiotics. However, four animals with only swelling in the dosing apparatus and catheter region did not receive antibiotic treatment (one control female, one male in the 1 mg/kg group, one female in the 6 mg/kg group, and one female in the 20 mg/kg group). One control male was euthanized due to pain from the catheterized leg. One male in the 20 mg/kg/week group that had swelling in the left leg inguinal region along the catheter tract was euthanized on day 308 due to a fractured left arm. All other animals were treated with systemic (intramuscular injection) or oral antibiotics (e.g. amoxicillin/clavulanate, enrofloxacin) during the dosing phase. Some animals were treated with non-steroidal anti-inflammatory drugs (e.g. flunixine meglumine and ibuprofen) during the dosing phase due to the clinical signs related to the catheter and dosing apparatus.

Body Weights

Reference ID: 3396720

Body weight was recorded once during the pre-dose phase, on the day prior to dosing and weekly thereafter, and weekly during the recovery phase.

Females in the 1 and 20 mg/kg/week groups had slight decreases in body weights (up to 9% and 12% reductions, respectively) during the treatment period. No significant difference in overall body weight change (Days 7 to 364 of the dosing phase) was observed when compared with controls.

Table 25: Body Weights in a 52-week Toxicity Study in Monkeys

Day	Body Weights (kg)							
	Groups (mg/kg/week)							
	Males				Females			
	0	1	6	20	0	1	6	20
Pre-dose 43	2.7	2.7	2.6	2.6	2.6	2.5	2.5	2.5
Study day 7	2.8	2.8	2.6	2.7	2.5	2.5	2.5	2.6
Study day 364	3.8	3.8	3.7	3.7	3.3	3	3.3	3
Recovery day 6	3.7	-	-	3.7	3.7	-	-	3.1
Recovery day 27	3.9	-	-	3.9	3.8	-	-	3.3

A significant difference in body weight change (either slight increase or slight decrease) was noted on one or more weeks in all groups. However, these differences were sporadic, incidental and not test article-related.

Feed Consumption

Qualitative food consumption was assessed once daily. There were no treatment-related changes.

Ophthalmoscopy

Ophthalmic examination was performed once during the pre-dose phase and during the final week of the dosing phase. There were no treatment-related changes.

ECG

ECG was recorded in animals anesthetized with ketamine, once during the pre-dose phase and during week 51 of the dosing phase. BMN 110 had no effects on RR, heart rate, QRS, PR, QT or QT_c (Friderecia's method).

Hematology

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Blood samples were collected for hematology and coagulation analysis at pre-study (days 22 and 37), on study days 22 (week 4), 85 (week 13), 176 (week 26), 267 (week 39), 295 (week 43), 330 (week 48), and 365 (week 53). Blood was also collect on day 393 (recovery phase, week 57). BMN 110 had no effects.

Clinical Chemistry

Blood samples were collected for clinical chemistry parameters on pre-dose days 22 and 37, on study days 22 (week 4), 85 (week 13), 176 (week 26), 267 (week 39), 295 (week 43), 330 (week 48), and 365 (week 53). Blood was also collected on day 393 (recovery phase, week 57). BMN 110 had no effects.

Urinalysis

Urine samples were collected for urinalysis at pre-dose (day 22), on days 22 (week 4), 85 (week 13), 176 (week 26), 267 (week 39), 274 (week 40), 295 (week 43), 330 (week 48), 365 (week 53), and 393 (recovery phase, week 57).

BMN 110 had no effects on urine volume, specific gravity, or pH.

Gross Pathology

All animals were subjected to necropsy (gross examination) at the end of the treatment period or at the end of the recovery period. There were no test article-related changes at the end of the treatment or recovery periods.

Organ Weights

At scheduled time points, the following organs were weighed: adrenal, brain, epididymis, heart, kidney, lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, uterus, and liver with gallbladder.

Decreased absolute thymus weight, thymus/bodyweight ratio, and thymus/brain weight ratio occurred in all treated females (absolute weight: ↓62%*, ↓32% and ↓42% for groups 2, 3 and 4 females, respectively; * $P \leq 0.05$). At the end of the recovery period, the absolute thymus weight in group 4 females was still 31% lower than that of the control females. Group 4 males had an approximately 2-fold increase in absolute spleen weight, spleen/bodyweight ratio, and spleen/brain weight ratio. No histopathologic changes were associated with the decreased thymus weight or increased spleen weight.

Histopathology

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Adequate Battery: Yes

Peer Review: No

The following tissues were collected from animals at scheduled sacrifice, and from animals euthanized at unscheduled intervals:

adrenal, aorta, brain, catheterization site, cecum, cervix, colon, duodenum, epididymis, esophagus, eye, femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, infusion site, jejunum, kidney, lacrimal gland, lesions, liver, lung with large, bronchi, lymph node (mandibular, mesenteric), mammary gland (females), optic nerve, ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

The tissues were preserved in 10% neutral-buffered formalin, except for the eyes, optic nerves, and testes, which were preserved in modified Davidson's fixative. All tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Histological Findings:

Chronic/chronic active Inflammation at the infusion site and catheter site was observed in control and treatment groups at a similar incidence. There were no drug-related changes in microscopic examination of the main study or recovery phase animals.

Special Evaluation

<u>Metric Long Bone (left tibia) Examinations</u>

The growth plates on left tibia were analyzed using radiography (conducted by (b) (4). Metric long bone (left tibia) examinations were performed on all animals once during the pre-dose phase and on the control and 20 mg/kg/week animals during week 51 of the dosing phase.

Pre-dose phase examination of radiographs confirmed that no skeletal abnormalities were present, and that all tibial epiphyseal plates were open (i.e. no complete closure/fusion). At the 51-week time point, all tibial epiphyseal plates were still open. Males in the 20 mg/kg/week groups had an approximately 13% increase in tibial growth. In contrast, females given 20 mg/kg/week had an approximately 37% decrease in tibial growth compared to the control females. This change in the average bone growth was due to two females which showed 0.3 and 0 cm tibial growth, compared to the respective pre-dose measurements. These two females had baseline tibial lengths that were substantially higher than those in other females, suggesting that tibial growth in

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these two females was mostly complete at the initiation of dosing. Therefore, the decrease in mean bone growth in the 20 mg/kg/week females is unlikely to be treatment-related. The group average tibial growth over the baseline value is summarized in the table below.

Table 26: Metric Long Bone Growth (Tibial Measurement) During Week 51 vs. Baseline in a 52-Week Toxicity Study in Monkeys

Gender	Groups	Group Average Growth (cm)	Group Average Growth (%)
Males	1 (0 mg/kg/week)	1.25	11.92
	4 (20 mg/kg/week)	1.38	13.22
Females	1 (0 mg/kg/week)	0.73	6.95
	4 (20 mg/kg/week)	0.46	4.26

Keratan Sulfate Degradation Products in Plasma

Blood samples were collected on days -7, 1, 8, 22, 36, 85, 176, 267, 358, and 393 (recovery phase) for measurement of KS (keratan sulfate) degradation products. Samples were collected at pre-dose and 24 hr post-infusion of drug during the main study.

(b) (4)

A combined (b) (4) standard was used as the reference standard for the analysis. The standard stock was prepared by digestion of bovine corneal keratan sulfate with keratanase II. The standard was characterized for (b) (4) by HPLC. Three major peaks from the reference standard were observed by strong anion exchange HPLC. Initially, Peak 2 was considered to be the monosulfated disaccharide and Peak 3 considered as the disulfated disaccharide. The disaccharide concentrations of the standard were therefore determined by quantitation of Peaks 2 and 3. Subsequent to the validation and testing of non-clinical samples, authentic standards for the monosulfated and disulfated disaccharides became available. The authentic and keratanase II-digested standards were analyzed by SAX-HPLC/RI to confirm the identity of the peaks by retention time. This analysis indicated that the monosulfated disaccharide peak was actually Peak 1, not Peak 2 as initially assumed for the digested standard. For additional confirmation, the digested bovine corneal KS standard peaks were collected and analyzed against the authentic standards by LC/MS/MS. The combined material from Peaks 1 and 2 of the digested standard had the same retention time (i.e. eluted in a single peak) and had the same mass as the monosulfated disaccharide in the authentic standard. Therefore, this material was confirmed to be the

monosulfated disaccharide based on comparison to the authentic standard. Peak 3 was confirmed as the disulfated disaccharide.

The study authors stated the following: "**When analyzed by LC/MS/MS, Peak 2 has a low response compared to Peak 1 (approximately 1% of Peak 1 response). Although Peaks 1 and 2 coelute under BN006 LC/MS/MS conditions, the impact of the inclusion of Peak 2 (if it is present in samples) in the calculation of (b) (4) concentration is insignificant**."

The authors provided the following assessment of the incorrect interpretation of the HPLC analysis of keratanase II-digested standard: **"Due to the misidentification of the monosulfated peak during standard characterization, the theoretical (b) (4) concentration in the standard was underestimated. All samples analyzed against this standard were therefore reported with a (b) (4) concentration lower than the actual concentration. It was decided with the Sponsor that the non-clinical study data will not be corrected. The same theoretical (b) (4) concentration was used for all validation and non-clinical study sample analyses and, although the reported (b) (4) and sum of disaccharide values may be lower than the actual values, the trend in overall disaccharide levels for the non-clinical studies can be observed using the uncorrected data. The data reported in the validation of method BN006 and the non-clinical study data, therefore, will not be corrected for (b) (4) concentration."**

Thus, the data presented in the study report is not accurate due to misidentification of the monosulfated peak during standard characterization. However, the Sponsor's justification for not correcting the data appears acceptable, and no further information is required. The data (not shown) indicates that there were no treatment-related changes in KS levels in monkey plasma.

<u>Anti-Drug Antibodies (including neutralizing antibodies)</u>

Blood samples were collected for measurement of total and neutralizing antibodies at pre-study (-7 days), on days 1, 50 (week 8), 85 (week 13), 176 (week 26), 267 (week 39), 295 (week 43), 330 (week 48), 358 (week 52), 365 (week 53), and 393 (week 57, recovery phase).

A validated immunoassay for detection of anti-GALNS antibodies in Cynomolgus monkey, human, and rat serum was used to determine total antibodies to rhGALNS (BMN 110).

Most animals treated with BMN 110 were positive for anti-drug antibodies (i.e. anti-rhGALNS or anti-BMN 110) at day 22, with a higher incidence in the 20 mg/kg group (see table below). Anti-drug titer levels increased with increasing treatment duration. There was no significant difference in the titer levels of anti-BMN 110 antibodies between dose groups.

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Neutralizing anti-drug antibodies (i.e. antibodies that neutralize the binding of rhGALNS to bovine soluble CI-M6PR were detected on day 22 in 4 animals treated with BMN 110. On day 50, most animals were positive for neutralizing anti-drug antibodies, with only 4 animals that were negative. One female in the 20 mg/kg group was positive for neutralizing anti-drug antibodies until day 85.

Anti-drug antibodies (including neutralizing antibodies) were still present during the recovery period in animals treated with BMN 110.

Table 27: Incidence and Titer Values of Anti-drug Antibodies in Monkeys Treated with BMN 110 at Day 22

Group # (Males)	Number of males with anti-drug antibodies	Titers	Group # (Females)	Number of females with anti-drug antibodies	Titers
2	2/4	30-2430	2	2/4	30-90
3	3/4	90-270	3	2/4	90-2430
4	6/7	30-270	4	6/7	30-2430

Pharmacodynamic Biomarker Measurement

Serum crosslinked C-telopeptide of type 1 collagen (CTx) as a bone resorption marker is a Type I collagen degradation product. The (b) (4) is an enzyme immunological test for quantification of degradation products of C-terminal telopeptides of type 1 collagen in serum. The lower limit of detection (LOD) is 0.069 ng/mL.

Serum samples were collected at pre-study (day -7), weeks 13 and 52 of the main study, and on week 57 (recovery phase) for the measurement of crosslinked C-telopeptide of type 1 collagen. However, only samples from the vehicle and high dose (20 mg/kg/week) groups that were collected pre-dose and on week 13 were analyzed. Samples from study weeks 52 and 57 were not analyzed because the Sponsor did not deem this to be necessary. No rationale was given. The incidence of needle dislodgements in the 20 mg/kg/week group during the 13-week treatment period was not provided. Therefore, the possible impact of needle dislodgements on the data cannot be assessed.

Therefore, the Sponsor concluded that BMN 110 at 20 mg/kg/week had no effect on the circulating levels of CTx, indicating that normal bone turnover in growing monkeys had not been disrupted after 13 weeks of treatment as shown in the Sponsor's table below.

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Table 28: Summary Data for Serum Crosslinked C-telopeptide of Type 1 Collagen (CTx)

			CTx ng/mL	
Group	**Sex**	**Data**	**Predose**	**Week 13**
1 M		Mean	2.336	1.586
(0 mg/kg/day)		SD	0.410	0.524
BMN110		n	7	6[a]
4 M		Mean	1.974	1.543
(20 mg/kg/day)		SD	0.141	0.353
BMN110		n	7	7
			n.s	n.s
1 F		Mean	1.981	1.228
(0 mg/kg/day)		SD	0.423	0.477
BMN110		n	7	7
4 F		Mean	2.220	1.265
(20 mg/kg/day)		SD	0.647	0.412
BMN110		n	7	7
			n.s	n.s

n.s.: not significantly different from vehicle-treated group of the same sex
a: Control male (Animal No. I06593) was euthanized on Day 67

Toxicokinetics

Blood samples were collected at pre-study (day -7), 30, 238, 245, 255, 270, 300, 340, and 420 min after the initiation of infusion on week 1; pre-dose and 238 min on weeks 2 and 4; pre-dose, 30, 238, 245, 255, 270, 300 and 340 min on weeks 6, 13, 19, 26, 39, and 52; and 238 min on weeks 27 to 38 and 40 to 51. Plasma concentrations of test article (BMN 110) were measured using an ELISA method. The lower limit of quantification (LOQ) was 30 ng/ml.

The Sponsor stated the following: "**Toxicokinetic parameters could not be derived for a number of monkeys, particularly for group 2 females at Doses 26 and 39, and for group 3 males at Doses 6 and 13, due to concentrations below lower limit of quantification or a not-well defined plasma profile. Therefore, very limited toxicokinetic information was obtained for these monkeys.**" Although the study report did not provide an explanation, it is plausible that needle dislodgement during the treatment period was one cause of the difficulty in data collection. Thus, the TK data should be interpreted with caution. The number of monkeys included in TK analysis is summarized in the Sponsor's table below.

Table 29: Number of Monkeys for Toxicokinetic Analysis

Dose Group	Dose Level (mg/kg)	Sex	No. of Monkeys						
			Week 1	Week 6	Week 13	Week 19	Week 26	Week 39	Week 52
2	1	M	2	4	3	3	4	3	3
		F	4	4	4	4	1	1	2
3	6	M	3	1	0	2	3	4	4
		F	4	3	3	2	3	3	4
4	20	M	6	6	5	2	6	6	3
		F	4	6	6	4	7	7	5

The increase in C_{max} and AUC_{0-t} was approximately dose proportional between the doses of 1 and 6 mg/kg BMN 110 on weeks 1, 6, and 13. Thereafter, the increase was greater than dose proportional between 1 and 6 mg/kg. The increase in C_{max} and AUC_{0-t} between 6 and 20 mg/kg was markedly greater than dose proportional at all time-points, especially during the latter part of the study (see table 29 below from study report). There was no gender-related difference in exposure to BMN 110 (C_{max} and AUC_{0-t}) in males and females given 20 mg/kg/week for 52 weeks. However, males administered 1 mg/kg at week 19 and 6 mg/kg at weeks 1 and 39 had greater than 2-fold higher exposure than in females.

In general, there was no apparent increase in exposure in male and female monkeys at 1 mg/kg/week and males at 6 mg/kg/week over 52 weeks. There was an increase in systemic exposure to BMN 110 in female monkeys in the 6 mg/kg/week group following weekly infusion over 52 weeks. After 26 weeks of treatment, animals in the 20 mg/kg group had a modest increase in exposure following repeated infusions. The change in exposure in males at 6 mg/kg on week 13 could not be determined due to the lack of toxicokinetic data in this group. Overall, plasma drug exposure was substantially increased with duration of treatment in the 6 and 20 mg/kg groups (see table 30 below from study report).

Mean $t_{1/2}$ values increased with the duration of treatment. Mean CL values decreased with dose level. Mean Vz (volume of distribution based on the terminal phase) values over 52 weeks ranged from 101 to 761 mL/kg for groups given 1 and 6 mg/kg/week, and from 39 to 154 mL/kg for groups given 20 mg/kg/week. The decreases in CL and Vz with increasing doses suggest that saturation of cellular uptake or other plasma clearance mechanisms for BMN 110 occurred at the tested doses.

The TK parameters are summarized in the Sponsor's tables below.

Table 30: Dose Proportionality of BMN 110 Exposure in Monkey Plasma

Study Week	Actual Dose Level Increase	Males		Females	
		C_{max} Dose Ratio	AUC_{0-t} Dose Ratio	C_{max} Dose Ratio	AUC_{0-t} Dose Ratio
1	1.0:6.0:20.0 - fold	1.0:6.7:102.0	1.0:6.2:78.2	1.0:3.4:96.8	1.0:3.9:79.2
6	1.0:6.0:20.0 - fold	1.0:7.4:71.8	1.0:7.4:61.9	1.0:8.5:99.8	1.0:6.9:79.1
13	1.0:6.0:20.0 - fold	1.0:NA:78.9	1.0:NA:76.1	1.0:6.5:90.2	1.0:10.1:105.4
19	1.0:6.0:20.0 - fold	1.0:7.1:84.1	1.0:7.9:102.4	1.0:18.4:149.9	1.0:20.0:165.5
26	1.0:6.0:20.0 - fold	1.0:8.3:99.2	1.0:7.0:101.9	1.0:31.0:246.5	1.0:32.3:267.2
39	1.0:6.0:20.0 - fold	1.0:25.7:108.4	1.0:21.3:114.5	1.0:21.0:372.5	1.0:19.0:338.3
52	1.0:6.0:20.0 - fold	1.0:16.9:353.9	1.0:14.1:356.1	1.0:20.6:389.5	1.0:23.8:455.5

Table 31: Toxicokinetic Parameters for BMN 110 in Monkeys (combined data for males and females)

Dose Group	Dose Level (mg/kg)	Week	AUC_{0-t} (ng*min/mL)	C_{eof} (ng/mL)	CL (mL/min/kg)	$t_{1/2}$ (min)
2	1	1	102193±25630	469±127	7.54	10.15
		52	70423±51499	443±272	21.69±7.30	29.56±24.98
3	6	1	524980±245155	2359±1389	13.74±6.52	9.93±2.32
		52	1149482±983845	7525±6769	11.47±10.25	37.81±12.95
4	20	1	8609700±3089975	51318±23606	2.58±0.85	10.58±3.06
		52	24457152±14962335	140475±84023	1.15±1.17	96.49±47.25

AUC_{0-t}, area under the plasma concentration-time curve from time 0 to the last measurable concentration, estimated by linear trapezoidal rule; C_{eof} , concentration at the end of infusion; CL, body clearance; $t_{1/2}$, terminal half-life.

Table 32: Changes in C_{max} and AUC Relative to Initial Dose after Repeated Dosing of BMN 110

Ratio	Dose Group	Dose Level (mg/kg)	Sex	C_{max} Exposure Ratio[a]	AUC_{0-t} Exposure Ratio[b]
Dose 6 / Dose 1	2	1	M	1.59±0.36	1.65±0.35
			F	1.56±0.32	1.68±0.38
	3	6	M	1.53[c]	1.44[c]
			F	3.99±2.18	3.10±0.97
	4	20	M	0.97±0.45	1.02±0.27
			F	1.75±1.28	1.70±1.02
Dose 13 / Dose 1	2	1	M	1.72±0.55	1.22±0.15
			F	1.44±0.62	1.10±0.27
	3	6	M	--[d]	--[d]
			F	3.52±2.94	2.35±1.86
	4	20	M	1.40±1.02	1.24±0.74
			F	1.51±0.75	1.73±0.59
Dose 19 / Dose 1	2	1	M	2.27±0.98	1.21±0.20
			F	0.98±0.51	0.69±0.29
	3	6	M	1.49[c]	0.93[c]
			F	6.58±4.13	4.03±0.84
	4	20	M	1.60±0.38	1.56±0.12
			F	1.98[c]	1.82[c]
Dose 26 / Dose 1	2	1	M	1.17±0.19	0.80±0.26
			F	1.04[c]	0.80[c]
	3	6	M	0.53±0.48	0.41±0.31
			F	7.90±2.30	5.19±0.90
	4	20	M	2.06±0.68	2.00±0.48
			F	2.36±0.58	2.19±0.48
Dose 39 / Dose 1	2	1	M	1.33±0.08	0.88±0.04
			F	1.23[c]	0.96[c]
	3	6	M	9.13±12.62	6.11±8.77
			F	5.85±3.83	3.48±2.44
	4	20	M	1.98±0.54	1.96±0.21
			F	4.32±0.64	3.30±2.06
Dose 52/ Dose 1	2	1	M	0.58±0.28	0.43±0.25
			F	0.89±0.03	0.60±0.01
	3	6	M	1.53±0.66	0.98±0.40
			F	4.10±4.21	2.57±2.69
	4	20	M	5.01±0.19	4.67±0.50
			F	3.05±2.41	3.29±2.39

AUC_{0-t}, area under the plasma concentration-time curve from time 0 to the last measurable concentration, estimated by linear trapezoidal rule; C_{max}, maximum concentration in plasma.

[a] C_{max} exposure ratio is C_{max} at Dose 6, 13, 19, 26, 39, or 52 over Dose 1.

[b] AUC_{0-t} exposure ratio is AUC_{0-t} at Dose 6, 13, 19, 26, 39, or 52 over Dose 1.

[c] Data from only one animal is available.

[d] No data is available for males at Dose 13 of the 6 mg/kg group due to a poorly defined plasma profile.

Dosing Solution Analysis

Dose formulations were prepared on each day of administration. All dose formulations were stored at room temperature until administration, within 12 hours of the start of preparation.

Concentration verification

Two sets of duplicate samples (1 ml each) from all dose preparations were collected on days 1, 85, 176, 218, 267, and 358 of the dosing phase. However, only one sample from each dose preparation on days 1 and 85 of the dosing phase was analyzed for test article content. The remaining samples were backup samples and/or retention samples.

Dose formulation samples measured in this study for the high-dose concentration of 3 mg/ml, mid-dose concentration of 0.9 mg/ml, and the low-dose concentrations of 0.15 mg/ml (day 85 only) were within 10% of the intended concentrations. However, due to the presence of precipitate in the 0.15 mg/ml sample from day 1 upon thawing, the analyzed concentrations in all samples were at 40 to 53% lower than target concentrations and were not compliant with the homogeneity criteria. The Sponsor stated the following to address this issue: "**Underestimation of the drug concentration in these samples was attributed to presence of the precipitate, which was likely an artifact of the storage. Dose solutions administered to the animals did not contain precipitate at time of dosing. Samples of the 0.15 mg/ml concentration prepared for all other analysis time points passed concentration verification and homogeneity criteria.**" The Sponsor's explanation is acceptable.

Homogeneity

Duplicate samples (1 ml each) were collected from the top, middle, and bottom of the 0.15 and 3 mg/ml dose formulations for administration on days 1 and 218 (week 32) to determine homogeneity. However, only one sample from the 0.15 and 3 mg/ml dose formulations (top, middle, and bottom) was analyzed for day 1. The Sponsor did not submit homogeneity data for the 0.15 mg/ml dose formulation from on day 1. No rationale was given, but the omission of this data was likely due to the presence of precipitate in samples upon thawing as described in the concentration verification section above. The concentrations of top, middle and bottom samples of the 3 mg/ml dose formulation on day 1 ranged from 100 to 103% of the intended concentration. The concentrations of top, middle and bottom samples of the 0.15 mg/ml dose formulation on day 218 ranged from 105 to 107% of the intended concentration. The concentrations of top, middle and bottom samples of the 3 mg/ml dose formulation on day 218 ranged from 100 to 102% of the intended concentration. Thus, the dose formulations for concentrations of 0.15 and 3 mg/ml were considered to be homogeneous.

Stability

Duplicate samples (1 ml each) of the 0.15 and 3 mg/ml dose formulations from days 1 and 218 were collected upon completion of dose preparation (time zero) and at the end

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of dosing to determine stability. All samples were stored at -60 to -80°C, until the samples were shipped to the Sponsor. The time for which samples were stored was recorded. The samples for stability analysis were stored under varying conditions as shown in the tables below.

Table 33: Storage Conditions for Samples in the Stability Analysis

Conc. mg/mL	Room Temp	Refrigerated 2°C to 8°C	Frozen -60°C to -80°C	Freeze/Thaw
0.15	6 hrs	1.5 months	>60 hrs; <1.5 months	N/A
3	6 hrs	6.5 months	>60 hrs; <1.5 months	4 cycles

Table 34: Stability Analysis of Two Different Dose Formulations Differing in Sodium Phosphate Concentration

Expected Conc. mg/mL	Formulation	Conc. (mg/mL) at 0 Hrs and RT	Average Conc. (mg/mL) at 12 Hrs and RT	% of Expected Conc.	Average Conc. (mg/mL) at 24 Hrs and RT	% of Expected Conc.
0.15	1 mM NaPO4	0.16	0.15	94	0.15	94
3	1 mM NaPO4	2.9	2.9	100	2.9	100
0.15	100 mM NaPO4	0.16	0.16	100	0.16	100
3	100 mM NaPO4	3.0	3.0	100	3.0	100

The study report stated the following: **"Based on a stability assessment done by the sponsor as described in the Stability Memos, low dose formulations (0.15 mg/mL) were stable for 24 hours at room temperature; 1.5 months when refrigerated (2 to 8°C); and for at least 60 hours, but less than 1.5 months when frozen (-60 to -80°C). High dose formulations (3 mg/mL) were stable for 24 hours at room temperature; 6.5 months when refrigerated (2 to 8°C); and for at least 60 hours, but less than 1.5 months when frozen (-60 to -80°C). Formulation at 3 mg/mL concentration was also tested for 4 freeze/thaw cycles. While some formulations were received at concentrations slightly higher than 3.0 mg/mL (up to 4.0 mg/mL), this concentration difference is very minor and therefore is not likely to have an impact on the stability of the dose formulations as received from the sponsor."**

In summary, cynomolgus monkeys were intravenously administered BMN 110 at doses of 1, 6 and 20 mg/kg/week for 42-51 weeks. BMN 110 had no effects on clinical signs, body weight, food consumption, ECG, hematology, clinical chemistry, or urinalysis. There were no treatment-related histopathologic findings. Bone growth, plasma levels of KS degradation products, and serum crosslinked C-telopeptide of type 1 collagen were not affected by BMN 110 treatment. No target organs of toxicity were identified.

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Anti-BMN 110 antibodies were detected in all drug-treated animals, and most animals were positive for neutralizing antibodies. Antibody titers were similar at all dose levels. The NOAEL was 20 mg/kg/week. However, given the high incidence of neutralizing antibodies, it is likely that the pharmacological activity of BMN 110 was inhibited in most of the animals. Therefore, the results of this study must be interpreted with caution.

7 Genetic Toxicology

N/A

8 Carcinogenicity

1. Background

Morquio A syndrome, also known as Mucopolysaccharidosis type IVA (MPS IVA), is an inherited autosomal recessive disorder caused by a deficiency in GALNS (N-acetylgalactosamine 6-sulfatase), which results in keratan sulfate (KS) accumulation in many tissues and organs, high circulating levels of KS, and KS excretion in urine. MPS IVA is a progressive disorder that affects multiple organ systems including the musculoskeletal, cardiopulmonary, and neurological systems. All patients have systemic skeletal dysplasia, which usually results in short stature and malformations of the knees, chest and spine.

BMN 110 (rhGALNS) will be administered by intravenous infusion as an enzyme replacement therapy for treatment of patients with Morquio A Syndrome. The active ingredient, rhGALNS, is expressed by and purified from Chinese Hamster Ovary (CHO) cells. BMN 110 is taken up by cells via the cation-independent mannose-6-phosphate receptor (CI-M6PR) followed by transfer and localization to the lysosomes. GALNS hydrolyzes the sulfate ester bonds in N-acetyl-galactosamine-6-sulfate or galactose-6-sulfate on the non-reducing ends of keratan sulfate and chondroitin 6-sulfate (C6S), which are two classes of glycosaminoglycans (GAGs) present in lysosomes. The native enzyme is proposed to be present in a 1.27-MDa complex that includes three other lysosomal hydrolases: β-galactosidase, α-neuraminidase, and cathepsin A. This enzyme complex appears to be essential for KS catabolism (Pshezhetsky and Potier, J Biol Chem, 271(45), pg. 28359-28365, 1996).

Cellular uptake and trafficking to lysosomes was demonstrated in cultured human Morquio fibroblast cells. Following intravenous administration to mice, rhGALNS was detected in the heart (mitral valves, atrium and septum), growth plate, articular cartilage, bone, bone marrow, and sinusoidal and Kupffer cells of the liver. Co-incubation of human Morquio chondrocytes with BMN 110 produced normalization of GALNS activity, cleared keratan sulfate from lysosomal stores, and restored expression of some key genes involved in chondrocyte mesenchymal differentiation and chondrocyte function.

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2. Assessment of Carcinogenicity Potential

The Sponsor submitted a written assessment of carcinogenicity potential, but did not conduct any carcinogenicity studies.

The enzymatic activity of BMN 110, a 110-kDa protein, is restricted to the lysosomal compartment where it specifically degrades KS and C6S. The lysosomal compartment provides an acidic pH environment that is necessary for enzymatic activity; BMN 110 lacks enzymatic activity at physiological pH (Masue M, et al, J Biochem, 110 (6), pg. 965-970, 1991). The mechanism of activity for BMN 110 does not raise a concern regarding the potential for neoplasm induction or tumor promotion. The carcinogenic potential of the KS catabolism products resulting from BMN 110 activity (b) (4) [redacted] has not been studied. However, given that KS degradation occurs routinely in normal individuals, there should be minimal concern about the carcinogenic potential of KS degradation products. The same view is also justified for C6S degradation products (b) (4) [redacted]

Most glycosaminoglycans are covalently attached to core proteins to form proteoglycans that bind to a large variety of ligands, thus regulating growth factor signaling, cellular behavior, inflammation, angiogenesis, and the proteolytic environment. Proteoglycans are classified based on the amino acid homology of their protein cores, their location (cell surface, basement membrane, or extracellular matrix), and their glycosaminoglycan substitution (Yip GW, Mol Cancer Ther, 5:2139-2148, 2006; Iozzo RV, et al, Mol Cells, 27(5):503-513, 2009). The four major classes of glycosaminoglycans identified are heparan sulfate, chondroitin sulfate/dermatan sulfate, keratan sulfate, and hyaluronan. Abnormal expression or deregulated function of these proteoglycans affect cancer and angiogenesis, and are critical for the evolution of the tumor microenvironment. The roles of heparan sulfate in cancer cell proliferation and growth have been widely investigated; however, only limited information on KS and chondroitin sulfate in tumorigenesis is available. KS is significantly expressed on the cell surface of malignant astrocytic tumors, suggesting that KS might be associated with the malignancy of this class of tumors (Kato Y, et al, Biochem Biophys Res Commun, 369(4):1041-1046, 2008). In addition, the keratan sulfate proteoglycan lumican inhibits melanoma progression (Vuillermoz B, et. al, Exp Cell Res, 296(2):294-306, 2004). Versican, a large aggregating chondroitin sulfate proteoglycan, is located primarily within the extracellular matrix and shows increased expression in several cancer types (breast, prostate, lung and ovary), and modified chondroitin sulfate abolished breast tumor growth in nude mice (Yip GW, Mol Cancer Ther, 5:2139-2148, 2006).

BMN 110 (rhGALNS) degrades glycosaminoglycans (GAGs) including KS and C6S in lysosomes, but had no effects on extracellular KS as shown by an *in vitro* pharmacology study in human Morquio chondrocytes. Although keratan sulfate proteoglycan and chondroitin sulfate proteoglycan may be involved in cancer-related processes, as

mentioned above, it is unlikely that rhGALNS can influence tumor growth or metastasis, due to the absence of pharmacological activity (i.e. degradation of KS and C6S) in the extracellular space. rhGALNS is targeted and delivered to lysosomes via the CI-M6PR, and has no effect on extracellular KS due to lack of enzyme activity at physiological pH.

There were no proliferative lesions in the 26-week intravenous toxicology study of BMN 110 in rats at doses up to 20 mg/kg/week, or in the 52-week intravenous juvenile monkey study at doses up to 20 mg/kg/week. However, given that neutralizing antibodies were detected in most animals in both of these studies, the absence of proliferative lesions must be interpreted with caution. Based on a weight-of-evidence approach, the carcinogenic potential of BMN 110 appears to be low, and therefore no additional studies to evaluate carcinogenic potential are needed.

CAC Concurrence (y/n): Yes.

CAC Recommendations: None.

Comments: None.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Intravenous Combined Fertility and Developmental Toxicity Study of BMN 110 in Rats

Study no.:	110-10-008
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 16, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	11615P81, 98%; specific activity (HPLC): 2.8 U/mg specific activity (fluorescence): 17.2 U/mg

Key Study Findings

In this combined segment 1 and 2 study, SD male and females rats were administered BMN 110 via intravenous bolus injection daily at doses of 1, 6 and 20 mg/kg before cohabitation, during cohabitation, the day before euthanasia (males only), and through day 20 of presumed gestation (GD 20). All study groups were administered 10 mg/kg diphenhydramine (DPH) via intraperitoneal injection before administration of the test

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article or vehicle on each day to prevent potential anaphylactic reactions. There were a total of four unscheduled deaths in females in BMN 110-treated groups, which may have been related to BMN 110 and/or DPH administration.

At 6 and 20 mg/kg/day, BMN 110 decreased body weight (up to 5%) and body weight gain (up to 68%) in males. Therefore, the paternal NOAEL is considered to be 1 mg/kg/day. Reduction in body weight gain in female rats was observed at all doses during the pre-cohabitation period (up to 58%, but not statistically significant). All female rats in the DPH or BMN 110 + DPH treatment groups had a slight reduction (< 10%) in body weight, and a slight (< 10%) to moderate (25%) reduction in body weight gain during pregnancy compared to the vehicle controls. Male and female rats treated with BMN 110 + DPH at all doses had statistically significant decreases in food consumption (up to 17%↓ in males, and up to 13%↓ in females) compared to rats treated with DPH alone. During pregnancy, females treated with DPH alone or BMN 110 + DPH had a reduction in absolute food consumption (up to 12%, $p \leq 0.05$). However, BMN 110 did not produce any significant inhibition of weight gain during pregnancy, compared to the DPH alone control group. Therefore, the maternal NOAEL during pregnancy was 20 mg/kg/day IV.

There were no BMN 110-related effects on mating and fertility parameters. The fertility indices (number of pregnancies per number of rats that mated) were similar across all study groups. BMN 110 had no effects on the number of corpora lutea, implantations, resorptions, or dams with resorptions. There was also no effect on the percentage of dead or resorbed conceptuses, litter sizes, live fetuses, percent live male and female fetuses, or fetal body weights. BMN 110 had no effects on sperm motility, count, or density. Gross fetal changes including short forelimbs and/or hindlimbs, short digits, and absent digits were considered as DPH-related, because the number of fetuses and litters with these effects was comparable across the groups administered DPH alone or DPH in combination with BMN 110. BMN 110 did not increase incidence of gross external, soft tissue, or skeletal alterations. Thus, the NOAEL for fertility and embryo-fetal development was 20 mg/kg/day IV, since changes in fertility parameters and embryo-fetal development were not considered as related to BMN 110 administration.

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Methods

Doses:	1, 6, 20 mg/kg
Frequency of dosing:	Daily
Dose volume:	6.67 ml/kg
Route of administration:	Intravenous bolus injection via lateral tail vein
Formulation/Vehicle:	BMN 110 was dissolved in the vehicle (20 mM sodium acetate, 50 mM sodium phosphate, 30 mM arginine hydrochloride, 2% sorbitol, 0.01% polysorbate 20, pH 5.4) at nominal concentrations of 0.15, 0.9 and 3 mg/ml.
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	25/sex/group for main study
Satellite groups:	3-6/sex/group for TK study
Study design:	SD male rats were administered BMN 110 via intravenous bolus injection daily at doses of 1, 6 and 20 mg/kg beginning 15 days before cohabitation, through cohabitation (maximum 21 days) and continuing through the day before euthanasia. SD female rats were administered BMN 110 via intravenous bolus injection daily at doses of 1, 6 and 20 mg/kg beginning 15 days before cohabitation (maximum of 21 days) and continuing through day 20 of presumed gestation (GD 20). Due to the rats experiencing an anaphylactic reaction in the 26-week repeat dose toxicology study, all study groups were administered 10 mg/kg diphenhydramine (DPH) via intraperitoneal injection, 10 to 20 minutes before administration of the test article or vehicle on each day of the dosing period to prevent potential anaphylactic reactions.
Deviation from study protocol:	Minor deviations had no impact on the outcome of the study.

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Table 35: Study Design for a Combined Fertility and Embryo-fetal Development Study in Rats

Treatment Group[a]	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Main Study	No of Animals TK Study
Vehicle (1)	0	6.67	0	25M/25F	3M/3F
Vehicle (2)[b,c]	0	6.67	0	25M/25F	3M/3F
Low (3)[c]	1	6.67	0.15	25M/25F	6M/6F
Mid (4)[c]	6	6.67	0.9	25M/25F	6M/6F
High (5)[c]	20	6.67	3	25M/25F	6M/6F

DPH, Diphenhydramine

[a] BMN 110 lot 11615P81; Vehicle prepared at CRL

[b] A additional vehicle group was added to the study to control for DPH administration.

[c] The animals received DPH , intraperitoneal 10 mg/kg, 10 to 20 minutes prior to dosing

The male rats received between 33 and 46 daily BMN 110 doses and the female rats received between 36 and 52 daily BMN 110 doses.

Observations and Results

Mortality

Morality was observed twice daily.

There were four unscheduled deaths. One TK male in the 20 mg/kg/day group died secondary to the bleeding procedure. Three main study females were found dead.

One female rat in the 1 mg/kg/day group was found dead on GD18. Clinical signs preceding death included the presence of a red perivaginal substance on GD14 along with decreased motor activity, cold to touch, pale front paws and hind paws, tachypnea and ataxia on GD18. This animal showed no changes in body weight, feed consumption, or macroscopic findings. The litter consisted of eight fetuses and seven early resorptions.

One female in the 6 mg/kg/day group was found dead on study day 17. Clinical signs preceding death included prostration and decreased motor activity on study day 9, and decreased motor activity on study day 10. All tissues appeared normal at necropsy.

One female in the 20 mg/kg/day group was found dead on study day 9. Clinical signs preceding death included prostration and decreased motor activity on study day 9. There were no treatment-related changes in body weight or feed consumption. The presence of tan areas on the lateral lobe of the liver was observed at necropsy.

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The Sponsor provided the following assessment: **"Although there was no obvious explanation for these deaths, the findings might be related to BMN 110 and/or DPH administration based on the clinical observations preceding their death (decreased motor activity, prostration) and the timing of their death after presumed antibody formation towards BMN 110 would have occurred."**

Clinical Signs

Clinical signs were observed daily and one or two hours after dose administration.

The common treatment-related clinical signs throughout the study across all BMN 110-treated groups (including the three female mortalities described above) consisted of decreased motor activity between study days 7 and 12 (1 mg/kg/day group: 1 male, 6 mg/kg/day group: 5 males/15 females, 20 mg/kg/day group: 10 males/20 females), prostration between study days 9 and 11 (6 mg/kg/day group: 18 females, 20 mg/kg/day group: 22 females) and ptosis on study day 10 (1 mg/kg/day group: 1 male, 6 mg/kg/day group: 8 males, 20 mg/kg/day group: 3 males). These clinical signs were generally resolved by the end of the day.

Rats receiving intraperitoneal injection of DPH only or in combination with BMN 110 had significantly increased incidences of scabs, masses (females only), and ulceration (females during gestation only) at the DPH injection site. Increased incidence of swelling at the DPH injection site occurred in female rats in the 20 mg/kg/day BMN 110 group ($p \leq 0.01$).

Body Weight

Body weights were recorded weekly during the pre-dose period (female rats), daily during the dose period, and at termination.

1. Male Rats

Compared to male rats in group 1 (vehicle only), male rats in group 2 (vehicle + DPH) had a slight decreases in body weight during the treatment period (up to ↓2%, not significant). However, compared to male rats in group 2 (vehicle + DPH), male rats in groups 4 and 5 (6 mg/kg/day BMN 110 + DPH and 20 mg/kg/day + DPH, respectively) had a slight but significant decrease in body weights on days 15 and 22 (on day 15, approximately 5% and 4% for groups 4 and 5, respectively; on day 22, 4% and 3% for groups 4 and 5, respectively). All males in the BMN 110 + DPH treatment groups had lower body weights during the days 1 to 43 compared to the DPH alone group, although the reduction of body weight was not BMN 110 dose-dependent.

Male rats treated with DPH alone (group 2) had a decrease in body weight gain on days 8-15 (↓18%, $p \leq 0.05$) compared to male rats treated with vehicle only (group 1). However, male rats in groups 4 and 5 had 63 to 68% decrease in body weight gains on days 8-15 ($p \leq 0.01$). On days 22-29, increased body weight gain was observed in

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males in groups 3 (↑16%) and 4 (↑34%, p ≤ 0.01) compared to group 2 males. Furthermore, decreased body weight was associated with reduced food consumption during the same treatment period. Although decreased body weight and body weight gain occurred sporadically during the treatment period, and no remarkable dose-dependency was observed, these changes were likely related to BMN 110 treatment. Bodyweight changes in males are summarized in the table below (taken from the study report).

Table 36: Body Weight Changes in Male Rats in a Combined Fertility and Developmental Toxicity study

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY)/TREATMENT		0 (VEHICLE)	0 (VEHICLE)/DPH	1/DPH	6/DPH	20/DPH
RATS TESTED	N	25	25	25	25	25
BODY WEIGHT CHANGE (G)						
DAYS 1 - 8	MEAN±S.D.	+27.7 ± 8.1	+26.1 ± 8.0	+24.4 ± 9.1	+25.3 ± 13.5	+26.9 ± 9.7
DAYS 8 - 15a	MEAN±S.D.	+26.8 ± 7.2	+21.9 ± 7.6‡	+17.0 ± 6.8	+8.1 ± 8.7**	+7.1 ± 11.3**
DAYS 1 - 15a	MEAN±S.D.	+54.6 ± 13.6	+48.0 ± 11.1	+41.4 ± 10.0	+33.4 ± 15.2**	+34.0 ± 15.2**
DAYS 22 - 29	MEAN±S.D.	+17.9 ± 6.8 [23]b	+19.6 ± 7.1 [24]b	+18.7 ± 6.8	+22.7 ± 7.2	+26.2 ± 9.2** [24]b
DAYS 29 - 36	MEAN±S.D.	+21.6 ± 5.4	+20.6 ± 6.0	+21.6 ± 6.1	+21.5 ± 6.3	+22.2 ± 5.9
DAYS 36 - 43	MEAN±S.D.	+17.4 ± 5.2	+16.7 ± 6.3	+13.4 ± 6.3	+15.7 ± 8.7	+16.2 ± 6.5
DAYS 1 - 43	MEAN±S.D.	+124.0± 23.4	+115.6± 19.9	+106.2± 20.1	+106.5± 25.7	+113.0± 24.1

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Last value recorded before cohabitation.
b. Excludes values for rats that were in cohabitation.
‡ Significantly different from the Group I value ($p \leq 0.05$).
** Significantly different from the Group II value ($p \leq 0.01$).

2. Female Rats

Pre-cohabitation:

DPH alone and BMN 110 + DPH combined treatment did not significantly decrease body weights in female rats during the treatment period of 1-15 days (pre-cohabitation period). However, female rats treated with DPH alone had a 15% decrease in body weight gain during the treatment days 1-15 compared to the vehicle control group. BMN 110 + DPH combined treatment resulted in non-significant decreases in body weight gains in female rats on days 1-8, 8-15, and 1-15 (pre-cohabitation period) compared to DPH treated females (Days 1-15: ↓43%, ↓58%, and ↓46% for females in groups 3, 4, and 5, respectively; see table below from study report). Although there was no statistically significant or dose-dependent reduction of body weight gain, this change appears to be related to BMN 110 treatment during the pre-cohabitation period.

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Table 37: Body Weight Changes in Female Rats during the Pre-cohabitation Period in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	25	25
BODY WEIGHT CHANGE (G)						
DAYS 1 - 8	MEAN±S.D.	+4.9 ± 5.6	+4.3 ± 5.2	+3.4 ± 6.2	+0.9 ± 5.8	+3.7 ± 5.0
DAYS 8 - 15b	MEAN±S.D.	+6.8 ± 5.4	+5.6 ± 6.1	+2.4 ± 5.0	+3.4 ± 4.8	+1.9 ± 6.8 [24]c
DAYS 1 - 15b	MEAN±S.D.	+11.8 ± 7.6	+10.0 ± 6.8	+5.7 ± 8.7	+4.2 ± 6.5	+5.4 ± 8.5 [24]c

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of presumed gestation.
b. Last value recorded before cohabitation.
c. Excludes a value for rat 6150, which was found dead on day 9 of study.

Gestation Days:

Body weight and body weight changes were comparable among the DPH alone and DPH + BMN 110 treatment groups. Although weight gain in the DPH alone group was generally lower than the vehicle alone group, BMN 110 did not enhance the DPH-induced reductions in body weight and body weight gain. Terminal body weights (on GD 21) and body weight change on GDs 18 to 21 were significantly reduced ($p \leq 0.05$) in the DPH only group compared to the vehicle group (see table below). Thus, body weight and body weight gain in females during gestation were not adversely affected by BMN 110.

Table 38: Body Weight Changes in Female Rats during the Gestation in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	24b	24b
PREGNANT	N	23	22	21	21	23
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 7	MEAN±S.D.	+23.7 ± 6.4	+21.7 ± 15.4	+18.8 ± 8.1	+19.3 ± 8.4	+17.8 ± 7.9
DAYS 7 - 14	MEAN±S.D.	+31.2 ± 5.8	+28.1 ± 7.2	+26.3 ± 6.1	+27.7 ± 6.3	+29.6 ± 6.0
DAYS 14 - 18	MEAN±S.D.	+47.9 ± 9.5	+45.2 ± 8.9	+41.8 ± 8.5	+43.3 ± 11.0	+48.4 ± 8.4
DAYS 18 - 21	MEAN±S.D.	+59.2 ± 11.3	+51.6 ± 12.9#	+51.0 ± 7.8 [20]c	+44.7 ± 14.1	+51.7 ± 10.6
DAYS 0 - 21	MEAN±S.D.	+162.0± 21.0	+146.7± 31.1	+138.7± 18.3 [20]c	+135.0± 23.5	+147.5± 20.9

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Excludes values for rats that were found dead prior to mating.
c. Excludes a value for dam 6098, which was found dead on day 18 of gestation.
Significantly different from the Group I value ($p \leq 0.05$).

Table 39: Body Weights in Female Rats during Selected Gestation Days in a Combined Fertility and Developmental Toxicity Study

Gestation Days	Groups				
	1	2	3	4	5
	0 (vehicle)	DPH	BMN 110 1 mg/kg/day + DPH	BMN 110 6 mg/kg/day + DPH	BMN 110 20 mg/kg/day + DPH
	Maternal body weight (g)				
0	259.8	254	250.6	249	254.9
1	265.6	257.7	255.3	253.3	259.5
10	296.9	286.3	279.3	278.5	284
18	362.6	349.1	337.4	339.2	350.7
21	421.8	400.7*	389.6	384	402.4

*p ≤0.05 compared to vehicle group

In summary, decreased body weight and body weight gain in animals treated with DPH alone or BMN 110 + DPH combination, and was associated with reduced food consumption (see Feed Consumption section below). Although there was no remarkable dose- or duration-dependent relationship, decreased body weight and body weight gain was considered as related to BMN 110 treatment in females in the pre-cohabitation period, and in males during treatment (1-43 days). Weight gain in females was not affected during pregnancy.

Feed Consumption

Food consumption values were recorded weekly during the dose period and on GDs 0, 7, 14 and 18 (female rats only).

Food consumption generally did not differ significantly between the group 1 (vehicle) and group 2 (vehicle + DPH) for both sexes during the dosing period (males: 1- 43 days; females: 1-15 days before cohabitation).

Males:

On days 8-15 and 1-15, male rats treated with BMN 110 + DPH at all doses had statistically significant, non-dose-dependent decreases in absolute food consumption (g/day, up to 17%↓, p≤ 0.01), compared to rats treated with DPH alone. Male rats in group 3 (1 mg/kg/day BMN 110 + DPH) had significant decreases in absolute food consumption on days 29-36 and 36-43 (↓6%, p≤ 0.01). Relative food consumption (g/kg/day) showed similar changes. The changes in absolute food consumption are summarized in the Sponsor's table below.

Table 40: Absolute Food Consumption in Male Rats during the Dose Period in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY)/TREATMENT		0 (VEHICLE)	0 (VEHICLE)/DPH	1/DPH	6/DPH	20/DPH
RATS TESTED	N	25	25	25	25	25
FEED CONSUMPTION (G/DAY)						
DAYS 1 - 8	MEAN±S.D.	24.2 ± 1.9	24.6 ± 1.8	23.4 ± 1.8	23.8 ± 2.0	23.9 ± 2.1
DAYS 8 - 15a	MEAN±S.D.	25.7 ± 1.8	25.5 ± 1.7	23.8 ± 1.6**	21.8 ± 2.1**	22.1 ± 2.2** [24]b
DAYS 1 - 15a	MEAN±S.D.	25.0 ± 1.7	25.1 ± 1.6	23.6 ± 1.4**	22.8 ± 1.8**	22.9 ± 1.7** [24]b
DAYS 22 - 29	MEAN±S.D.	23.6 ± 3.3 [20]b,c	24.0 ± 3.9 [24]c	22.9 ± 4.0	24.2 ± 1.7	25.1 ± 2.1 [24]c
DAYS 29 - 36	MEAN±S.D.	27.0 ± 2.0 [23]c	27.5 ± 2.1	25.9 ± 1.8**	26.1 ± 2.0*	27.1 ± 1.8 [23]c
DAYS 36 - 43	MEAN±S.D.	26.7 ± 1.8	27.1 ± 1.4	25.5 ± 1.8**	26.1 ± 2.2	26.3 ± 1.8
DAYS 1 - 43	MEAN±S.D.	25.6 ± 1.9	25.7 ± 1.6	24.3 ± 1.5**	24.4 ± 1.7**	24.9 ± 1.7

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Last value recorded before cohabitation.
b. Excludes values that could not be calculated as well as those associated with spillage.
c. Excludes values for rats that were in cohabitation.
* Significantly different from the Group II value (p≤0.05).
** Significantly different from the Group II value (p≤0.01).

Females (pre-cohabitation):

On days 8-15, female rats treated with 6 or 20 mg/kg/day BMN 110 + DPH had statistically significant decreases in absolute food consumption (g/day), and these decreases were dose-dependent (↓5.2%, ↓7.5%*, ↓13%*, for groups 3, 4, and 5, respectively, *p≤ 0.01), as compared to rats treated with DPH alone. Female rats in groups 4 (6 mg/kg/day BMN 110 + DPH) and 5 (20 mg/kg/day BMN 110 + DPH) had a significant, dose-dependent decrease in absolute food consumption on days 1-15 (↓3.4%, ↓4.6%*, ↓9.7%*, for groups 3, 4, and 5, respectively, *p≤ 0.01). Relative food consumption showed similar changes. The changes in absolute food consumption are summarized in the Sponsor's table below.

Table 41: Absolute Food Consumption in Female Rats during the Pre-Cohabitation Period in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY)/TREATMENT a		0 (VEHICLE)	0 (VEHICLE)/DPH	1/DPH	6/DPH	20/DPH
RATS TESTED	N	25	25	25	25	25
FEED CONSUMPTION (G/DAY)						
DAYS 1 - 8	MEAN±S.D.	18.5 ± 1.4 [24]b	17.8 ± 2.1	17.4 ± 1.4	16.6 ± 1.7	16.9 ± 1.2 [23]b
DAYS 8 - 15c	MEAN±S.D.	17.7 ± 1.6 [24]b	17.2 ± 2.0	16.3 ± 1.6	15.9 ± 1.8**	14.9 ± 1.0** [23]b,d
DAYS 1 - 15c	MEAN±S.D.	18.1 ± 1.4 [24]b	17.5 ± 2.0	16.9 ± 1.3	16.2 ± 1.6**	15.8 ± 1.0** [23]b,d

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of presumed gestation.
b. Excludes values that were associated with spillage.
c. Last value recorded before cohabitation.
d. Excludes a value for rat 6150, which was found dead on day 9 of study.
** Significantly different from the Group II value (p≤0.01).

Females (gestation period):

During the gestation period, females treated with DPH alone or BMN 110 + DPH had a reduction in absolute food consumption (up to 12%↓, $p \leq 0.05$). Absolute food consumption was significantly reduced (↓6.5%, $p \leq 0.05$) in the DPH group on GDs 0-21 as compared to the vehicle group. Groups 4 and 5 showed reduced absolute food consumption during the first week (GDs 0-7). Similar reductions in relative food consumption occurred in groups 4 and 5 on GDs 0-7. Absolute and relative food consumption in female rats during gestation is summarized in the Sponsor's tables below.

Table 42: Absolute Food Consumption in Female Rats during Gestation in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	24b	24b
PREGNANT	N	23	22	21	21	23
MATERNAL FEED CONSUMPTION (G/DAY)						
DAYS 0 - 7	MEAN±S.D.	21.3 ± 2.4	20.2 ± 2.6	19.5 ± 2.0	18.7 ± 1.5	18.7 ± 2.4
DAYS 7 - 14	MEAN±S.D.	23.0 ± 2.6	21.4 ± 2.5	20.0 ± 1.8	20.7 ± 2.0	20.8 ± 2.3 [22]c
DAYS 14 - 18	MEAN±S.D.	25.0 ± 3.5	23.1 ± 3.1	21.6 ± 2.0	22.8 ± 1.5	23.9 ± 2.6
DAYS 18 - 21	MEAN±S.D.	24.3 ± 2.7	22.7 ± 3.3	21.6 ± 2.6 [20]d	21.9 ± 1.7	23.7 ± 8.3
DAYS 0 - 21	MEAN±S.D.	23.0 ± 2.4	21.5 ± 2.4#	20.5 ± 1.6 [20]d	20.6 ± 1.3	21.2 ± 2.7

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Excludes values for rats that were found dead prior to mating.
c. Excludes a value that was associated with spillage.
d. Excludes a value for dam 6098, which was found dead on day 18 of gestation.
Significantly different from the Group I value ($p \leq 0.05$).

Table 43: Relative Food Consumption in Female Rats during Gestation Days in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	24b	24b
PREGNANT	N	23	22	21	21	23
MATERNAL FEED CONSUMPTION (G/KG/DAY)						
DAYS 0 - 7	MEAN±S.D.	77.7 ± 6.0	76.2 ± 6.0	74.6 ± 5.3	72.0 ± 5.9*	70.6 ± 6.8**
DAYS 7 - 14	MEAN±S.D.	76.6 ± 5.4	74.1 ± 5.4	71.0 ± 3.9	73.4 ± 5.7	72.9 ± 4.9 [22]c
DAYS 14 - 18	MEAN±S.D.	74.1 ± 7.3	71.1 ± 7.0	69.0 ± 5.4	72.4 ± 5.0	73.5 ± 4.9
DAYS 18 - 21	MEAN±S.D.	62.4 ± 5.6	60.8 ± 5.9	59.8 ± 6.6 [20]d	60.9 ± 4.8	62.9 ± 19.0
DAYS 0 - 21	MEAN±S.D.	73.6 ± 4.7	71.5 ± 4.2	69.5 ± 3.5 [20]d	70.4 ± 3.9	70.4 ± 5.9

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Excludes values for rats that were found dead prior to mating.
c. Excludes a value that was associated with spillage.
d. Excludes a value for dam 6098, which was found dead on day 18 of gestation.
* Significantly different from the Group II value ($p \leq 0.05$).
** Significantly different from the Group II value ($p \leq 0.01$).

In general, decreased food consumption was more evident in animals treated with BMN 110 + DPH, compared to DPH alone. However, this effect was not clearly dose-dependent or duration-dependent (i.e. the effect was limited to the first week of pregnancy. The decreased food consumption was considered as related to BMN 110.

Clinical Pathology (Hematology and Clinical Chemistry)

Prior to day 1 and on the day of scheduled euthanasia, blood samples for clinical pathology assessments were collected from six main study rats per sex per dose group (three rats for hematology and three rats for clinical chemistry). Daily IV administration of BMN 110 had no effects on hematology or clinical chemistry parameters.

Estrous Cycling

There were no BMN 110-related effects on female estrus cycling.

Toxicokinetics

TK blood samples were collected on the first and next to last day of dosing (GD 19). Samples were collected prior to dosing and at approximately 1, 5, 15, 30 and 60 minutes post-dose from three rats per sex per time point in groups 3 through 5 (i.e., each rat was bled three times on both days). The TK rats receiving vehicle (groups 1 and 2) were bled on the first and next to last day of dosing at 1 minute post-dose. Plasma concentrations of test article (BMN 110) were measured by the BioAnalytical Testing department at BioMarin (Novato, CA) using a validated ELISA method. The lower limit of quantitation (LLOQ) was 100 ng/mL.

After 35-43 days of once daily IV administration of BMN 110 in rats, the systemic exposure (AUC and C_{max}) was comparable between males and females at all doses. Mean $t_{1/2}$ values increased with dose and treatment duration. Increases in BMN 110 C_{max} were roughly dose proportional between 1 and 20 mg/kg/day at the first dose and greater than dose proportional at the next to last dose. Increases in AUC_{0-t} were greater than dose proportional between 1 and 20 mg/kg/day on both days of TK measurement. There was no change in exposure between the first and next to last doses at the 6 and 20 mg/kg/day dose levels, whereas a 56-67% decrease in exposure was observed at the 1 mg/kg/day dose level. The TK data is summarized in the Sponsor's tables below.

Reference ID: 3396720

Table 44: Mean Toxicokinetic Parameters for BMN 110 in Rat Plasma on the First Day of Dosing

Dose Group	Dose Level (mg/kg)	Sex	AUC_{0-t} (min*ng/mL)	$AUC_{0-\infty}$ (min*ng/mL)	C_{max} (ng/mL)	T_{max}[a] (min)	$t_{1/2}$ (min)	CL (mL/min/kg)	Vz (mL/kg)
III	1	M	189400	194309	29200	1	2.9	5.1	21.8
		F	159059	160570	24833	1	2.3	6.2	21.0
		Combined	174237	177350	27017	1	2.7	5.6	21.8
IV	6	M	3722854	3724676	172000	1	5.0	1.6	11.7
		F	3281044	3287691	203333	1	6.4	1.8	16.9
		Combined	3501944	3505981	187667	1	5.8	1.7	14.2
V	20	M	22401494	27143747	663333	5	24.5	0.7	26.1
		F	22514994	25923858	677000	1	22.4	0.8	24.9
		Combined	22458256	26424799	668833	5	23.0	0.8	25.1

[a] Median value is listed for T_{max}.

Table 45: Mean Toxicokinetic Parameters for BMN 110 in Rat Plasma on the Next to Last Day of Dosing

Dose Group	Dose Level (mg/kg)	Sex	AUC_{0-t} (min*ng/mL)	$AUC_{0-\infty}$ (min*ng/mL)	C_{max} (ng/mL)	T_{max}[a] (min)	$t_{1/2}$ (min)	CL (mL/min/kg)	Vz (mL/kg)
III	1	M	63046	81505	11217	1	NA[b]	NA[b]	NA[b]
		F	56093	64789	10867	1	NA[b]	NA[b]	NA[b]
		Combined	59581	68265	11042	1	NA[b]	NA[b]	NA[b]
IV	6	M	2953978	2990447	122633	1	9.2	2.0	26.5
		F	2839488	3190144	126800	1	18.1	1.9	49.0
		Combined	2896738	3063869	124717	1	14.3	2.0	40.4
V	20	M	23978332	33004101	677000	5	32.9	0.6	28.7
		F	15844780	20081393	574000	1	28.0	1.0	40.2
		Combined	19346272	25954032	581333	1	31.0	0.8	34.5

[a] Median value is listed for T_{max}.

[b] PK parameters were not available due to a low squared correlation coefficient ($R^2 < 0.8$).

Antibody Analysis

Prior to day 1 and on the last day of dosing, blood samples for antibody analysis were collected pre-dose from all rats in the TK groups. A bridging electrochemiluminescence assay format with rhGALNS was used to determine the titer of total antibodies to rhGALNS (BMN 110).

No anti-BMN 110 antibodies were detected in the animals prior to dosing or in the vehicle treated animals, with the exception of one male in the vehicle only group on study day 44. Anti-BMN 110 antibodies were detected in all BMN 110-treated animals at the end of the study (GD 20 for females and study day 44 for males), with the exception of three animals given 20 mg/kg/day (one male and 2 females). Overall, no gender-related differences were observed. Greater titers were present in animals given 1 mg/kg/day (see table below), which could be responsible for the decrease in systemic exposure observed in this group.

Table 46: Anti-BMN 110 Antibody Titers in Rat Plasma in a Combined Fertility and Developmental Toxicity Study

Groups	Titers and numbers of rats				
3	2430 (n =2)	21900 (n = 1)	65600 (n = 2)	197000 (n = 3)	> 590000 (n = 4)
4	7290 (n = 2)	21900 (n = 3)	65600 (n = 6)	197000 (n = 1)	
5	2430 (n = 1)	7290 (n = 5)	21900 (n = 1)	65600 (n = 1)	Negative (n = 3)

Dosing Solution Analysis

Test article solutions were prepared at least once daily at the Testing Facility and then once weekly after stability was established, and were stored at 2 to 8°C. Duplicate samples were taken from the first preparation, at the mid-point of the study period and on the last day of dosing (0.15, 0.9, and 3 mg/ml). All samples were analyzed within the established stability period (10 days). Mean measured concentrations for all dose formulations ranged from 98.7 to 105% of the nominal concentrations, and were within the acceptable limits (≤ 10% difference from nominal concentration). The dose formulation was considered stable. No homogeneity data was submitted.

Necropsy

Male rats were euthanized after completion of the cohabitation period (study days 34-47) and a gross necropsy was performed. Male reproductive organs were weighed (testis, epididymis, right epididymis, seminal vesicles and prostate). Sperm concentration and motility were evaluated using computer-assisted sperm analysis (CASA). Sperm samples were collected from the vas deferens of each testis. Tissue samples for histological examination were taken from the remaining portion of the left epididymis (corpus and caput), the right epididymis, prostate, seminal vesicles, and testes.

Female rats were C-sectioned on GD 21 and the fetuses were removed. The number and distribution of corpora lutea in ovaries, implantation sites, live and dead fetuses, and resorptions (early and late) in each rat were examined and recorded. In addition, a gross necropsy examination of main study rats was conducted. The body weight of each fetus was recorded. Fetuses were examined for sex, gross external alterations, soft tissue alterations, and skeletal alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations, and the remaining fetuses were examined for skeletal alterations after staining with alizarin red S. Uteri of apparently non-pregnant rats were examined to confirm the absence of implantation sites. Placentae were examined for size, color and shape.

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Fertility Parameters (Mating/Fertility Index, see Cesarean Section Data section for Corpora Lutea, Preimplantation Loss, etc.)

There were no BMN 110-related effects on mating and fertility parameters in female and male rats. The fertility indices (number of pregnancies per number of rats that mated) were 91.7%, 88%, 84%, 87.5%, and 95.6% for groups 1, 2, 3, 4, and 5, respectively. Although male rats receiving DPH only or in combination with BMN 110 at 1 or 6 mg/kg/day had slight decreases in fertility index, these changes did not reach a statistically significant level and were not dose-dependent. Data for mating and fertility in male rats is summarized in the Sponsor's table below.

Table 47: Mating and Fertility Summary Data in Male Rats

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS IN COHABITATION	N	25	25	25	25	24a
INCLUDED IN ANALYSES	N	25	25	25	24b	24
DAYS IN COHABITATION c,d MEAN±S.D.		3.4 ± 2.7	3.1 ± 2.2	2.1 ± 1.4	2.4 ± 1.4	3.2 ± 2.7
RATS THAT MATED d	N(%)	24(96.0)	25(100.0)	25(100.0)	24(100.0)	23(95.8)
FERTILITY INDEX e,f	N/N	22/24	22/25	21/25	21/24	22/23
	(%)	(91.7)	(88.0)	(84.0)	(87.5)	(95.6)
RATS WITH CONFIRMED MATING DATES	N	24	25	25	24	23
MATED WITH FEMALE g						
DAYS 1-7	N(%)	23(95.8)	24(96.0)	25(100.0)	24(100.0)	23(100.0)
DAYS 8-14	N(%)	1(4.2)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION f	N/N	22/25	22/25	21/25	21/24	22/24
	(%)	(88.0)	(88.0)	(84.0)	(87.5)	(91.7)

a. Excludes values for rat 6025, which was not assigned to cohabitation because there were no available female rats.
b. Excludes values for rat 5995; cohort female rat 6120 was found dead on day 17 of study (day 2 of cohabitation).
c. Restricted to rats with a confirmed mating date and rats that did not mate.
d. Includes only one mating for each male rat.
e. Number of pregnancies/number of rats that mated.
f. Includes only one pregnancy for each rat that impregnated more than one female rat.
g. Restricted to rats with a confirmed mating date.

The percent motile sperm was slightly reduced ($p \leq 0.05$) in males treated with DPH only compared to group 1 males (vehicle only). When compared to vehicle control rats (group 1), male rats receiving DPH only or in combination with BMN 110 at 1, 6, and 20 mg/kg/day had decreases (not significant) in sperm parameters including the number of motile sperm, percent motile sperm, static count, and total counts for sperm from the vas deferens and sperm count and density for sperm from the cauda epididymis. However, overall, all these sperm parameters in animals receiving DPH in combination with BMN 110 are comparable to males treated with DPH alone. Thus, it is likely that the observed decreases in sperm parameters are DPH treatment-related rather than BMN 110 treatment-related. Data for sperm motility, count and density in male rats is summarized in the Sponsor's table below.

Reference ID: 3396720

Table 48: Sperm Motility, Count and Density in Male Rats in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY)/TREATMENT		0 (VEHICLE)	0 (VEHICLE)/DPH	1/DPH	6/DPH	20/DPH
RATS TESTED	N	25	25	25	25	25
VAS DEFERENS SPERM MOTILITY						
NUMBER MOTILE	MEAN±S.D.	505.5 ± 197.1	480.7 ± 184.7	510.8 ± 199.5	452.5 ± 204.5 [24]a	488.2 ± 200.1
MOTILE PERCENT	MEAN±S.D.	93.4 ± 2.7	88.9 ± 12.8#	89.2 ± 12.1	90.0 ± 9.1 [24]a	88.4 ± 10.7
STATIC COUNT (NONMOTILE)	MEAN±S.D.	35.2 ± 17.6	57.6 ± 62.8	62.8 ± 70.7	57.7 ± 63.6 [24]a	60.0 ± 48.4
TOTAL COUNT b	MEAN±S.D.	540.6 ± 210.6	538.3 ± 183.5	573.6 ± 244.0	510.2 ± 233.5 [24]a	548.2 ± 205.5
CAUDA EPIDIDYMAL SPERM COUNT						
SPERM COUNT c	MEAN±S.D.	139.6 ± 65.0	129.5 ± 51.9	126.7 ± 61.6	112.0 ± 42.3	119.4 ± 53.4
SPERM DENSITY d	MEAN±S.D.	1295.08 ± 486.38	1223.60 ± 492.05	1183.03 ± 495.10	1068.04 ± 374.44	1142.10 ± 469.07

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rat 5983, which had motility data that reflected drifting debris.

b. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.

c. Sperm count used in the calculation of sperm density. Ten fields were evaluated.

d. The sperm density was calculated by dividing the sperm count by the volume in the image area (38.3×10^{-6} mL), multiplying by 2 (dilution factor + 1) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table A15 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

Significantly different from the Group I value ($p \leq 0.05$).

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Compared to the vehicle control group (group 1), females in group 2 (DPH only) had significant increases in the incidences of resorptions, late resorptions, dams with resorptions, percentage of dead or resorbed conceptuses, and decreased female fetal body weights. Similar changes were observed in animals treated with DPH combined with BMN 110 at 1, 6, and 20 mg/kg/day groups. Thus, these changes were considered as related to DPH administration rather than BMN 110 treatment. Female rats in group 3 had a significant increase ($p \leq 0.05$) in pre-implantation loss compared to dams in group 2, but not to dams in group 1. In addition, one rat in the 6 mg/kg/day BMN 110 + DPH group (# 6109) had a litter consisting entirely of resorbed conceptuses; this finding was not considered to be related to BMN 110, because the overall incidence of resorptions was not increased in other animals treated with BMN 110. Therefore, BNM 110 had no effects on corpora lutea, implantations, total resorptions, early and late resorptions, number of dams with resorptions, percentage of dead or resorbed conceptuses, litter sizes, live fetuses, percentage of live male and female fetuses, or fetal body weights. Data from caesarean section observations is summarized in the Sponsor's tables below.

Table 49: Caesarean Section Observations in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	24b	24b
PREGNANT	N(%)	23(92.0)	22(88.0)	21(84.0)	21(87.5)	23(95.8)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	22	20	21	23
CORPORA LUTEA	MEAN±S.D.	15.3 ± 2.1	15.1 ± 2.3	15.4 ± 1.6	13.7 ± 3.7	14.5 ± 2.4
IMPLANTATIONS	MEAN±S.D.	14.0 ± 2.5	14.8 ± 1.9	14.3 ± 1.8	12.6 ± 3.8	14.0 ± 2.0
% PREIMPLANTATION LOSS	MEAN±S.D.	7.9 ± 14.7	2.0 ± 4.7	6.8 ± 7.5*	9.3 ± 18.8	3.5 ± 6.4
LITTER SIZES	MEAN±S.D.	13.1 ± 2.3	12.3 ± 2.6	11.8 ± 1.9	10.2 ± 3.8	12.2 ± 2.0
LIVE FETUSES	N	301	271	234	214	280
	MEAN±S.D.	13.1 ± 2.4	12.3 ± 2.6	11.7 ± 1.9	10.2 ± 3.8	12.2 ± 2.0
DEAD FETUSES	N	1	0	1	0	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.9 ± 1.0	2.4 ± 1.4##	2.6 ± 0.9	2.4 ± 1.6	1.8 ± 1.3
EARLY RESORPTIONS	N	19	22	26	21	21
	MEAN±S.D.	0.8 ± 1.0	1.0 ± 1.2	1.3 ± 1.0	1.0 ± 1.1	0.9 ± 1.4
LATE RESORPTIONS	N	1	32	25	30	20
	MEAN±S.D.	0.0 ± 0.2	1.4 ± 1.1##	1.2 ± 0.6	1.4 ± 0.9	0.9 ± 0.7
% POSTIMPLANTATION LOSS	MEAN±S.D.	6.3 ± 6.7	17.1 ± 10.4##	18.4 ± 6.4	24.6 ± 23.4	12.7 ± 8.8

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Excludes values for rats that were found dead prior to mating.
Significantly different from the Group I value (p≤0.01).
* Significantly different from the Group II value (p≤0.05).

Table 50: Caesarean Section Observations in a Combined Fertility and Developmental Toxicity Study (Continued)

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RATS TESTED	N	25	25	25	24b	24b
PREGNANT	N(%)	23(92.0)	22(88.0)	21(84.0)	21(87.5)	23(95.8)
FOUND DEAD	N(%)	0(0.0)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	22	20	21	23
DAMS WITH ANY RESORPTIONS	N(%)	12(52.2)	21(95.4)##	20(100.0)	19(90.5)	21(91.3)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	22(100.0)	20(100.0)	20(95.2)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	22(100.0)	20(100.0)	21(100.0)	23(100.0)

a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Excludes values for rats that were found dead prior to mating.
Significantly different from the Group I value (p≤0.01).

Table 51: Litter Observations in a Combined Fertility and Developmental Toxicity Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	22	20	20	23
IMPLANTATIONS	MEAN±S.D.	14.0 ± 2.5	14.8 ± 1.9	14.3 ± 1.8	13.2 ± 2.8	14.0 ± 2.0
LIVE FETUSES	N	301	271	234	214	280
	MEAN±S.D.	13.1 ± 2.4	12.3 ± 2.6	11.7 ± 1.9	10.7 ± 3.0	12.2 ± 2.0
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.8 ± 12.7	51.4 ± 11.8	50.6 ± 10.6	50.1 ± 20.1	50.8 ± 15.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.64 ± 0.44	5.45 ± 0.22	5.44 ± 0.37	5.60 ± 0.34	5.55 ± 0.31
MALE FETUSES	MEAN±S.D.	5.72 ± 0.52	5.58 ± 0.30	5.61 ± 0.38	5.84 ± 0.33 [19]b	5.70 ± 0.33
FEMALE FETUSES	MEAN±S.D.	5.53 ± 0.39	5.33 ± 0.20#	5.26 ± 0.34	5.42 ± 0.35	5.43 ± 0.31
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	6.3 ± 6.7	17.1 ± 10.4##	18.3 ± 6.4	20.8 ± 16.2	12.7 ± 8.8

[ ] = NUMBER OF VALUES AVERAGED
a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Litter 6104 had no male fetuses.
Significantly different from the Group I value (p≤0.05).
Significantly different from the Group I value (p≤0.01).

Offspring (Malformations, Variations, etc.)

There were a total of two dead fetuses. One in the vehicle group (group 1) showed meningocele, short trunk, neck edema, right eye bulge depressed, short snout and gastroschisis. This fetus also showed the following soft tissue alterations: constricted right nasal turbinate, dilated left nasal turbinate, protrusion of all liver lobes, stomach, spleen, pancreas and small intestines through an abdominal opening, irregularly shaped brain, microphthalmia of the right eye, diaphragmatic hernia with the left kidney protruding into the thoracic cavity, absent right kidney and right ureter, and malpositioned left adrenal. One dead fetus in group 3 (1 mg/kg/day BMN 110 + DPH) had both eye bulges depressed at gross external examination, with no abnormal findings in skeletal examination.

An increased incidence in total alterations (gross external, soft tissue, or skeletal) among fetuses and litters was observed in dams receiving DPH treatment only (p≤0.01) or in combination with BMN 110 (not significant), compared to the vehicle only group. However, litters and fetuses of dams treated with BMN 110 + DPH groups (groups 3-5) had a smaller increase in these parameters when compared to the DPH only treatment group (group 2). Thus, BMN 110 did not produce an increase in the incidence of total alterations. Several changes observed in gross external examination were considered as related to DPH administration, including short forelimbs and/or hindlimbs, short digits, and absent digits. DPH administration also resulted in a significant reduction (p≤0.01) in the number of ossified tarsals in group 2, as compared to group 1. In summary, BMN 110 had had no effects on gross, soft tissue or skeletal observations. The fetal observations are listed in the tables below (taken from the study report).

Reference ID: 3396720

Table 52: Fetal Gross External Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	302	271	235	214	280
LIVE	N	301	271	234	214	280
DEAD b	N	1	0	1	0	0
SNOUT: CLEFT						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SNOUT: SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.4)q
EYE: BULGE DEPRESSED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	1(0.4)k	1(0.5)n	0(0.0)
FORE AND/OR HINDLIMBS: SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	4(18.2)##	1(5.0)	1(5.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	4(1.5)##d,f,g,i	1(0.4)k	2(0.9)n,o	1(0.4)q
FORE AND/OR HINDLIMBS: ROTATED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)j	0(0.0)	0(0.0)
FORE AND/OR HINDLIMBS: FLEXED						
LITTER INCIDENCE	N(%)	0(0.0)	2(9.1)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(0.7)e,h	0(0.0)	0(0.0)	0(0.0)
FORE AND/OR HINDLIMBS: DIGIT SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	4(18.2)##	3(15.0)	3(15.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	4(1.5)##d,g,i	3(1.3)j,k	4(1.9)m,n,o,p	2(0.7)q
FORE AND/OR HINDLIMBS: DIGITS ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	3(15.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.4)d	2(0.8)j,k	3(1.4)n,p	0(0.0)

Significantly different from the Group I value (p≤0.01).

Table 53: Fetal Gross External Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study--Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	302	271	235	214	280
LIVE	N	301	271	234	214	280
DEAD b	N	1	0	1	0	0
BODY: UMBILICAL HERNIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
BODY: EDEMA						
LITTER INCIDENCE	N(%)	1(4.3)	2(9.1)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	2(0.7)e,f	1(0.4)	1(0.5)L	0(0.0)
BODY: GASTROSCHISIS						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)
BODY: DISCOLORATION						
LITTER INCIDENCE	N(%)	0(0.0)	3(13.6)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	3(1.1)e,h	0(0.0)	1(0.5)L	0(0.0)
BODY: ANAL OPENING LARGE						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.4)f	0(0.0)	0(0.0)	0(0.0)
BODY: GENITAL TUBERCLE SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.4)f	0(0.0)	0(0.0)	0(0.0)
TAIL: SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	2(10.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)	2(0.9)m,p	1(0.4)q

FOOTNOTES:

a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Dead fetus(es) were excluded from summarization and statistical analyses. Observations for these conceptus(es) are cited on Table B29.
c. Fetus 6048-1 had other gross external alterations.
d. Fetus 6054-11 had other gross external alterations.
e. Fetus 6060-15 had other gross external alterations.
f. Fetus 6061-10 had other gross external alterations.
g. Fetus 6062-14 had other gross external alterations.
h. Fetus 6063-12 had other gross external alterations.
i. Fetus 6075-4 had other gross external alterations.
j. Fetus 6076-9 had other gross external alterations.
k. Fetus 6089-8 had other gross external alterations.
L. Fetus 6102-7 had other gross external alterations.
m. Fetus 6111-13 had other gross external alterations.
n. Fetus 6116-8 had other gross external alterations.
o. Fetus 6116-14 had other gross external alterations.
p. Fetus 6125-5 had other gross external alterations.
q. Fetus 6135-8 had other gross external alterations.

Table 54: Fetal Soft Tissue Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	19	23
FETUSES EVALUATED	N	146	130	113	102	135
LIVE	N	145	130	113	102	135
DEAD b	N	1	0	0	0	0
EYES: MICROPHTHALMIA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(1.0)	0(0.0)

a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table B29.

Table 55: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study

DOSE GROUP		I	II	III	IV	V
DOSE (MG/KG/DAY)/TREATMENT a		0 (VEHICLE)	0 (VEHICLE)/DPH	1/DPH	6/DPH	20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
SKULL: MAXILLA, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: PALATE, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: EYE SOCKET, SMALL						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	1(0.8)j	0(0.0)	0(0.0)
SKULL: PARIETAL, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
SKULL: SQUAMOSAL, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
SKULL: MANDIBLE, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
SKULL: NASAL, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)h	0(0.0)	0(0.0)	0(0.0)
SKULL: FRONTAL, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)h	0(0.0)	0(0.0)	0(0.0)
SKULL: FRONTALS, SUTURE LARGE						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.7)p

Table 56: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study-Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
CERVICAL VERTEBRAE: CERVICAL RIB, PRESENT AT 7TH CERVICAL VERTEBRA						
LITTER INCIDENCE	N(%)	1(4.3)	2(9.1)	0(0.0)	1(5.0)	2(8.7)
FETAL INCIDENCE	N(%)	1(0.6)d	2(1.4)	0(0.0)	1(0.9)m	2(1.4)q
CERVICAL VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	3(13.6)	1(5.0)	0(0.0)	4(17.4)
FETAL INCIDENCE	N(%)	1(0.6)	4(2.8)h	3(2.5)k,L	0(0.0)	6(4.1)p
CERVICAL VERTEBRAE: ARCH, 6TH HAS THE APPEARANCE LIKE THE 7TH						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	2(10.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	2(1.6)j	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)d	0(0.0)	0(0.0)	0(0.0)	0(0.0)
THORACIC VERTEBRAE: CENTRUM, BIFID						
LITTER INCIDENCE	N(%)	3(13.0)	3(13.6)	1(5.0)	3(15.0)	2(8.7)
FETAL INCIDENCE	N(%)	3(1.9)	4(2.8)	1(0.8)	4(3.6)	3(2.1)
THORACIC VERTEBRAE: CENTRUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
LUMBAR VERTEBRAE: ARCH, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
LUMBAR VERTEBRAE: CENTRUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
SACRAL VERTEBRAE: CENTRUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 57: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study-Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
CAUDAL VERTEBRAE: CENTRUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
RIBS: BENT						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)	0(0.0)	0(0.0)	0(0.0)
RIBS: WAVY						
LITTER INCIDENCE	N(%)	1(4.3)	2(9.1)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	3(2.1)g,h	1(0.8)k	0(0.0)	0(0.0)
RIBS: INCOMPLETELY OSSIFIED (HYPOPLASTIC)						
LITTER INCIDENCE	N(%)	1(4.3)	1(4.5)	1(5.0)	0(0.0)	1(4.3)
FETAL INCIDENCE	N(%)	1(0.6)c	2(1.4)g,h	1(0.8)k	0(0.0)	1(0.7)p
MANUBRIUM: LARGE						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	2(10.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	2(1.6)i,j	0(0.0)	0(0.0)

Table 58: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study-Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
STERNAL CENTRA: SUMMARIZATION (SUMMARIZATION OF INCOMPLETELY OSSIFIED, LARGE AND FUSED)						
LITTER INCIDENCE	N(%)	1(4.3)	2(9.1)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.4)	2(1.6)	1(0.9)	0(0.0)
STERNAL CENTRA: INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.3)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	1(0.7)	0(0.0)	0(0.0)	0(0.0)
STERNAL CENTRA: LARGE						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	2(1.6)i,j	1(0.9)m	0(0.0)
STERNAL CENTRA: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	2(1.6)i,j	1(0.9)m	0(0.0)
XIPHOID: LARGE						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	2(1.6)i,j	1(0.9)m	0(0.0)
XIPHOID: IRREGULARLY SHAPED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	2(1.6)i,j	1(0.9)m	0(0.0)
SCAPULAE: BODY AND ALA, BENT						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Table 59: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study-Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
FORELIMB: METACARPAL, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)L	0(0.0)	0(0.0)
FORELIMB: PHALANX, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(10.0)	2(10.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	2(1.6)j,L	2(1.8)m,o	1(0.7)q
FORELIMB: HUMERUS, SHORT						
LITTER INCIDENCE	N(%)	1(4.3)	1(4.5)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	1(0.7)e	1(0.8)j	1(0.9)m	0(0.0)
FORELIMB: RADIUS, SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)j	1(0.9)m	0(0.0)
FORELIMB: ULNA, SHORT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)j	1(0.9)m	0(0.0)
FORELIMB: PHALANX, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	1(0.8)j	1(0.9)m	0(0.0)
FORELIMB: HUMERUS, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)h	0(0.0)	0(0.0)	0(0.0)
FORELIMB: DIGIT, ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)e	1(0.8)j	1(0.9)o	0(0.0)

Table 60: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Combined Fertility and Developmental Toxicity Rat Study-Continued

DOSE GROUP DOSE (MG/KG/DAY)/TREATMENT a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS EVALUATED	N	23	22	20	20	23
FETUSES EVALUATED	N	156	141	122	112	145
LIVE	N	156	141	121	112	145
DEAD b	N	0	0	1	0	0
FORELIMB: RADIUS, BENT						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FORELIMB: ULNA, BENT						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)
PELVIS: SUMMARIZATION (SUMMARIZATION OF ISCHIUM AND PUBIS, INCOMPLETELY OSSIFIED)						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)	0(0.0)	0(0.0)
PELVIS: ISCHIUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)k	0(0.0)	0(0.0)
HINDLIMB: PHALANX, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	2(9.1)	1(5.0)	2(10.0)	1(4.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.4)e,f	1(0.8)j	2(1.8)m,n	1(0.7)q
HINDLIMB: PHALANX, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	2(9.1)	0(0.0)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.4)e,f	0(0.0)	2(1.8)m,n	0(0.0)
HINDLIMB: METATARSALS, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)o	0(0.0)
HINDLIMB: FEMUR, TIBIA, FIBULA, BENT						
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)c	0(0.0)	0(0.0)	0(0.0)	0(0.0)

FOOTNOTES:

a. Dosing occurred on day 1 of study through day 20 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table B29.
c. Fetus 6045-4 had other skeletal alterations.
d. Fetus 6048-1 had other skeletal alterations.
e. Fetus 6054-11 had other skeletal alterations.
f. Fetus 6059-10 had other skeletal alterations.
g. Fetus 6070-1 had other skeletal alterations.
h. Fetus 6070-17 had other skeletal alterations.
i. Fetus 6080-8 had other skeletal alterations.
j. Fetus 6089-8 had other skeletal alterations.
k. Fetus 6092-5 had other skeletal alterations.
L. Fetus 6092-11 had other skeletal alterations.
m. Fetus 6116-14 had other skeletal alterations.
n. Fetus 6119-11 had other skeletal alterations.
o. Fetus 6125-5 had other skeletal alterations.
p. Fetus 6131-14 had other skeletal alterations.
q. Fetus 6135-10 had other skeletal alterations.

In summary, SD male and female rats were administered BMN 110 via intravenous bolus injection daily at doses of 1, 6 and 20 mg/kg before cohabitation, through cohabitation, the day before euthanasia (males only), and through day 20 of presumed gestation (GD 20). All study groups (except for vehicle only group) were administered 10 mg/kg diphenhydramine (DPH) via intraperitoneal injection before administration of the test article or vehicle on each day to prevent potential anaphylactic reactions. There were a total of four unscheduled deaths that may be related to BMN 110 and/or DPH administration. BMN 110 at 6 and 20 mg/kg/day decreased body weight and body

weight gain in males. The decrease in body weight and body weight gain was associated with decreased food consumption. Therefore, the paternal NOAEL was considered to be 1 mg/kg/day IV due to decreased body weight, body weight gain and food consumption.

During the pregnancy, BMN 110 had no significant effect on body weight or body weight gain in females. Therefore, the maternal NOAEL during pregnancy was 20 mg/kg/day IV.

There were no BMN 110-related effects on mating and fertility parameters in female and male rats. BMN 110 had no effects on corpora lutea, implantations, total resorptions, early and late resorptions, number of dams with resorptions, the percentage of dead or resorbed conceptuses, litter sizes, live fetuses, percent live male and female fetuses or fetal body weights. BMN 110 had no effects on sperm motility, count, or density. Gross fetal changes including short forelimbs and/or hindlimbs, short digits, and absent digits were considered as DPH-related, because the number of fetuses and litters with these effects was comparable across the groups administered DPH alone or DPH in combination with BMN 110. Therefore, it appears that BMN 110 did not increase the incidence of gross external, soft tissue or skeletal alterations. Thus, the NOAEL for fertility and embryo-fetal development was 20 mg/kg/day IV.

9.2 Embryonic Fetal Development

Intravenous Dose-Range Developmental Toxicity Study of BMN 110 in Rabbits (Study report BMN110-10-008)

This was a dose-range finding study for an embryo-fetal toxicity and teratogenic potential study in rabbits. BMN 110 was administered daily via IV infusions to presumed-pregnant female rabbits [Hra:(NZW)SPF]. All animals were presumed pregnant at first dose on gestation day 7 (GD 7). The rabbits weighed 2.7 to 3.6 kg each on GD 0 and were approximately 6 months of age. BMN 110 or vehicle was administered once daily by 4 hr IV infusion via the marginal ear vein on days 7 through 20 of the presumed gestation period at dose levels of 0 (vehicle), 1, 6, and 20 mg/kg/day. The study design is summarized in the Sponsor's table below.

Reference ID: 3396720

Table 61: Study Design for Intravenous Dose-Range Finding Developmental Toxicity Study of BMN 110 in Rabbits

Treatment Group[a]	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
				Main Study Animals	TK Animals
Vehicle (1)	0	6.67	0	8F	2F
Vehicle (2)[b]	0	6.67	0	8F	2F
Low (3)	1	6.67	0.15	8F	3F
Mid (4)	6	6.67	0.9	8F	3F
High (5)	20	6.67	3	8F	3F

TK, toxicokinetic

[a] BMN 110 lot 11615P78; Vehicle prepared at CRL

[b] An additional vehicle group was added to the study to control for DPH administration in case the animals needed to be treated with DPH due to anaphylactoid-type reactions. No anaphylactoid-type reactions were observed and therefore this group was not treated with DPH

Clinical observations, body weight and food consumption were recorded daily. Blood samples were collected on days 1 and 23 for analysis of hematology and clinical chemistry parameters. Blood samples were collected for TK analysis at pre-dose, during infusion, and after the end of infusion. Fetuses were collected by C-section and fetal blood samples for TK analysis were collected. On GD 7 and GD 19, blood samples were collected from all TK animals prior to dosing for antibody measurement. On GD 29, the main study rabbits were C-sectioned. Examination of rabbits included number and distribution of the corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. The live fetuses were weighed and examined for gross external alternations and sex determination. The main study rabbits were subjected to a gross necropsy examination.

All animals survived until the scheduled euthanasia (GD 29) with the exception of two animals. One doe in the vehicle group aborted and was euthanized on GD 19. One doe in 20 mg/kg/day group delivered on GD 29 and was euthanized on the same day. This doe had an observation of scant feces on GDs 19, 20, 22-25, and 27, and thin body condition on GDs 28 and 29. Food consumption and body weight were reduced after GD 9. There were no macroscopic findings at necropsy. The litter consisted of 6 live and 5 dead fetuses, partially cannibalized.

Female rabbits treated with 20 mg/kg/day BMN 110 showed clinical signs of thin body condition and the presence of scant feces (2 and 5 females, respectively). At the end of the study, body weight (up to 16%↓ on GD 29) and food consumption (up to 52%↓) were reduced in the females given 20 mg/kg/day. There were no BMN 110-treatment related changes in hematology or clinical chemistry parameters.

Reference ID: 3396720

Females in the 6 and 20 mg/kg/day groups had numerous pitted areas or tan areas of the liver (6/8 and 3/8 females, respectively). One female in the 20 mg/kg/day group had pale heart. A decrease in fetal body weight was observed in the 20 mg/kg/day group (e.g., 12%↓ in females relative to DPH alone females, not statistically significant), which could be secondary to maternal toxicity or indicative of direct fetal toxicity (see table below from study report). At all doses, BMN 110 increased the percentage of post-implantation loss (2%, 7.1%, 5.1%, and 9.2%, for the control, 1, 6, and 20 mg/kg/day groups, respectively; see table below). The percentage of does with any resorptions increased in BMN 110 treatment groups (20%, 37%, 50%, and 57% for the control, 1, 6, and 20 mg/kg/day groups, respectively; see Sponsor's table below). Although not statistically significant, adverse effects on embryo-fetal development (i.e. increased incidence of resorptions) were observed in all groups treated with BMN 110, especially the high-dose group. No other drug-related changes in the C-section or litter parameters were observed. All fetuses appeared normal at gross external examination.

Table 62: Caesarean-Section Observations in a Dose Range-Finding Study in Rabbits

DOSE GROUP DOSE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
RABBITS TESTED	N	8	8	8	8	8
PREGNANT	N(%)	6(75.0)	7(87.5)	8(100.0)	6(75.0)	8(100.0)
ABORTED AND EUTHANIZED	N(%)	1(16.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DELIVERED AND EUTHANIZED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(12.5)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	5	7	8	6	7
CORPORA LUTEA	MEAN±S.D.	11.4 ± 1.7	10.6 ± 1.9	8.8 ± 2.1	9.7 ± 1.9	9.4 ± 1.3
IMPLANTATIONS	MEAN±S.D.	10.2 ± 1.5	10.3 ± 2.4	8.4 ± 2.4	9.7 ± 1.9	9.1 ± 1.5
% PREIMPLANTATION LOSS	MEAN±S.D.	10.2 ± 9.5	3.6 ± 9.4	5.8 ± 9.1	0.0 ± 0.0	3.2 ± 5.5
LITTER SIZES	MEAN±S.D.	10.0 ± 1.6	10.3 ± 2.4	8.0 ± 2.7	9.2 ± 1.8	8.3 ± 1.7
LIVE FETUSES	N	50	72	64	55	58
	MEAN±S.D.	10.0 ± 1.6	10.3 ± 2.4	8.0 ± 2.7	9.2 ± 1.8	8.3 ± 1.7
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.0 ± 0.0	0.4 ± 0.5	0.5 ± 0.5	0.8 ± 1.1
EARLY RESORPTIONS	N	1	0	2	3	2
	MEAN±S.D.	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.5	0.5 ± 0.5	0.3 ± 0.5
LATE RESORPTIONS	N	0	0	1	0	4
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.6 ± 1.1
% POSTIMPLANTATION LOSS	MEAN±S.D.	2.0 ± 4.5	0.0 ± 0.0	7.1 ± 11.8	5.1 ± 5.8	9.2 ± 11.8
DOES WITH ANY RESORPTIONS	N(%)	1(20.0)	0(0.0)	3(37.5)	3(50.0)	4(57.1)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred on days 7 through 20 of gestation.

Table 63: Litter Observations (Caesarean-Delivered Fetuses) in a Dose Range-Finding Study in Rabbits

DOSE GROUP DOSE (MG/KG/DAY)a		I 0 (VEHICLE)	II 0 (VEHICLE)/DPH	III 1/DPH	IV 6/DPH	V 20/DPH
LITTERS WITH ONE OR MORE LIVE FETUSES	N	5	7	8	6	7
IMPLANTATIONS	MEAN±S.D.	10.2 ± 1.5	10.3 ± 2.4	8.4 ± 2.4	9.7 ± 1.9	9.1 ± 1.5
LIVE FETUSES	N	50	72	64	55	58
	MEAN±S.D.	10.0 ± 1.6	10.3 ± 2.4	8.0 ± 2.7	9.2 ± 1.8	8.3 ± 1.7
LIVE MALE FETUSES	N	20	37	30	28	32
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	38.6 ± 22.6	52.2 ± 18.0	47.8 ± 16.1	48.1 ± 24.6	55.5 ± 6.3
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	43.48 ± 5.13	38.57 ± 4.22	40.78 ± 7.31	40.71 ± 4.39	34.83 ± 7.56
MALE FETUSES	MEAN±S.D.	44.44 ± 5.04	38.38 ± 4.44	41.65 ± 8.53	41.06 ± 4.87	35.69 ± 8.54
FEMALE FETUSES	MEAN±S.D.	42.98 ± 5.20	38.69 ± 5.51	40.78 ± 6.71	40.43 ± 4.54	34.00 ± 6.57
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	2.0 ± 4.5	0.0 ± 0.0	7.1 ± 11.8	5.1 ± 5.8	9.2 ± 11.8
		NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION				

a. Dose administration occurred on days 7 through 20 of gestation.

BMN 110 concentrations were below the limit of quantitation (< 75 ng/mL) for the majority of post-infusion PK samples in the 1 mg/kg/day group. The increase in C_{max} and AUC_{0-t} values was dose proportional between 1 and 6 mg/kg/day and greater than dose proportional between 6 and 20 mg/kg/day on GD 7 (dose 1). The increase in C_{max} and AUC_{0-t} was greater than dose proportional between 1 and 20 mg/kg on GD 19 (dose 13). No apparent increase in exposure was observed for rabbits at 1 and 20 mg/kg from GD 7 to GD 19. However, there was an increase in exposure at 6 mg/kg on GD 19. The TK data is summarized in the Sponsor's table below.

Table 64: Mean Toxicokinetic Parameters for BMN 110 in Rabbit Plasma in a Dose-Range Finding Study

Study Day	Dose Group	Dose Level (mg/kg)		AUC_{0-t} (min*ng/mL)	$AUC_{0-\infty}$ (min*ng/mL)	C_{max} (ng/mL)	C_{eof} (ng/mL)	T_{max} (min)	$t_{1/2}$ (min)	CL (mL/min/kg)	Vz (mL/kg)
GD7	3	1	Mean	72875	NA	344	344	230	NA	NA	NA
			SD	12419	NA	64	65	NA	NA	NA	NA
			N	3	0	3	3	3	0	0	0
	4	6	Mean	572434	712276	2796	2796	230	6.98	8.42	84.86
			SD	151320	NA	919	919	NA	NA	NA	NA
			N	3	1	3	3	3	1	1	1
	5	20	Mean	11367364	11369127	68408	67908	230	6.25	2.56	20.13
			SD	8828386	8827742	56712	56996	NA	1.96	1.64	8.73
			N	3	3	3	3	3	3	3	3
GD19	3	1	Mean	78440	NA	420	393	30	NA	NA	NA
			SD	17708	NA	53	70	NA	NA	NA	NA
			N	3	0	3	3	3	0	0	0
	4	6	Mean	2473789	2534143	16893	16893	230	25.38	4.53	116.01
			SD	1770365	1817458	12370	12370	NA	10.99	4.77	61.59
			N	3	3	3	3	3	3	3	3
	5	20	Mean	9176893	9203248	45012	44432	245	16.84	2.28	57.13
			SD	2308714	2290841	9595	9230	NA	2.93	0.64	26.51
			N	3	3	3	3	3	3	3	3

NA indicated TK parameter or statistics could not be calculated.

Median value was listed for T_{max}.

Low levels of BMN 110 (94 ng/ml) were detected 15 minutes after the end of infusion in rabbit fetal plasma at the 20 mg/kg/day dose level. These data demonstrated that BMN 110 crossed the placental barrier, presumably due to the expression of CI-M6PR in the placenta.

No anti-BMN 110 antibodies were detected at GD 7 in any of the does. Anti-BMN 110 antibodies were detected in all drug-treated does at GD 19.

In conclusion, maternal toxicity was observed in pregnant rabbits given BMN 110 at 6 and 20 mg/kg/day. Pitted or tan areas in the liver were observed in does given 6 and 20 mg/kg/day BMN 110. Decreased food consumption, body weight, thin body condition, and scant feces were also observed in females in the 20 mg/kg/day group. Decreased fetal body weight was noted at the 20 mg/kg/day dose level. At all doses, BMN 110 increased the percentage of post-implantation loss. The percentage of does with resorptions increased in BMN 110 treatment groups. Therefore, the Sponsor determined that the BMN 110 dose levels in the pivotal developmental toxicology study in rabbits should be 1, 3, and 10 mg/kg/day. Although not statistically significant, adverse effects on embryo-fetal development (i.e. increased incidence of resorptions) were observed in all groups treated with BMN 110, especially the high-dose group.

Study title: Intravenous Developmental Toxicity Study of BMN 110 in Rabbits

Study no.:	BMN110-10-061
Study report location:	N/A
Conducting laboratory and location:	(b) (4) [redacted] TK and anti-BMN 110 antibody analysis: BioAnalytical Testing (BAT), BioMarin Pharmaceutical Inc. Novato, CA
Date of study initiation:	May 7, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	11780P15, purity: 99.8% specific activity: 8.1 U/mg (fluorescence) specific activity: 3.4 U/mg (HPLC)

Key Study Findings

In this segment 2 study, time-mated rabbits were administered BMN 110 via intravenous infusion daily at doses of 1, 3 and 10 mg/kg through gestation days 7 to 20. There were no drug-related deaths, clinical signs, or changes in body weight or food consumption. All dose levels of BMN 110 produced red, tan, mottled, raised and/or pitted areas on the liver. Thus, the maternal NOAEL was < 1 mg/kg/day IV. BMN 110 had no effects on corpora lutea, implantations, resorptions, number of does with resorptions, the percentage of dead or resorbed conceptuses, litter sizes, live fetuses, percent live male and female fetuses, or fetal body weights. BMN 110 did not increase the incidence of gross external alterations, soft tissue alterations, or skeletal alterations. Thus, the NOAEL for rabbit embryonic and fetal development was 10 mg/kg/day IV.

Methods

Doses:	1, 3, and 10 mg/kg
Frequency of dosing:	Daily
Dose volume:	6.67 ml/kg
Route of administration:	IV infusions via the marginal ear vein for 4 hours
Formulation/Vehicle:	BMN 110 was formulated in 20 mM sodium acetate, 50 mM sodium phosphate, 30 mM arginine hydrochloride, 2% sorbitol, 0.01% polysorbate 20, pH 5.4 at nominal concentrations of 0.15, 0.45, and 1.5 mg/ml
Species/Strain:	NZW[Hra:SPF] rabbits
Number/Sex/Group:	20 time-mated female rabbits/group

Reference ID: 3396720

Satellite groups: Not applicable

Study design: BMN 110 or vehicle was administered intravenously to time-mated female rabbits (6 months old) on GD 7 through 20 at dose levels of 0 (vehicle), 1, 3, and 10 mg/kg/day. An additional vehicle group (group 2) was included in the study design to control for the administration of diphenhydramine (DPH) if required to mitigate any anaphylactic reactions in the BMN 110-treated rabbits. However, no DPH was administered since there was no anaphylactic reaction. On GD 29, rabbits were C-sectioned and the fetuses were removed for examination. Two groups of rabbits were therefore treated with vehicle only (groups 1 and 2) and were combined for reporting purposes.

Deviation from study protocol: Minor deviations had no impact on the outcome of the study or the interpretation of the results.

The study design is summarized in the Sponsor's table below.

Table 65: Study Design for Embryo-fetal Developmental Toxicity Study in Rabbits

Treatment Group[a]	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Main Study
Vehicle (1)	0	6.67	0	20F
Vehicle (2)[b]	0	6.67	0	20F
Low (3)	1	6.67	0.15	20F
Mid (4)	3	6.67	0.45	20F
High (5)	10	6.67	1.5	20F

DPH, Diphenhydramine

[a] BMN 110 lot 11780P15; Vehicle prepared at CRL

[b] An additional vehicle group was added to the study to control for DPH administration in case the animals needed to be treated with DPH due to anaphylactoid-type reactions. No anaphylactoid-type reactions were observed and therefore this group was not treated with DPH

Observations and Results

Mortality

Mortality was observed twice daily. There were no drug-related deaths. One doe in the vehicle group 1 (# 8008) aborted and was euthanized on GD (gestation day) 26 and one doe in vehicle group 2 (# 8032) delivered early and was euthanized on GD 29.

Rabbit 8008 in the vehicle group 1 had adverse clinical signs including soft or liquid feces, ungroomed coat, red snout, red perivaginal substance, red substance in cage pan, body weight loss, and reduced food consumption. Necropsy revealed red and dark red mottled lungs, a red substance in the stomach, and a bi-lobed median lobe of the liver (3.1 cm x 1.7 cm x 1.5 cm). The litter consisted of two dead fetuses and five late resorptions. There was one empty implantation site that was presumed to have been a cannibalized conceptus. Rabbit 8032 in the vehicle group 2 had soft or liquid feces, scant feces, increased body weight, and reduced food consumption. All tissues appeared normal at necropsy. The litter consisted of eight live and three dead fetuses.

Clinical Signs

Clinical signs were observed daily. There were no drug-related clinical signs.

Body Weight

Body weight was recorded daily. BMN-110 had no effects on body weight or body weight gain.

Feed Consumption

Food consumption was recorded daily. BMN 110 had no effects on food consumption.

Toxicokinetics

On GD 20, blood samples were collected prior to dosing and at approximately 10 minutes before the end of the infusion. An ELISA method using an electrochemi-luminescence assay was used to detect rhGALNS in rabbit plasma. The lower limit of quantitation was 75 ng/mL.

On GD 20, the mean plasma concentration of BMN 110 at approximately 10 min before the end of the infusion was 749 ng/mL (range of 248 to 6540 ng/mL) for the 1 mg/kg group, 2620 ng/mL (range of 538 to 6860 ng/mL) for the 3 mg/kg group, and 12,320 ng/mL (range of 444 to 38,700 ng/mL) for the 10 mg/kg group.

Antibody analysis

Blood samples were collected prior to dosing on GD 7 and before euthanasia on GD 29 for analysis of anti-BMN 110 antibodies by immunoassay. All animals in the 1, 3 and 10 mg/kg/day dose groups had detectable antibodies to BMN 110 on GD 29, with the

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exception of one rabbit in the 10 mg/kg/day dose group due to an insufficient sample for analysis.

Dosing Solution Analysis

Concentrations:

Solutions of the test article were prepared at least once daily at the testing facility and then once weekly after stability was established. The dosing solutions were stored at 2 to 8°C. Duplicate samples were taken from the first preparation, at the mid-point of the study period, and on the last day of dosing (0.15, 0.45, and 1.5 mg/ml). All samples (one sample from each preparation) were analyzed within the established stability period (at 5 ± 3°C for 10 days). The mean measured concentrations for all dose formulations ranged from 97.8% to 106% of the nominal concentrations, and were within the acceptable limits (≤ 10% difference from nominal concentration).

Stability:

The study report stated the following: "**Stability data for prepared test article formulations bracketing the range of concentrations in this study are being determined during the conduct of Protocol 0020001917 and will not be determined during the conduct of this study."** No data was submitted.

Homogeneity:

The study report stated the following: "**Homogeneity analyses of the prepared test article formulations will not be conducted. Information is on file with the Sponsor to document the solubility of the test article in the vehicle components over the range of concentrations to be used in this study; this information will be provided to the Study Director.**" No data was submitted.

Necropsy

All surviving rabbits were euthanized on GD 29. Cesarean-section and a gross necropsy of the thoracic, abdominal and pelvic viscera were performed. The median lobe of the liver in each rabbit was excised and retained in neutral buffered 10% formalin for possible future evaluation. Uteri of non-pregnant rabbits were examined for the absence of implantation sites. Uteri and ovaries of apparently non-pregnant rabbits were retained in neutral buffered 10% formalin. Placentae were examined for size, color and shape. The number and distribution of corpora lutea in ovaries, implantation sites, live and dead fetuses, and early and late resorptions in uterus of each rabbit were examined and recorded. The body weight of each fetus was recorded. Fetuses were examined for sex, gross alterations, soft tissue alterations, and skeletal alterations. Approximately, one-half of the fetuses in each litter were examined for soft tissue alterations, and the remaining fetuses were examined for skeletal alterations after staining with Alizarin red S.

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Red, tan, raised, or pitted areas on the liver were observed in all BMN 110-treated groups (1, 2, and 8 females in 1, 3, and 10 mg/kg/day groups, respectively). Two other rabbits at 3 mg/kg/day had tan and red mottled liver. The Sponsor indicated that these findings were considered as drug-related and could be due to the species susceptibility to protein loading and/or to the daily schedule of administration.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Pregnancy occurred in 17 to 20 does in each dose group. Cesarean-section observations were based on 34, 17, 19 and 20 pregnant does with one or more live fetuses on GD 29 in groups 1/2 through 5, respectively. BMN 110 had no effects on corpora lutea, implantations, litter sizes, sex ratio, live or dead fetuses, early and late resorptions, percentage of resorbed conceptuses, or fetal body weights. All placentae appeared normal.

Offspring (Malformations, Variations, etc.)

In groups 1/2, 3, 4, and 5, the number of litters with alterations were 13/34 (38.2%), 7/17 (41.2%), 14/19 (73.7%) and 9/20 (45.0%), respectively. The number of fetuses with alterations was 26 (8.3%), 12 (7.4%), 29 (16.5%) and 12 (6.6%) and the percentage of fetuses with any alteration per litter was 8, 7.5, 17.1 and 7.2 in these same respective dose groups. A significant increase in the number of fetuses with any alterations (16.5% vs. 8.3% in the control group) and in the number of fetuses with skeletal alterations per litter (17.1 ± 15.9% vs. 8.0 ± 12.5% in the control group) was observed in the 3 mg/kg/day group. However, the litter or fetal incidence of soft tissue or skeletal alterations in the 3 mg/kg/day dose group was within the historical control data range, and there was no dose-dependent increase in these alterations. Thus, the soft tissue and skeletal alterations in the 3 mg/kg/day dose group were not considered as drug-related. Fetal gross alterations, soft tissue alterations, and skeletal alterations are summarized in the Sponsor's tables below.

Table 66: Fetal Gross External Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	163	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	1	0	0
EYE(S): BULGE DEPRESSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)

a. Dosing occurred on days 7 through 20 of gestation.
b. Dead fetus and late resorptions were excluded from summarization and statistical analyses. Observations for these conceptuses are cited on Table 22.

Table 67: Fetal Soft Tissue Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	314	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
EYES: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.9)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	1(0.5)
EYES: CIRCUMCORNEAL HEMORRHAGE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
HEART: INTERVENTRICULAR SEPTAL DEFECT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
VESSELS: PERSISTENT TRUNCUS ARTERIOSIS					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)d	0(0.0)
LUNGS: INTERMEDIATE LOBE ABSENT					
LITTER INCIDENCE	N(%)	4(11.8)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	7(2.2)	4(2.5)	0(0.0)**	0(0.0)**
LIVER: EXTRA LOBE					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
LIVER: MISSHAPEN					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	0(0.0)	0(0.0)
GALLBLADDER: ABSENT					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)c	0(0.0)	0(0.0)	0(0.0)

a. Dosing occurred on days 7 through 20 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 22.
c. Fetus 8002-6 had other soft tissue alterations.
d. Fetus 8079-8 had other soft tissue alterations.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table 68: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	0	0	0
SKULL: IRREGULAR OSSIFICATION c (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL d; INDIVIDUAL SUBCATEGORIES CITED BELOW)					
LITTER INCIDENCE	N(%)	4(11.8)	2(11.8)	6(31.6)	3(15.0)
FETAL INCIDENCE	N(%)	6(1.9)	3(1.9)	10(5.7)	4(2.2)
SKULL: NASAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF INTERNASAL, MIDLINE SUTURE DISPLACED AND NASAL-FRONTAL SUTURE IRREGULAR)					
LITTER INCIDENCE	N(%)	3(8.8)	2(11.8)	5(26.3)	2(10.0)
FETAL INCIDENCE	N(%)	5(1.6)	3(1.9)	5(2.8)	3(1.6)
SKULL: NASALS, CONTAIN AN INTERNASAL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.1)i
SKULL: NASALS, MIDLINE SUTURE DISPLACED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.9)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)	0(0.0)	1(0.5)
SKULL: NASAL - FRONTAL, SUTURE IRREGULAR					
LITTER INCIDENCE	N(%)	3(8.8)	1(5.9)	5(26.3)**	0(0.0)
FETAL INCIDENCE	N(%)	5(1.6)	1(0.6)	5(2.8)	0(0.0)
SKULL: FRONTAL(S), IRREGULAR OSSIFICATION (SUMMARIZATION OF FUSED AND INTERFRONTAL)					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	1(5.3)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	2(1.1)	1(0.5)
SKULL: FRONTALS, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	2(1.1)	0(0.0)

** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table 69: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study-Continued

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	0	0	0
SKULL - OTHER ALTERATIONS:					
SKULL: PARIETAL, CONTAINS A HOLE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
SKULL: PARIETAL, CONTAINS AN INTRAPARIETAL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
SKULL: INTERPARIETALS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	4(2.3)**	0(0.0)
SKULL: SUPRAOCCIPITALS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	4(2.3)**	0(0.0)
SKULL: EYE SOCKET, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j
SKULL: ANTERIOR FONTANELLE, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	4(11.8)	1(5.9)	3(15.8)	4(20.0)
FETAL INCIDENCE	N(%)	4(1.3)f	1(0.6)	4(2.3)	4(2.2)k
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)

** Significantly different from the vehicle control group value ($p \leq 0.01$).

Table 70: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study-Continued

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	0	0	0
THORACIC VERTEBRAE: CENTRA, FUSED					
LITTER INCIDENCE	N(%)	1(2.9)	1(5.9)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.3)	1(0.6)g	0(0.0)	1(0.5)k
THORACIC VERTEBRAE: CENTRUM, BIFID					
LITTER INCIDENCE	N(%)	2(5.9)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	2(0.6)	0(0.0)	0(0.0)	2(1.1)k,L
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.1)k,L
THORACIC VERTEBRAE: ARCHES, FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k
THORACIC VERTEBRAE: HEMIVERTEBRA					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
SACRAL VERTEBRAE: ARCH, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k
CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	1(5.3)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.3)f	0(0.0)	1(0.6)	1(0.5)k

Table 71: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study-Continued

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	0	0	0
RIBS: FLAT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j
RIBS: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	2(1.1)k
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.3)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	1(0.5)
RIBS: PROXIMATE					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
RIBS: SPLIT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.9)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)g	0(0.0)	0(0.0)
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	2(10.5)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	6(3.4)**h	1(0.5)j
MANUBRIUM: LARGE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j

** Significantly different from the vehicle control group value (p≤0.01).

Table 72: Fetal Skeletal Alterations (Caesarean-Delivered Live Fetuses) in a Developmental Toxicity Rabbit Study-Continued

DOSE GROUP		I/II	III	IV	V
DOSE (MG/KG/DAY)a		0 (VEHICLE)	1	3	10
LITTERS EVALUATED	N	34	17	19	20
FETUSES EVALUATED	N	315	162	176	183
LIVE	N	314	161	176	183
DEAD b	N	0	1	0	0
LATE RESORPTIONS b	N	1	0	0	0
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	2(5.9)	1(5.9)	4(21.0)	3(15.0)
FETAL INCIDENCE	N(%)	2(0.6)e	1(0.6)	4(2.3)h	3(1.6)i,k
STERNAL CENTRA: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.3)e	0(0.0)	0(0.0)	1(0.5)j
STERNAL CENTRA: LARGE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)j
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.9)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)	0(0.0)	1(0.5)j
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.5)k
XIPHOID: FUSED					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)e	0(0.0)	0(0.0)	0(0.0)
XIPHOID: LARGE					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)e	0(0.0)	0(0.0)	0(0.0)
XIPHOID: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(2.9)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)e	0(0.0)	0(0.0)	0(0.0)

FOOTNOTES:

a. Dosing occurred on days 7 through 20 of gestation.
b. Dead fetus and late resorption were excluded from summarization and statistical analyses. Observations for these conceptuses are cited on Table 22.
c. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.
d. Includes all alterations noted for the skull except hyoid, ala, angulated, small eye socket and irregularly shaped anterior fontanelle. These categories are excluded because these alterations do not result from irregular ossification.
e. Fetus 8007-9 had other skeletal alterations.
f. Fetus 8009-5 had other skeletal alterations.
g. Fetus 8053-7 had other skeletal alterations.
h. Fetus 8072-5 had other skeletal alterations.
i. Fetus 8082-2 had other skeletal alterations.
j. Fetus 8089-6 had other skeletal alterations.
k. Fetus 8093-1 had other skeletal alterations.
L. Fetus 8093-7 had other skeletal alterations.

In conclusion, time-mated rabbits were administered BMN 110 via intravenous infusion daily at doses of 1, 3 and 10 mg/kg through gestation days 7 to 20. Tan, raised, or pitted areas or tan and red mottled areas were observed in liver of does in the 1, 3, and 10 mg/kg/day groups. Thus, the maternal NOAEL was < 1 mg/kg/day based on the gross changes in liver. BMN 110 had no effects on embryo-fetal development. Therefore, the embryo-fetal developmental NOAEL was 10 mg/kg/day/day.

9.3 Prenatal and Postnatal Development

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of BMN 110 by Intravenous Injection in Rats

Study no.:	BMN110-12-013
Study report location:	N/A
Conducting laboratory and location:	(b) (4) [redacted] TK and antibody analysis: BioAnalytical Testing BioMarin Pharmaceutical Inc. Novato, CA
Date of study initiation:	March 27, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	P4014-10009 specific activity: 5 U/mg purity: 99%

Key Study Findings

In this pre-/postnatal developmental study, rats were administered BMN 110 daily at doses of 1, 6 and 20 mg/kg IV from gestation day (GD) 7 through lactation day (LD) 20. Diphenhydramine (DPH) was administered intraperitoneally at 10 to 20 minutes prior to

Reference ID: 3396720

BMN 110 or control article administration to prevent anaphylactic reactions. A small number of deaths occurred during pregnancy in the 1, 6, and 20 mg/kg/day + DPH groups. However, mortality was not dose dependent, and 3/7 deaths were preceded by signs of anaphylaxis or DPH toxicity. Given the small number and dubious nature of these deaths, the interpretation of the offspring observations is not substantially impacted, although the possible involvement of maternal toxicity in the adverse effects in offspring cannot be excluded. Treatment of F0 females with 20 mg/kg/day BMN 110 + DPH produced a reduction in body weight (up to 3.7%) and body weight gain (up to 21%) during pregnancy, compared to the DPH alone group. BMN 110 at doses of 6 and 20 mg/kg/day significantly decreased food consumption in dams (F0 generation) (up to 12%↓ and 23.5%↓, respectively) during gestation. Treatment with DPH alone produced a reduction in litter size. The 6 and 20 mg/kg/day BMN 110 + DPH groups had a statistically significant decrease in the percentage of liveborn fetuses and a statistically significant increase in the percentage of stillbirths, compared to the DPH alone group. These effects were dose-dependent. The increase in percentage of stillbirths at 6 and 20 mg/kg/day exceeded the range of historical control values from the testing laboratory. The percent survival of pups during postpartum days 1-4 (viability index) was significantly reduced in the 20 mg/kg/day BMN 110 + DPH group (94.4% compared to 98.7% in the DPH alone group). The reduction in viability index was correlated with a statistically significant increase in pup mortality during postpartum days 2-4 at 20 mg/kg/day (3.8% compared to 0.6% in the DPH alone group). There were no BMN 110 treatment-related gross lesions in F0 or F1 generations. All F0 females in the BMN 110 + DPH treatment groups were positive for anti-drug antibodies. BMN 110 was detected in milk of F0 dams treated at doses of 6 and 20 mg/kg/day, with a dose-dependent incidence. Plasma drug concentration was approximately 1.6-3 times lower on LD 14 compared to GD 7. The maternal NOAEL was 6 mg/kg/day IV based on significantly reduced body weight gain and reduced food consumption at 20 mg/kg/day. The NOAEL for pre- and post-postnatal development was 1 mg/kg/day IV, based on the increase in percentage of stillbirths at 6 and 20 mg/kg/day, and the increase in postnatal deaths at 20 mg/kg/day.

F1 generation:

Body weight, body weight gain, food consumption, sexual maturity (the age of preputial separation and vaginal patency), and learning and memory functions were not adversely affected by maternal treatment with BMN 110. The reproductive parameters for the F1 generation (mating and fertility, C-section and litter parameters, F2 generation survival, and external evaluation) were not affected by BMN 110 treatment. Although not statistically significant, a reduction in pregnancy rate was observed in the offspring of the maternal 20 mg/kg/day BMN 110 + DPH group. It is noted that several developmental tests to evaluate behavior, locomotor activity, sensory functions, and reflex development (e.g. static righting reflex, cliff aversion, startle response) were not conducted. Since the omitted tests are routinely included in pre- and postnatal developmental studies, the evaluation of F1 generation development was suboptimal in this study, and this should be conveyed in the Pregnancy section of the labeling.

Reference ID: 3396720

Methods

Doses:	1, 6, and 20 mg/kg
Frequency of dosing:	Daily
Dose volume:	6.9 ml/kg
Route of administration:	IV bolus injection (lateral tail vein)
Formulation/Vehicle:	BMN 110 was formulated in vehicle (20 mM sodium acetate, 50 mM sodium phosphate, 30 mM arginine hydrochloride, 2% sorbitol, 0.01% polysorbate 20, pH 5.4) at nominal concentrations of 0.15, 0.87, and 2.9 mg/ml.
Species/Strain:	Sprague-Dawley rats [Crl:CD(SD)]
Number/Sex/Group:	25 time-mated females/group
Satellite groups:	TK: 3-6 females/group
Study design:	See table below from study report
Deviation from study protocol:	Minor deviations had no impact on the outcome of the study.

F0 generation:

F0 generation female rats were administered vehicle, DPH (intraperitoneal), and BMN 110 (IV bolus) once daily on GD (gestation day) 7 through day 20 postpartum (rats assigned to natural delivery that delivered a litter) or GD 24 (rats assigned to natural delivery that did not deliver a litter). The study design is summarized in the Sponsor's table below.

Table 73: Study Design for Peri-/Postnatal Developmental Toxicity Study in Rats (F0 Generation)

Treatment Group[a]	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals Main Study	No. of Animals TK Study
Vehicle (1)	0	6.9	0	25F	3F
Vehicle + DPH (2)[b,c]	0	6.9	0	25F	6F
Low (3)[c]	1	6.9	0.15	25F	6F
Mid (4)[c]	6	6.9	0.87	25F	6F
High (5)[c]	20	6.9	2.90	25F	6F

DPH: Diphenhydramine

[a] BMN 110 lot P4014-10009; Vehicle (20 mM sodium acetate, 50 mM sodium phosphate, 30 mM arginine hydrochloride, 2% sorbitol, 0.01% polysorbate 20, pH5.4) prepared at CRL.

[b] A additional vehicle group was added to the study to control for diphenhydramine (DPH) administration.

[c] The animals received DPH , intraperitoneally at 10 mg/kg, 10 to 20 minutes prior to BMN 110 administration.

F1 generation:

F1 generation pups were not directly dosed with the test or control articles, but may have been exposed to the test and/or control articles during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period.

Group No.	No. of F1 Generation Rats/Sex	Maternal Test Material	Maternal Dose Level (mg/kg/day)	Assigned Animal Numbers	
				Males	Females
1	25	Control Article	0	801-825	1001-1025
2	25	Control Article/DPH	0/10	826-850	1026-1050
3	25	BMN 110/DPH	1/10	851-875	1051-1075
4	25	BMN 110/DPH	6/10	876-900	1076-1100
5	25	BMN 110/DPH	20/10	901-925	1101-1125

Observations and Results

F0 dams:

Survival:

There were a total of 7 unscheduled deaths. All deaths occurred in the BMN 110 + DPH treatment groups (1, 4 and 2 dams in the 1, 6 and 20 mg/kg/day BMN 110 groups, respectively). Three dams were euthanized on GDs 16, 17, and 24. Three dams were found dead on GDs 14 and 20. One dam was aborted and euthanized on GD 19.

On GD 24, one dam (#9062) in the 1 mg/kg/day BMN 110 + DPH dose group had not completed delivery and was euthanized due to adverse clinical observations that were secondary to the difficult parturition. Clinical signs and necropsy findings included dehydration, cold to touch, bradypnea, thin body condition, hunched posture, hyperpnea, whole body pale, cloudy red fluid in abdominal cavity, and abdominal adipose and pancreas adhered to left horn of uterus during GDs17-24.

One dam (#9076) in the 6 mg/kg/day BMN 110 + DPH group was aborted and euthanized on GD 19. This dam had soft or liquid feces, mucoid feces, red perivaginal substance, chromorhinorrhea, and mild dehydration during GDs 18-19.

Two dams (# 9097 and 9098) in the 6 mg/kg/day BMN 110 + DPH group on GDs 14-17 and one dam (#9123) in the 20 mg/kg/day BMN 110 + DPH group on GDs 15-16 had clinical observations indicative of anaphylactic reactions following BMN 110 administration (i.e. pale body, ears and extremities and decreased motor activity) or DPH-related toxicity (i.e. ptosis, bradypnea and ataxia).

One dam (#9083) in the 6 mg/kg/day BMN 110 + DPH group and one dam (#9122) in the 20 mg/kg/day BMN 110 + DPH group were found dead on GD 20. Anaphylactic

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reactions due to BMN 110 administration or DPH-related toxicity were not observed in these two dams. Red fluid in abdominal cavity and numerous black areas on mucosal surface in stomach pyloric region were observed in dam # 9083. The uterine contents for these dams are summarized in the table below.

Table 74: Uterine Contents for Unscheduled Deaths

Parameters	Groups						
	3	4				5	
Dam #	9062	9076	9083	9097	9098	9122	9123
Day of death	GD 24	GD 19	GD 20	GD 14	GD 17	GD 20	GD 16
Mode of death	UE	AE	FD	FD	UE	FD	UE
Uterine contents	4A, 1B, 4L, 4M	12L	13L	16U	14L	12D, 1L	10U, 1E

GD = Gestation Day
UE = Unscheduled Euthanasia
FD = Found Dead
AE = Aborted and Euthanized
A = Alive, delivered
B = Born alive, found dead
D = Dead fetus
L = Late Resorption
M = Missing, presumed cannibalized
U = Viability could not be determined due to the death of the dam

The Sponsor provided the following assessment of these deaths: (b) (4)

The Sponsor's conclusion is not acceptable. (b) (4)

Clinical signs:

Gestation period: Clinical signs described in the mortality section were observed in unscheduled sacrificed dams during the gestation period. These clinical signs were likely related to co-administration of DPH and BMN 110 or DPH alone. A significantly increased number of rats with sparse hair coats on limbs in the 1 mg/kg/day BMN 110 + DPH dose group (5 rats vs. 1 rat in the vehicle control group) was not considered as related to BMN 110 because of the lack of dose dependency.

Lactation period: A significantly increased number of rats with sparse hair coats was observed in all groups treated with DPH only or DPH in combination with BMN 110, compared to animals treated with vehicle only (group 1). However, the number of animals with sparse hair coats was comparable among animals treated with DPH only or DPH + BMN 110. Thus, this change was likely related to DPH, and not BMN 110.

Scabs at the injection site occurred at a higher frequency in all groups administered DPH compared to vehicle control group (group 1). However, the incidence of scabs at the injection site was comparable between animals treated with DPH only and animals treated with DPH combined with BMN 110. Thus, this change is not likely to be related to BMN 110.

Body Weight and Body Weight Changes (gestation period):

Overall, dams treated with DPH alone or DPH + BMN 110 had slightly decreased body weight (up to 6.1%↓ in the 20 mg/kg/day BMN 110 + DPH group on GD 20) during gestation compared to the vehicle control dams. Dams in the DPH + BMN 110 treatment groups had lower body weights compared to the DPH alone group. Dams in the 20 mg/kg/day BMN 110 + DPH group had the largest average reduction of body weight, although the reduction did not reach statistical significance (up to 3.7% on GD 17 compared to the DPH alone group).

Body weight gain in all groups administered DPH in combination with BMN 110 was lower than the vehicle alone group and the DPH alone group during gestation (up to 29%↓ and 21%↓, respectively). In addition, body weight gain was significantly reduced (up to 21%↓ $p \leq 0.01$) on GDs 15 to 18 in the 20 mg/kg/day BMN 110 + DPH dose group compared to the DPH alone group. BMN 110 at 20 mg/kg/day + DPH produced a decrease in body weight and body weight gain, compared to DPH treatment alone. The body weight gains in dams are summarized in the Sponsor's table below.

Reference ID: 3396720

Table 75: F0 Body Weight Gains during Gestation

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 0 - 7	MEAN±S.D.	+46.6 ± 8.3	+46.8 ± 9.4	+43.6 ± 9.8	+44.1 ± 8.7	+44.0 ± 8.5
DAYS 7 - 10	MEAN±S.D.	+14.3 ± 5.9	+11.6 ± 4.7	+13.2 ± 5.8	+13.8 ± 7.8	+12.0 ± 6.6
DAYS 10 - 12	MEAN±S.D.	+11.7 ± 3.7	+9.8 ± 5.0	+9.9 ± 3.4	+10.8 ± 7.7	+10.5 ± 4.1
DAYS 12 - 15	MEAN±S.D.	+20.0 ± 5.3	+21.8 ± 5.0	+18.2 ± 4.8	+18.0 ± 5.3 [24]b	+19.0 ± 6.0
DAYS 15 - 18	MEAN±S.D.	+38.0 ± 8.0	+34.1 ± 6.0	+32.1 ± 7.4	+30.1 ± 9.2 [23]b	+27.0 ± 11.5** [21]b
DAYS 18 - 20	MEAN±S.D.	+29.0 ± 5.8	+23.9 ± 8.7# [24]c	+21.7 ± 8.2	+24.5 ± 9.8 [21]b	+23.1 ± 8.7 [20]b
DAYS 7 - 20	MEAN±S.D.	+113.0± 13.1	+101.3± 15.0## [24]c	+95.1 ± 17.5	+99.2 ± 17.7 [21]b	+90.6 ± 18.7 [20]b
DAYS 0 - 20	MEAN±S.D.	+159.6± 15.0	+148.4± 17.7# [24]c	+138.8± 20.4	+144.3± 20.7 [21]b	+135.4± 20.5 [20]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion.
c. Excludes values for dams that were in the process of delivering or had delivered.
Significantly different from the Group 1 value (p≤0.05).
Significantly different from the Group 1 value (p≤0.01).
** Significantly different from the Group 2 value (p≤0.01).

Body Weight and Body Weight Changes (lactation period):

Body weight and body weight gain during the lactation period were generally comparable among the study groups. A statistically significant (p≤0.05) increase in body weight gain on lactation days (LDs) 10 to 14 occurred in the 6 and 20 mg/kg/day BMN 110 + DPH groups. However, this effect was transient and therefore was not considered as related to BMN 110 or DPH. The body weight changes are summarized in the Sponsor's table below.

Table 76: F0 Body Weight Gains during Lactation

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
INCLUDED IN ANALYSES	N	24	25	24	21b	20b
DELIVERED A LITTER	N	24	25	23c	21	20
MATERNAL BODY WEIGHT CHANGE (G)						
DAYS 1 - 4	MEAN±S.D.	-0.6 ± 14.3	-2.1 ± 12.4	+0.6 ± 14.1	+1.1 ± 11.7	-0.4 ± 10.0
DAYS 4 - 7	MEAN±S.D.	+22.4 ± 12.4	+20.9 ± 9.3	+19.5 ± 13.8	+18.5 ± 8.8	+16.2 ± 7.9
DAYS 7 - 10	MEAN±S.D.	+11.7 ± 10.2	+11.4 ± 10.0	+9.9 ± 12.5 [22]d	+11.3 ± 10.8	+7.8 ± 9.9 [19]d
DAYS 10 - 14	MEAN±S.D.	+6.5 ± 11.8	+4.7 ± 8.7	+8.1 ± 9.6 [22]d	+12.5 ± 11.3*	+12.5 ± 10.0* [19]d
DAYS 14 - 21	MEAN±S.D.	+12.0 ± 15.2	+15.4 ± 13.6	+14.3 ± 14.6 [22]d	+12.5 ± 16.1	+16.7 ± 15.5 [19]d
DAYS 1 - 21	MEAN±S.D.	+52.1 ± 19.8	+50.2 ± 19.6	+54.8 ± 18.5 [22]d	+55.9 ± 18.6	+53.3 ± 15.4 [19]d

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion during the gestation period.
c. Dam 9062 partially delivered a litter and was euthanized on Day 24 of gestation.
d. Excludes values for dams that were euthanized due to no surviving pups.
* Significantly different from the Group 2 value (p≤0.05).

Food Consumption

Gestation period:

Absolute (g/day) and relative (g/kg/day) food consumption in dams in the DPH alone group were not significantly different from the vehicle group.

In general, absolute food consumption was comparable among the vehicle, DPH alone, and 1 mg/kg/day BMN 110 + DPH groups. Absolute food consumption was significantly reduced ($p \leq 0.01$) in the 20 mg/kg/day BMN 110 + DPH group during the GDs 7 to 20 (↓9.6%) and GDs 0 to 20 (↓7%), compared to the DPH alone group. Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) of absolute food consumption also occurred in the 6 and 20 mg/kg/day BMN 110 + DPH groups during GDs 15 to 18 compared to the DPH alone group (↓12% and ↓23.5%, respectively).

DPH treatment had no effects on relative food consumption during gestation. Relative food consumption was slightly reduced ($p \leq 0.05$ or $p \leq 0.01$) in all groups administered DPH in combination with BMN 110 for GDs 0 to 20 and 7 to 20, compared to the DPH alone group. The largest reduction in relative food consumption occurred in the 20 mg/kg/day BMN 110 + DPH group. Thus, reduced food consumption during the gestation period is related to BMN 110 treatment. The changes in food consumption are summarized in the Sponsor's tables below.

Table 77: Absolute Food Consumption (g/day) in F0 Dams during Gestation

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
MATERNAL FOOD CONSUMPTION (G/DAY)						
DAYS 0 - 7	MEAN±S.D.	21.6 ± 1.4	22.4 ± 3.0	21.2 ± 1.9	21.5 ± 1.6	21.7 ± 2.1
DAYS 7 - 10	MEAN±S.D.	22.2 ± 1.9	21.4 ± 1.9	21.5 ± 2.4	20.8 ± 3.0	20.8 ± 2.5
DAYS 10 - 12	MEAN±S.D.	24.2 ± 1.8	23.2 ± 2.4	22.7 ± 3.4	23.6 ± 3.0	23.3 ± 2.2
DAYS 12 - 15	MEAN±S.D.	24.0 ± 1.8	24.3 ± 2.3	22.7 ± 4.6	22.7 ± 2.2 [24]b	23.1 ± 2.6
DAYS 15 - 18	MEAN±S.D.	24.4 ± 3.3	23.8 ± 3.4	21.7 ± 5.8	20.9 ± 3.6* [23]b	18.2 ± 3.6** [21]b
DAYS 18 - 20	MEAN±S.D.	23.4 ± 3.1	21.8 ± 3.6	21.4 ± 3.6	21.2 ± 4.8 [21]b	19.4 ± 4.3 [20]b
DAYS 7 - 20	MEAN±S.D.	23.6 ± 1.6	23.0 ± 1.8	22.0 ± 2.6	22.1 ± 2.1 [21]b	20.8 ± 2.0** [20]b
DAYS 0 - 20	MEAN±S.D.	22.9 ± 1.2	22.8 ± 1.8	21.7 ± 2.1	22.0 ± 1.7 [21]b	21.2 ± 1.7** [20]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion.
* Significantly different from the Group 2 value (p≤0.05).
** Significantly different from the Group 2 value (p≤0.01).

Table 78: Relative Food Consumption (g/kg/day) in F0 Dams during Gestation

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
MATERNAL FOOD CONSUMPTION (G/KG/DAY)						
DAYS 0 - 7	MEAN±S.D.	86.2 ± 4.8	89.5 ± 10.7	85.4 ± 7.0	86.2 ± 5.2	87.0 ± 5.9
DAYS 7 - 10	MEAN±S.D.	79.3 ± 6.1	77.0 ± 5.2	77.9 ± 6.5	75.1 ± 8.6	75.6 ± 8.0
DAYS 10 - 12	MEAN±S.D.	82.4 ± 5.3	79.8 ± 8.2	78.4 ± 9.5	81.1 ± 9.1	80.6 ± 4.2
DAYS 12 - 15	MEAN±S.D.	77.5 ± 5.9	79.4 ± 6.3	75.0 ± 15.2	74.4 ± 6.5* [24]b	76.5 ± 7.9
DAYS 15 - 18	MEAN±S.D.	72.1 ± 9.5	71.4 ± 8.6	66.3 ± 16.9	63.1 ± 9.5* [23]b	56.2 ± 11.0** [21]b
DAYS 18 - 20	MEAN±S.D.	63.0 ± 7.9	60.0 ± 8.7 [24]c	60.0 ± 7.0	58.2 ± 11.6 [21]b	55.2 ± 11.4 [20]b
DAYS 7 - 20	MEAN±S.D.	74.4 ± 4.8	73.7 ± 3.5 [24]c	71.2 ± 6.2	70.1 ± 4.8* [21]b	67.6 ± 5.5** [20]b
DAYS 0 - 20	MEAN±S.D.	73.6 ± 3.2	74.4 ± 3.7 [24]c	71.6 ± 4.4*	71.0 ± 3.9** [21]b	70.0 ± 3.9** [20]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion.
c. Excludes values for dams that were in the process of delivering or had delivered.
* Significantly different from the Group 2 value (p≤0.05).
** Significantly different from the Group 2 value (p≤0.01).

Lactation period:

Food consumption was evaluated from LD 1 to 14, because it was assumed that pups started consuming maternal food after day 14 of lactation.

Compared to vehicle and DPH alone groups (1 and 2, respectively), the 20 mg/kg/day BMN 110 + DPH dams exhibited slight reductions (not significant) in absolute and relative food consumption during the lactation period (↓6% on LDs 1-14, and ↓12% on LDs 4-7).

Table 79: Absolute Food Consumption (g/day) in F0 Dams during Lactation Period

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
INCLUDED IN ANALYSES	N	24	25	24	21b	20b
DELIVERED A LITTER	N	24	25	23c	21	20
MATERNAL FOOD CONSUMPTION (G/DAY)						
DAYS 1 - 4	MEAN±S.D.	30.5 ± 6.1 [23]d	28.6 ± 5.5	29.1 ± 6.7	30.7 ± 4.8	28.9 ± 4.7
DAYS 4 - 7	MEAN±S.D.	49.5 ± 6.4	46.1 ± 6.7	42.8 ± 9.9	45.4 ± 6.7	40.8 ± 7.6
DAYS 7 - 10	MEAN±S.D.	60.9 ± 6.1 [23]d	57.3 ± 8.4	55.6 ± 8.4 [22]e	56.8 ± 8.4	53.1 ± 7.0 [19]e
DAYS 10 - 14f	MEAN±S.D.	61.4 ± 13.3	62.2 ± 7.5	60.0 ± 7.4 [22]e	62.5 ± 8.7 [20]d	58.0 ± 8.6 [19]e
DAYS 1 - 14f	MEAN±S.D.	51.2 ± 6.1	49.6 ± 6.0	48.4 ± 6.6 [22]e	50.2 ± 6.8 [20]d	46.6 ± 5.7 [19]e

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion during the gestation period.
c. Dam 9062 partially delivered a litter and was euthanized on Day 24 of gestation.
d. Excludes values that could not be calculated as well as those associated with spillage.
e. Excludes values for dams that were euthanized due to no surviving pups.
f. Because it is presumed that the pups begin to consume maternal food after day 14 of lactation, maternal food consumption values were not tabulated on days 14 to 21 of lactation.

Table 80: Relative Food Consumption (g/kg/day) in F0 Dams during Lactation Period

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS TESTED	N	25	25	25	25	25
PREGNANT	N	24	25	24	25	22
INCLUDED IN ANALYSES	N	24	25	24	21b	20b
DELIVERED A LITTER	N	24	25	23c	21	20
MATERNAL FOOD CONSUMPTION (G/KG/DAY)						
DAYS 1 - 4	MEAN±S.D.	103.2 ± 19.5 [23]d	98.9 ± 18.4	101.5 ± 23.4	104.2 ± 15.2	98.6 ± 13.2
DAYS 4 - 7	MEAN±S.D.	159.0 ± 14.7	152.9 ± 16.6	143.1 ± 31.0	148.7 ± 19.6	135.1 ± 20.9
DAYS 7 - 10	MEAN±S.D.	187.4 ± 14.1 [23]d	180.4 ± 19.6	176.8 ± 22.4 [22]e	176.4 ± 22.4	167.4 ± 19.6 [19]e
DAYS 10 - 14f	MEAN±S.D.	184.8 ± 40.5	190.4 ± 16.4	185.5 ± 18.8 [22]e	188.6 ± 20.7 [20]d	178.0 ± 22.6 [19]e
DAYS 1 - 14f	MEAN±S.D.	161.2 ± 18.0	160.0 ± 14.4	157.5 ± 19.0 [22]e	159.6 ± 17.9 [20]d	149.6 ± 15.2 [19]e

DAYS = DAYS OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion during the gestation period.
c. Dam 9062 partially delivered a litter and was euthanized on Day 24 of gestation.
d. Excludes values that could not be calculated as well as those associated with spillage.
e. Excludes values for dams that were euthanized due to no surviving pups.
f. Because it is presumed that the pups begin to consume maternal food after day 14 of lactation, maternal food consumption values were not tabulated on days 14 to 21 of lactation.

Uterine contents

Litter Observations (Naturally Delivered Pups):

A total of 24, 25, 24, 25 and 22 dams were pregnant in groups 1, 2, 3, 4, and 5, respectively. The number of females delivering was 24, 25, 24, 21, and 20 in the same respective dose groups. Some rats in groups 4 and 5 died during the gestation period.

Treatment with DPH alone produced a reduction in litter size. The 6 and 20 mg/kg/day BMN 110 + DPH groups had a statistically significant decrease in the percentage of liveborn fetuses and a significant increase in the percentage of stillbirths, compared to the DPH alone group. These effects were dose-dependent. The percent survival in pups during postpartum days 1-4 was significantly reduced in the 20 mg/kg/day BMN 110 + DPH group (94.4% compared to 98.7% in the DPH alone group).
The natural delivery observations are summarized in the Sponsor's tables below.

Table 81: Natural Delivery Observation –F0 Generation Females

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25	25
PREGNANT	N(%)	24(96.0)	25(100.0)	24(96.0)	25(100.0)	22(88.0)
INCLUDED IN ANALYSES	N	24	25	24	21b	20b
DELIVERED A LITTER	N(%)	24(100.0)	25(100.0)	23(100.0)c	21(100.0)	20(100.0)
DURATION OF GESTATION d	MEAN±S.D.	22.1 ± 0.4	22.4 ± 0.6#	22.6 ± 0.5	22.5 ± 0.6	22.4 ± 0.5
IMPLANTATION SITES	N	349	361	323	299	287
PER DELIVERED LITTER	MEAN±S.D.	14.5 ± 1.8	14.4 ± 2.0	14.0 ± 2.1	14.2 ± 2.0	14.4 ± 1.9
DAMS WITH STILLBORN PUPS	N(%)	2(8.3)	3(12.0)	5(21.7)	6(28.6)	6(30.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX e	%	100.0	100.0	100.0	84.0	90.9
	N/N	24/ 24	25/ 25	23/ 23	21/ 25	20/ 22
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	1(4.3)	0(0.0)	1(5.0)

a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations or abortion during the gestation period.
c. Excludes dam 9062, which partially delivered a litter and was euthanized on Day 24 of gestation.
d. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the day the first pup was delivered.
e. Number of rats with live offspring/number of pregnant rats.
Significantly different from the Group 1 value (p≤0.05).

Table 82: Natural Delivery Observation –F1 Generation Litters

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
BMN 110 DOSE LEVEL (MG/KG/DAY)a		0	0	1	6	20
DPH DOSE LEVEL (MG/KG/DAY)a		-	10	10	10	10
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	25	23	21	20
PUPS DELIVERED (TOTAL)	N	332	312	277	269	226
	MEAN±S.D.	13.8 ± 1.5	12.5 ± 2.4#	12.0 ± 2.5	12.8 ± 2.2	11.3 ± 2.8
LIVEBORN	MEAN±S.D.	13.7 ± 1.6	12.3 ± 2.5#	11.7 ± 2.6	12.1 ± 2.8	10.6 ± 3.3
	N(%)	328(98.8)	308(98.7)	270(97.5)	254(94.4)**	213(94.2)**
STILLBORN	MEAN±S.D.	0.1 ± 0.4	0.2 ± 0.5	0.3 ± 0.6	0.5 ± 1.0	0.6 ± 1.1
	N(%)	3(0.9)	4(1.3)	7(2.5)	11(4.1)**	13(5.8)**
UNKNOWN VITAL STATUS b	N	1	0	0	4	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1	N/N(%)	0/328(0.0)	2/308(0.6)	3/270(1.1)	0/254(0.0)	4/213(1.9)
DAYS 2- 4	N/N(%)	1/328(0.3)	2/306(0.6)	0/267(0.0)	6/254(2.4)	8/209(3.8)**
DAYS 5- 7	N/N(%)	1/327(0.3)	1/304(0.3)	16/267(6.0)**	0/248(0.0)	1/201(0.5)
DAYS 8-14	N/N(%)	2/326(0.6)	0/303(0.0)	0/251(0.0)	0/248(0.0)	2/200(1.0)**
DAYS 15-18	N/N(%)	2/324(0.6)	1/303(0.3)	1/251(0.4)	0/248(0.0)	0/198(0.0)
DAYS 19-21	N/N(%)	0/322(0.0)	0/302(0.0)	0/250(0.0)	0/248(0.0)	0/198(0.0)
VIABILITY INDEX c	%	99.7	98.7	98.9	97.6	94.4**
	N/N	327/328	304/308	267/270	248/254	201/213
LACTATION INDEX d	%	98.5	99.3	93.6**	100.0	98.5
	N/N	322/327	302/304	250/267	248/248	198/201

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Maternal cannibalization precluded identification of vital status at birth.
c. Number of live pups on Day 4 postpartum/number of liveborn pups on Day 1 postpartum.
d. Number of live pups on Day 21 (weaning) postpartum/number of live pups on Day 4 postpartum.
Significantly different from the Group 1 value (p≤0.05).
** Significantly different from the Group 2 value (p≤0.01).

Necropsy observations

F0 Generation:

One female in the 1 mg/kg/day BMN 110 + DPH group (# 9068) had four lesions at scheduled necropsy: the pancreas was adhered to the left horn of the uterus; the left horn of uterus was adhered to the abdominal wall; uterus contained a green caseous material and two late resorptions. Other lesions occurred in dams that that died prematurely, which were described above in the F0 Survival section. No gross lesions were considered related to BMN 110 because none occurred in more than one or two dams in any group.

F1 Generation Pups

There were no gross lesions related to DPH or BMN 110 in pups found dead or pups that survived to scheduled euthanasia. Among the pups found dead, the number of pups with no milk in the stomach was significantly increased (p≤0.01) and the number of pups that appeared normal were significantly decreased (p≤0.01) in the 1 mg/kg/day BMN 110 + DPH group, compared to the DPH alone group. These changes were not considered as BMN 110-related because of the lack of dose-dependency.

Toxicokinetics

Blood samples were collected from each F0 generation rat in the TK groups at approximately 1 minute post dose on GD 7 and LD 14. Plasma concentrations of test article (BMN 110) were measured by the BioAnalytical Testing department at BioMarin (Novato, CA) using a validated electrochemiluminescence (ECLA) method. The lower limit of quantitation was 100 ng/mL.

BMN 110 was detected in all F0 females on GD 7 and LD 14, except for one female in the 1 mg/kg/day group on LD 14. On GD 7, the mean concentrations of BMN 110 in rat plasma were 22,333 ± 924 ng/mL, 161,167 ± 15,105 ng/mL and 539,000 ± 38,283 ng/mL for the 1, 6 and 20 mg/kg/day dose levels, respectively. On LD 14, the mean concentrations were 7695 ± 9031 ng/mL, 100,983 ± 16,780 ng/mL and 335,400 ± 109,372 ng/mL for the 1, 6 and 20 mg/kg/day dose levels, respectively. The plasma concentration of BMN 110 was approximately 1.6-3 times lower on LD 14 compared to GD 7.

Dosing Solution Analysis

Concentrations:

The dosing formulations were prepared at least once every 10 days, stored at 2 to 8°C, and dispensed daily. Analysis of the dose formulations occurred prior to use of the formulations for dose administration. During the study, duplicate middle samples for analysis were taken from each formulation (including the control article) prepared for the initial dosing, the third dose, and the final dose. The mean measured concentrations for all dose formulations ranged from 98.4% to 106% of the nominal concentrations (0.15, 0.87, and 2.9 mg/ml), and were within the acceptable limits (≤ 10% difference from nominal concentration). The concentrations of dose formulations were acceptable.

Stability:

The Sponsor stated the following: "**Stability analyses performed previously in conjunction with** (b) (4) **Study No. 20001917 demonstrated that the test article is stable in the control article when prepared and stored under the same conditions at concentrations bracketing those used in the present study. Stability data have been retained in the study records for 20001917. Prior to study commencement, stability data were generated by the** (b) (4) **for 10 days for formulations stored at 2°C to 8°C over the concentration range 0.070 to 3.2 mg/mL under Study No. 0020001917."** No data was submitted.

Homogeneity:

The study report stated the following: "**The Sponsor has provided data that demonstrate that the test article is soluble in the control article when prepared under the same mixing conditions at concentrations bracketing those used in the present study**". No data was submitted.

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Other

Anti-BMN 110 antibody analysis:

Blood samples were collected from each F0 generation rat in the main study groups at one day before termination. Samples were analyzed to determine the titer of antibodies to BMN 110 using a bridging electrochemiluminescence assay.

All F0 females in the BMN 110 treatment groups were positive for anti-BMN 110 antibodies. However, the results of the titer assay showed an inverse correlation between dose and titer levels. Higher anti-drug antibody (ADA) titers occurred in the lowest dose group (1 mg/kg/day BMN 110, mean titer = 1,434,134), while samples from the higher dose groups had lower titer values (6 mg/kg/day, mean titer = 176,462; 20 mg/kg/day, mean titer = 85,719). The Sponsor provided the following comment: "**this assay is sensitive to the presence of BMN110 in serum samples: BMN110 concentrations > 10 ng/mL in neat serum interfered with the detection of anti-drug antibody (ADA) when tested in the assay method validation BMN110-12-031.**" Thus, the Sponsor provided the following evaluation of the anti-BMN 110 titer data: "**the data suggest that high doses of BMN 110 may dampen total anti-BMN 110 antibody responses, it is also possible that this effect was an artifact of interference from BMN 110 in the serum samples for the higher dose groups. In the absence of BMN 110 concentration data for these samples, interference from the free drug in the serum cannot be ruled out. Therefore, the relative ADA levels observed for each group may not be accurate."**

Milk Analysis:

Milk samples (at least 100 µl per dam) were collected from the first 5 main study F0 generation rats per group (rats with a litter) on LD 14 at approximately 0.5 hours post-dose. An ELISA method using electrochemiluminescence (ECLA) was used to detect BMN 110 (rhGALNS) in rat milk. The minimum level of quantitation was 60 ng/ml.

No BMN 110 was detected in milk samples from dams administered 1 mg/kg/day. However, BMN 110 was detected in one of five samples from dams administered 6 mg/kg/day (132 ng/ml) and in four of five dams administered 20 mg/kg/day (102 - 212 ng/ml). Thus, there was a dose-dependent increase in the number of rats with detectable levels of BMN 110 in milk.

F1 Generation

All F1 generation rats were weaned on day 21 postpartum. At weaning, a table of random units was used to select 25 male and 25 female pups per group, resulting in a total of 250 F1 generation rats (125 per sex) chosen for continued evaluation of sexual maturation (vaginal opening, and preputial separation), behavior (learning and memory), and reproductive and fertility parameters. The latter was evaluated by mating animals within treatment groups (1 male: 1 female) at approximately 90 days of age. Mated F1

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females were euthanized on GD 21 and evaluated for number of corpora lutea, implantation sites, placentae (size, color or shape), live and dead fetuses, and resorptions. After completion of the 16-day cohabitation period, all surviving F1 male generation rats were euthanized. All F1 animals were subjected to a complete necropsy and examined for gross lesions.

Survival (males):

There were five unscheduled sacrifices (3, 1, and 1 F1 males in the 1, 6, and 20 mg/kg/day BMN 110 + DPH maternal dose groups, respectively). Three males in group 3 (1 mg/kg/day) and one male in group 5 (20 mg/kg/day) were euthanized on postpartum days 81, 84, 92 and 24, respectively, due to adverse clinical signs. One male in group 4 (6 mg/kg/day) was found dead on postpartum day 67. All three males in group 3 had chromodacryorrhea, chromorhinorrhea, and swollen snout. Two of them had broken palate and one had a hole in palate. One male had dark red lobes in the lungs. The male in group 4 did not show adverse clinical signs, and had no macroscopic findings. In the group 5 male, necropsy revealed a distended urinary bladder, fluid-filled cysts on the right kidney that extended into the parenchyma, and slight pelvic dilation in both kidneys. The deaths do not appear to be treatment-related.

Survival (females):

There were four unscheduled sacrifices (1 and 3 F1 females in the 6 and 20 mg/kg/day BMN 110 + DPH maternal dose groups, respectively). The 4 F1 females were euthanized on GDs 16, 17, and 21, and postpartum day 75. None of the unscheduled sacrifices were treatment-related since the reasons for euthanasia were due to early delivery (F #1104), mis-timed pregnancy (F #1123), ruptured right eye and exophthalmos (F #1122), and a broken palate (F #1098).

Clinical signs: There were no treatment-related clinical signs.

Body weight (males):

F1 males in the DPH alone group (#2) had slight reductions (up to 4.4%) of body weight during the postpartum days 36 to 92. The body weight of F1 males in the BMN 110 + DPH groups (#3-5) were comparable to F1 males in group 1 (vehicle only group). Body weights on postpartum day 127 (last day of weighing prior to sacrifice) were 95.2%, 97%, 101% and 98.4 % of the vehicle only group in the groups 2, 3, 4, and 5, respectively.

Body weight gain in F1 males in the DPH alone group was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$, up to 13%↓), compared to the F1 males in the vehicle alone group on postpartum days 50 to 57, 85 to 92, 22 to 92, 113 to 120 and 22 to 127. Compared to the vehicle control group, body weight gain was significantly increased ($p \leq 0.05$ or $p \leq 0.01$, up to 20%↑) on postpartum days 78 to 85 in the 1 and 6 mg/kg/day BMN 110 + DPH groups F1 males. However, the increases were not considered as related to BMN

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110, because they were not dose dependent. Therefore, body weight gain in F1 males was comparable among the groups administered BMN 110 + DPH and the group administered vehicle alone.

Body weight (females):

Body weight and body weight gain for the post-weaning period through pregnancy in the F1 females were comparable among the groups. On postpartum day 92 (last weight measured prior to cohabitation), body weights were 99%, 102.9%,101.5% and 100.4% of the group 1 (vehicle alone) body weight in groups 2, 3, 4, and 5, respectively. Body weights on GD 21 were 100.5%, 101.7%, 102.3% and 102.2% of the group 1 body weight for the same respective dose groups. Thus, DPH alone or in combination with BMN 110 had no effects on body weight or body weight gain of F1 females during the postpartum (weaning to day 92) and gestation periods.

Food consumption (males):

Compared to the vehicle only group, absolute food consumption (g/day) by F1 males in the DPH alone group was significantly reduced ($p \leq 0.05$ to $p \leq 0.01$, up to ↓5.5%) for postpartum days 64 to 71, 71 to 78, 78 to 85, 85 to 92, and 120 to 127. However, relative food consumption (g/kg/day) was comparable among the study groups. BMN 110 had no effects on food consumption in F1 males.

Food consumption (females):

Absolute and relative food consumption for the post-weaning period through gestation in the F1 female rats was comparable among groups. DPH alone or in combination with BMN 110 had no effects on food consumption in the F1 females during the postpartum and gestation periods.

Sexual Maturation:

Sexual maturation was evaluated daily starting on days 28 and 39 postpartum for females and males, respectively, until the criterion was achieved.

DPH alone or in combination with BMN 110 had no effects on the age of preputial separation or vaginal patency.

Neurological assessment:

The Sponsor conducted memory and learning tests in this study, but did not perform other behavioral tests (e.g. static righting reflex, cliff aversion, startle response, and motor activity) and tests for sensory functions that are routinely included in pre-/ postnatal development studies. An information request was sent to the Sponsor on May 28, 2013 to ask for justification for not performing evaluations of behavior, locomotor activity, sensory functions, and reflex development in the F1 generation in this study.

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The Sponsor responded on June 13, 2013, but the justification is not acceptable (see appendix). Since the omitted development tests are routinely included in pre- and postnatal developmental studies, the evaluation of F1 generation development was suboptimal in this study, and this should be conveyed in the Pregnancy section of the labeling.

Learning and Memory:

One male and one female rat from each litter were selected on postpartum day 24 ± 1 for evaluation in the passive avoidance test. Female F1 rats from maternal groups treated with DPH alone or BMN 110 + DPH had significantly prolonged latency (up to 41%) in trial 1 of the second test session, when compared to the vehicle only group females. Prolonged latency (up to 41%) in trial 1 of the second test session was also observed in male rats from maternal groups treated with DPH alone or BMN 110 + DPH, except for males in group 3. The change in test performance in males did not reach statistical significance. The data indicates that BMN 110 had no effect on performance in the passive avoidance test, whereas DPH produced impaired performance (see table below from study report).

Table 83: Summary Data of Passive Avoidance Test in F1 Generation

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
MALE RATS						
SESSION 1a	N	24	25	22	21	18b
TRIALS TO CRITERION	MEAN±S.D.	6.4 ± 2.8	5.7 ± 3.2	7.3 ± 3.0*	5.4 ± 1.6	6.8 ± 4.2
LATENCY TRIAL 1c	MEAN±S.D.	5.8 ± 2.6	5.6 ± 4.5	4.8 ± 3.2	4.8 ± 2.9	4.7 ± 4.6
LATENCY TRIAL 2c	MEAN±S.D.	18.0 ± 19.5	24.0 ± 22.4	5.9 ± 4.8**	11.1 ± 13.1	16.6 ± 21.6
FAILED TO LEARN d	N(%)	1(4.2)	2(8.0)	1(4.5)	0(0.0)	3(16.7)
SESSION 2a	N	23	23	21	21	15
TRIALS TO CRITERION	MEAN±S.D.	3.8 ± 2.3	3.4 ± 1.3	4.5 ± 3.8	4.2 ± 3.7	3.0 ± 0.5
LATENCY TRIAL 1c	MEAN±S.D.	22.4 ± 22.3	31.7 ± 24.9	21.4 ± 24.0	27.5 ± 24.4	30.1 ± 22.2
FEMALE RATS						
SESSION 1a	N	24	25	22	21	19
TRIALS TO CRITERION	MEAN±S.D.	6.9 ± 3.3	6.0 ± 3.2	6.4 ± 2.2	5.9 ± 2.4	6.0 ± 2.0
LATENCY TRIAL 1c	MEAN±S.D.	5.3 ± 3.8	6.1 ± 4.8	4.0 ± 3.6	5.0 ± 3.4	4.6 ± 2.0
LATENCY TRIAL 2c	MEAN±S.D.	15.0 ± 18.6	16.8 ± 16.0	7.7 ± 8.5	10.6 ± 13.0	13.0 ± 15.9
FAILED TO LEARN d	N(%)	1(4.2)	2(8.0)	0(0.0)	1(4.8)	0(0.0)
SESSION 2a	N	23	23	22	20	19
TRIALS TO CRITERION	MEAN±S.D.	3.6 ± 1.2	3.5 ± 2.7	3.0 ± 1.2	4.1 ± 3.2	3.8 ± 1.5
LATENCY TRIAL 1c	MEAN±S.D.	18.0 ± 18.4	35.9 ± 22.4##	35.5 ± 24.6	29.4 ± 22.3	24.7 ± 21.4

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. Excludes values for rat 912, which was euthanized on Day 24 postpartum due to adverse clinical observations.
c. The latency was recorded in seconds.
d. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
Significantly different from the Group 1 value ($p \leq 0.01$).
* Significantly different from the Group 2 value ($p \leq 0.05$).
** Significantly different from the Group 2 value ($p \leq 0.01$).

One male and one female rat from each litter were selected on postpartum day 70 ± 2 for evaluation in the water-filled M-maze test. Prolonged latency (up to 44%) in trial 2 of the first test session was observed in male and female rats from maternal groups

treated with DPH alone or BMN 110 + DPH, except for males in group 3. This effect did not reach statistical significance. Latency was also prolonged in females from maternal groups treated with DPH alone or BMN 110 + DPH, in trial 1 of test session 2. The data shows that BMN 110 had no effect on performance in the water-filled M-maze test, whereas DPH produced impaired performance (see table below from study report).

Table 84: Summary Data of Water-Filled M Maze Test in F1 Generation

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
MALE RATS						
SESSION 1a	N	24	25	22	21	18b
TRIALS TO CRITERION	MEAN±S.D.	8.7 ± 2.8	9.3 ± 3.0	10.2 ± 3.1	9.0 ± 2.8	9.8 ± 2.8
ERRORS PER TRIAL	MEAN±S.D.	0.38 ± 0.22	0.47 ± 0.31	0.50 ± 0.36	0.42 ± 0.30	0.51 ± 0.27
LATENCY TRIAL 2c	MEAN±S.D.	14.0 ± 12.2	16.3 ± 11.3	15.4 ± 12.0	19.8 ± 17.7	17.6 ± 10.7
FAILED TO LEARN d	N(%)	0(0.0)	3(12.0)	3(13.6)	1(4.8)	1(5.6)
SESSION 2a	N	24	22	19	19e	17
TRIALS TO CRITERION	MEAN±S.D.	6.4 ± 1.8	6.4 ± 2.2	6.4 ± 1.6	5.9 ± 2.0	7.0 ± 2.9
ERRORS PER TRIAL	MEAN±S.D.	0.13 ± 0.16	0.10 ± 0.16	0.12 ± 0.14	0.06 ± 0.10	0.19 ± 0.24
LATENCY TRIAL 1c	MEAN±S.D.	10.6 ± 7.0	9.0 ± 5.1	11.3 ± 5.3	8.4 ± 4.9	10.6 ± 6.6
FEMALE RATS						
SESSION 1a	N	24	25	22	21	19
TRIALS TO CRITERION	MEAN±S.D.	8.8 ± 2.9	9.0 ± 3.2	8.6 ± 2.7	10.4 ± 3.2	10.7 ± 3.1
ERRORS PER TRIAL	MEAN±S.D.	0.44 ± 0.27	0.39 ± 0.20	0.39 ± 0.21	0.45 ± 0.23	0.54 ± 0.25
LATENCY TRIAL 2c	MEAN±S.D.	13.5 ± 9.4	15.5 ± 12.2	15.6 ± 12.8	14.0 ± 10.2	19.5 ± 10.0
FAILED TO LEARN d	N(%)	2(8.3)	2(8.0)	0(0.0)	3(14.3)	2(10.5)
SESSION 2a	N	22	23	22	18	16
TRIALS TO CRITERION	MEAN±S.D.	7.3 ± 3.2	8.3 ± 3.3	8.2 ± 3.3	8.3 ± 4.3	7.2 ± 2.9
ERRORS PER TRIAL	MEAN±S.D.	0.14 ± 0.16	0.23 ± 0.23	0.26 ± 0.24	0.24 ± 0.28	0.22 ± 0.25
LATENCY TRIAL 1c	MEAN±S.D.	9.2 ± 3.5	12.3 ± 5.9#	12.0 ± 8.2	11.6 ± 6.4	11.1 ± 6.1

a. Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b. Excludes values for rat 912, which was euthanized on Day 24 postpartum due to adverse clinical observations.
c. The latency was recorded in seconds.
d. Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
e. Excludes values for rat 883, which was tested with the incorrect goal in Session 2 (Retention Phase).
Significantly different from the Group 1 value ($p \leq 0.05$).

Based on the outcome of the passive avoidance and water-filled M-maze tests, it appears that BMN 110 had no effects on learning and memory in the F1 generation.

Mating and Fertility:

Pregnancy occurred in 23 (92%), 24 (96%), 24 (96%), 23 (92%) and 21 (87%) mated pairs from maternal groups 1 (vehicle only), 2 (DPH alone), 3 (1 mg/kg/day BMN 110 + DPH), 4 (6 mg/kg/day BMN 110 + DPH), and 5 (20 mg/kg/day BMN 110 + DPH), respectively. Although not significant, there was a reduction in pregnancy rate in the offspring of maternal treatment group 5. Overall, there were no statistically significant differences among the study groups for number of days in cohabitation, rats that mated, fertility index, or time to mating. Mating and fertility of the F1 generation females were not affected by BMN 110 or DPH treatment. The reproductive and fertility parameters are summarized in the Sponsor's table below.

Table 85: Mating and Fertility Parameters in F1 Generation Females

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
RATS IN COHABITATION	N	25	25	25	25	24a
DAYS IN COHABITATION b	MEAN±S.D.	4.0 ± 3.8	3.2 ± 2.3	3.7 ± 2.0	3.1 ± 2.0	4.1 ± 3.4 [23]c
RATS THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)	24(100.0)
FERTILITY INDEX d	N/N	23/25	24/25	24/25	23/25	21/24
	(%)	(92.0)	(96.0)	(96.0)	(92.0)	(87.5)
RATS WITH CONFIRMED MATING DATES	N	25	25	25	25	23c
MATED BY FIRST MALE e						
DAYS 1-7	N(%)	23(92.0)	23(92.0)	23(92.0)	23(92.0)	19(82.6)c
DAYS 8-14	N(%)	0(0.0)	2(8.0)	2(8.0)	2(8.0)	3(13.0)c
MATED BY SECOND MALE e						
DAYS 15-21	N(%)	2(8.0)	0(0.0)	0(0.0)	0(0.0)	1(4.3)c
RATS PREGNANT/RATS IN COHABITATION	N/N	23/25	24/25	24/25	23/25	21/24
	(%)	(92.0)	(96.0)	(96.0)	(92.0)	(87.5)

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 1122, which was euthanized on Day 75 postpartum due to adverse clinical observations.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Excludes values for rat 1123; mating date was incorrectly identified.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.

Caesarean Section and Litter Data:

Pregnancy occurred in 23, 24, 24, 23 and 21 females from maternal groups 1 (vehicle alone), 2 (DPH alone), 3 (1 mg/kg/day BMN 110 + DPH), 4 (6 mg/kg/day BMN 110 + DPH), and 5 (20 mg/kg/day BMN 110 + DPH), respectively. Two dams delivered on GD 21 in group 5. Caesarean section observations were based on 23, 24, 24, 22 and 19 pregnant dams in the five respective dose groups. BMN 110 had no effects on corpora lutea, implantations, percent preimplantation loss, litter size, live fetuses, dead fetuses, resorptions (early and late), postimplantation loss, number of dams with any resorptions, or number of dams with viable fetuses. Administration of DPH alone (group 2) appeared to produce an increase in the number and percent of dams with resorptions compared to group 1, whereas the other c-section parameters were unaffected. Two litters in group 3 had one or more large placentae. The following tables from the study report show the reproductive and fertility parameters for the F1 generation.

Table 86: Caesarean-Section Observations for F1 Generation Females

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
RATS TESTED	N	25	25	25	25	24a
PREGNANT	N(%)	23(92.0)	24(96.0)	24(96.0)	23(92.0)	21(87.5)
DELIVERED AND EUTHANIZED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(9.5)
UNSCHEDULED EUTHANASIA	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	24	24	22	19
CORPORA LUTEA	MEAN±S.D.	16.3 ± 3.3	16.5 ± 2.0	15.9 ± 3.1	16.2 ± 2.5	15.7 ± 2.1
IMPLANTATIONS	MEAN±S.D.	15.2 ± 2.8	14.8 ± 2.8	14.7 ± 4.0	14.8 ± 2.6	14.9 ± 1.6
% PREIMPLANTATION LOSS	MEAN±S.D.	6.0 ± 10.6	10.5 ± 15.5	10.2 ± 19.3	8.5 ± 13.0	4.9 ± 5.6
LITTER SIZES	MEAN±S.D.	14.7 ± 2.9	14.0 ± 3.2	14.3 ± 4.0	13.8 ± 2.8	13.8 ± 2.1
LIVE FETUSES	N	338	337	343	304	263
	MEAN±S.D.	14.7 ± 2.9	14.0 ± 3.2	14.3 ± 4.0	13.8 ± 2.8	13.8 ± 2.1
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.5 ± 0.8	0.8 ± 0.7	0.4 ± 0.6	1.0 ± 1.6	1.0 ± 1.8
EARLY RESORPTIONS	N	12	18	10	21	20
	MEAN±S.D.	0.5 ± 0.8	0.8 ± 0.7	0.4 ± 0.6	1.0 ± 1.6	1.0 ± 1.8
LATE RESORPTIONS	N	0	0	0	0	0
% POSTIMPLANTATION LOSS	MEAN±S.D.	3.7 ± 5.6	6.6 ± 10.2	2.7 ± 4.3	6.5 ± 10.2	6.8 ± 11.1

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Excludes values for rat 1122, which was euthanized on Day 75 postpartum due to adverse clinical observations.

Table 87: Caesarean-Section Observations for F1 Generation Females (continued)

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
RATS TESTED	N	25	25	25	25	24a
PREGNANT	N(%)	23(92.0)	24(96.0)	24(96.0)	23(92.0)	21(87.5)
DELIVERED AND EUTHANIZED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(9.5)
UNSCHEDULED EUTHANASIA	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.3)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	24	24	22	19
DAMS WITH ANY RESORPTIONS	N(%)	8(34.8)	15(62.5)	8(33.3)	9(40.9)	10(52.6)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	24(100.0)	24(100.0)	22(100.0)	19(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	24(100.0)	22(91.7)	22(100.0)	19(100.0)

a. Excludes values for rat 1122, which was euthanized on Day 75 postpartum due to adverse clinical observations.

Necropsy Observations:

F1 male rats in the DPH alone group had a 5% reduction in terminal body weight ($p \leq 0.05$) compared to the vehicle alone group. The absolute and relative (%bodyweight) weight of testes and epididymides was comparable among the study groups. F1 males

in the BMN 110 + DPH treatment groups had the following gross lesions: a broken palate, dark red lobes of the lungs, enlarged spleen, inguinal hernia, renal lesions (small size, adhered to the pancreas, calculi, large size, tan area, slight to moderate dilation, clear fluid filled cysts), urinary bladder lesions (thickened wall, calculi, distended with yellow fluid), slight dilation of the ureter, small epididymides, and flaccid or small testes. Two F1 females in group 4 had slight dilation of the renal pelvis. However, these gross lesions were not considered related to BMN 110 or DPH, because the lesions were not dose-dependent and occurred in only one or two rats in any group. Thus, maternal treatment with BMN 110 + DPH did not result in gross lesions in the F1 generation rats.

F2 Generation

The number of live fetuses, fetal weight, sex ratio, and percent live male fetuses per litter were not affected by treatment of F0 maternal groups with DPH alone or BMN 110 + DPH. The percent resorbed conceptuses/litter was highly variable, but no clear treatment related effects were observed. BMN 110 had no effects on external evaluation observations. The following tables show the F2 survival, body weight and external evaluation data (taken from the study report).

Table 88: F2 Generation Litter Observations (C-section Delivered Fetuses)

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	24	24	22	19
IMPLANTATIONS	MEAN±S.D.	15.2 ± 2.8	14.8 ± 2.8	14.7 ± 4.0	14.8 ± 2.6	14.9 ± 1.6
LIVE FETUSES	N	338	337	343	304	263
	MEAN±S.D.	14.7 ± 2.9	14.0 ± 3.2	14.3 ± 4.0	13.8 ± 2.8	13.8 ± 2.1
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.6 ± 11.8	45.8 ± 17.4	42.1 ± 15.7	49.3 ± 12.1	47.3 ± 14.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.42 ± 0.31	5.56 ± 0.31	5.36 ± 0.38	5.55 ± 0.37	5.47 ± 0.34
MALE FETUSES	MEAN±S.D.	5.54 ± 0.35	5.75 ± 0.32# [23]a	5.55 ± 0.47 [23]b	5.72 ± 0.35	5.61 ± 0.40
FEMALE FETUSES	MEAN±S.D.	5.29 ± 0.30	5.41 ± 0.27	5.24 ± 0.37	5.39 ± 0.39	5.34 ± 0.32
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.7 ± 5.6	6.6 ± 10.2	2.7 ± 4.3	6.5 ± 10.2	6.8 ± 11.1

[ ] = NUMBER OF VALUES AVERAGED
a. Litter 1041 had no male fetuses.
b. Litter 1052 had no male fetuses.
Significantly different from the Group 1 value ($p \leq 0.05$).

Table 89: External Fetal Alterations in F2 Generation Litters

MATERNAL GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	CONTROL ARTICLE/DPH	BMN 110/DPH	BMN 110/DPH	BMN 110/DPH
MATERNAL BMN 110 DOSE LEVEL (MG/KG/DAY)		0	0	1	6	20
MATERNAL DPH DOSE LEVEL (MG/KG/DAY)		-	10	10	10	10
LITTERS EVALUATED	N	23	24	24	22	19
LITTERS WITH LIVE FETUS(ES)	N	23	24	24	22	19
FETUSES EVALUATED	N	338	337	343	304	263
LIVE	N	338	337	343	304	263
SNOUT: CLEFT						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)a	0(0.0)	0(0.0)	0(0.0)
JAW: AGNATHIA						
LITTER INCIDENCE	N(%)	0(0.0)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.3)a	0(0.0)	0(0.0)	0(0.0)

a. Fetus 1029-9 had other gross external alterations.

10 Special Toxicology Studies

N/A

11 Integrated Summary and Safety Evaluation

Morquio A syndrome, also known as Mucopolysaccharidosis type IVA (MPS IVA), is an inherited autosomal recessive disorder caused by mutations in the GALNS (N-acetyl-galactosamine 6-sulfatase) gene. GALNS functions in the degradation of keratan sulfate (KS) and chondroitin-6-sulfate (C6S), which are both glycosaminoglycans (GAGs). Mutations in the GALNS gene reduce or eliminate the enzyme activity. The deficiency in GALNS activity results in KS accumulation in many tissues and organs, high circulating levels of KS, and KS excretion in urine. MPS IVA is a progressive disorder that affects multiple organ systems including the musculoskeletal, cardiopulmonary, and neurological systems. All patients have systemic skeletal dysplasia, which usually results in short stature and malformations of the knees, chest and spine. The persistent and progressive accumulation of KS leads to significant morbidities and multi-systemic clinical impairments resulting in impaired functional capacity, decreased endurance, poor quality of life, and early mortality.

VIMIZIM™ (elosulfase alfa) is an enzyme replacement therapy (ERT) for treatment of patients with Morquio A Syndrome. The active ingredient in VIMIZIM™ drug product (concentrate for infusion) is rhGALNS (recombinant human N-acetylgalactosamine-6-sulfatase) expressed in Chinese Hamster Ovary (CHO) cells. Elosulfase alfa is taken up by cells via the cation-independent mannose-6-phosphate receptor (CI-M6PR) and subsequently transferred and localized to lysosomes. N-acetylgalactosamine-6-sulfatase is a lysosomal enzyme that hydrolyzes sulfate from either galactose-6-sulfate or N-acetyl-galactosamine-6-sulfate on the non-reducing ends of the glycosaminoglycans KS and C6S in lysosomes.

The proposed indication for VIMIZIM™ is for patients with Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome). The recommended dose is 2 mg/kg administered once a week as an intravenous infusion over approximately 4 hours.

CI-M6PR, the receptor for elosulfase alfa, is conserved across species (81%, 80%, 79%, and 94% protein sequence identity in the mouse, rat, rabbit, and cynomolgus monkey, respectively, compared to humans). The GALNS protein sequence identity also confers a phylogenic similarity to human (84%, 84%, 85%, and 97% protein sequence identity in the mouse, rat, rabbit, and cynomolgus monkey, respectively, compared to humans). Therefore, mouse, rat, rabbit, and cynomolgus monkey were selected as relevant species to evaluate the pharmacodynamic (PD), pharmacokinetic (PK), and toxicity of elosulfase alfa.

In vivo PD studies using an animal model of disease were not conducted, because no animal models exhibiting the human disease phenotype for MPS IVA were available. Although three mouse models have been reported, none of these exhibit skeletal abnormalities, a clinical sign that is observed in all MPS IVA patients. The failure of mouse models to recapitulate the skeletal dysplasia seen in humans with MPS IVA is likely due to the lack KS expression in mouse skeletal tissue.

The nonclinical PD, PK, and toxicity of BMN 110 (elosulfase alfa) were evaluated in five *in vitro* studies and eleven *in vivo* studies. Respiratory and CNS safety pharmacology parameters were assessed in rats, and cardiovascular parameters were assessed in monkeys via telemetry. The nonclinical PK assessment was conducted in conjunction with the toxicity studies in rats and monkeys, and tissue distribution was evaluated in mice. Four toxicity studies with TK measurement were conducted, including a combined single-dose/CNS safety pharmacology study in rats, a 26-week repeat-dose study in rats, a 28-day repeat dose study in monkeys, and a 52-week repeat-dose study in monkeys. The developmental and reproductive toxicity program included four studies: a combined fertility/embryo-fetal development study in rats; a pre-/postnatal development study in rats; a dose range-finding embryo-fetal development study in rabbits, and a definitive embryo-fetal development study in rabbits.

BMN 110 was taken up by the human Morquio fibroblast cell line (b) (4). The calculated K_{uptake} was approximately 2.5 nM, and the intracellular half-life of rhGALNS was approximately 6 days. Cellular uptake of BMN 110 and localization to lysosomes was demonstrated in cultured chondrocytes from patients with MPS IVA. Treatment of human primary Morquio chondrocytes (MQCH) with BMN 110 at 1 and 10 nM restored GALNS activity within the cells, and cleared lysosomal KS storage. Extracellular KS was not affected by BMN 110 treatment, indicating that BMN 110 activity was restricted to the lysosome. BMN 110 also produced an increase in the expression of chondrogenic genes including collagen II, collagen X, aggrecan and sox 9 in MQCH cells. The abnormal expression of collagen I was reduced by BMN 110.

Following intravenous administration to mice, rhGALNS was detected in the heart (mitral valves, atrium and septum), growth plate, articular cartilage, bone, bone marrow,

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and sinusoidal and Kupffer cells of the liver. BMN 110 at doses of 1, 6, and 20 mg/kg had no effects on CNS (rats), respiratory function (rats), ECG (monkeys), or hemodynamic parameters (monkeys).

The characterization of the pharmacokinetics of BMN 110 was limited to the toxicity studies in rats and monkeys. It should be noted that neutralizing antibodies were detected in all BMN 110-treated groups in the chronic studies in rats and monkeys, based on the observed inhibition of binding to soluble CI-M6PR. The $t_{1/2}$ increased with increasing dose in both species. Following the initial IV bolus administration of BMN 110 in rats, the $t_{1/2}$ was about 2 minutes at 1 mg/kg, 9 minutes at 6 mg/kg, and 7 minutes at 20 mg/kg. At dose 26, the $t_{1/2}$ in the 20 mg/kg group was increased to 26 minutes. Following repeated IV (weekly) infusion of BMN 110 in monkeys, the $t_{1/2}$ values increased from approximately 10 minutes at dose 1 to 30-90 minutes at dose 52. Systemic exposure (C_{max} and AUC_{0-t}) in monkeys was increased at week 52, compared with the initial dose (e.g. 2 to 3-fold increase in the 6 and 20 mg/kg groups). Mean clearance and distribution (V_z) values decreased with increasing doses in both species. The reductions in CL and V_z with increasing doses suggest saturation of the BMN 110 clearance mechanism(s), such as protease-mediated degradation or receptor-mediated uptake.

The manufacturing process for BMN 110 was modified during the development period. The Sponsor conducted a 28-day study in monkeys to evaluate the toxicity and TK parameters of the three different lots (one lot of Phase 1/2 material and 2 lots of Phase 3 material). The treatment groups were all given 20 mg/kg from one of the three lots, administered as a weekly 4-hr IV infusion. No adverse effects were observed with any of the tested lots. There was an increase of approximately 1.5-fold in systemic exposure (AUC_{0-t}) to BMN 110 for both Phase 3 lots, compared to the Phase 1/2 lot. Anti-BMN 110 antibodies were detected in all treated animals. The no observed adverse effect level (NOAEL) was 20 mg/kg/week for each of the tested lots.

In the 26-week IV toxicity study in rats, DPH (diphenhydramine) was administered to groups treated with BMN 110 to prevent or reduce anaphylactic reactions. SD rats were treated once weekly with 0 (vehicle), 0 (vehicle + DPH), 1 (+ DPH), 6 (+ DPH), or 20 mg/kg BMN 110 (+ DPH); BMN 110 was given via IV bolus injection. There were no BMN 110 treatment-related changes in body weight gain, food consumption, hematology, clinical chemistry, macroscopic observations, or microscopic observations. The main findings were related to anaphylactic reactions to BMN 110, including three deaths. These types of reactions were generally mitigated by the DPH pretreatment, which was initiated on week 3. BMN 110 at 6 and 20 mg/kg/week increased urine volume, pH, and decreased urine specific gravity. However, there were no associated histopathologic changes in kidneys. All BMN 110-treated animals, with one exception, were positive for anti-BMN 110 antibodies during the dosing phase and the recovery period. Furthermore, neutralizing antibodies were detected in most BMN110-treated animals during the dosing phase and the recovery period. No target organ of toxicity was identified. The tolerated dose was 20 mg/kg/week IV in rats, based on the changes in urinalysis parameters. However, given the high incidence of neutralizing antibodies,

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it is likely that the pharmacological activity of BMN 110 was inhibited in most of the animals. Therefore, the results of this study must be interpreted with caution.

In the 52-week toxicity study in cynomolgus monkeys, BMN 110 was administered once weekly at doses of 0, 1, 6, or 20 mg/kg via IV infusion. No anaphylactic-type reactions were observed. There were no drug-related deaths or clinical signs. BMN 110 had no effects on body weight gain, food consumption, ophthalmic examination, ECG, hematology, clinical chemistry, urinalysis, macroscopic findings, or microscopic findings. BMN 110 had no effects on long bone growth or plasma levels of keratan sulfate degradation products. The bone resorption marker, serum crosslinked C-telopeptide of type 1 collagen, was not affected by administration of BMN 110 at a dose of 20 mg/kg/week. Almost all animals treated with BMN 110 were positive for anti-drug antibodies. All but 4 animals in the treatment groups were positive for neutralizing anti-drug antibodies. No target organs of toxicity were identified. The NOAEL was 20 mg/kg/week.

In the combined segment 1 and 2 study, male and female SD rats were administered BMN 110 via intravenous bolus injection daily at doses of 1, 6 and 20 mg/kg before cohabitation, during cohabitation, the day before euthanasia (males only), and through day 20 of presumed gestation. The study included a control group treated with the vehicle for BMN 110, and a control group treated with the vehicle and DPH (referred to as DPH alone). DPH was administered at 10 mg/kg/day via intraperitoneal injection to all rats in the BMN 110 treatment groups to prevent anaphylactic reactions. The effects of BMN 110 for all study parameters were evaluated based on comparison to the DPH alone control group. It is noteworthy that the dose frequency (daily) in this study was different from that of the 26-week IV rat toxicity study (weekly), although the same dose levels were used. A small number of deaths in the absence of anaphylactic reactions occurred in females in all BMN 110 + DPH treatment groups in the segment 1/2 study. These deaths may have been related to BMN 110 and/or DPH administration. Clinical signs that preceded death included prostration, decreased motor activity, cold to touch, pale front paws and hind paws, tachypnea and ataxia.

At 6 and 20 mg/kg/day, BMN 110 produced a decrease in body weight (↓up to 5%) and body weight gain (↓up to 68%) in males. BMN 110 + DPH treatment resulted in non-significant decreases in body weight gains in female rats during the pre-cohabitation days (↓43%, ↓58%, and ↓46% for the 1, 6 and 20 mg/kg/day + DPH groups, respectively) compared to DPH treated females. Although these effects were not statistically significant or dose-dependent, the reduction of female body weight gain prior to mating was most likely related to BMN 110 treatment. Absolute and relative food consumption in male rats was reduced (up to 17%) throughout the study in the BMN 110 + DPH dose groups, as compared to the DPH alone group. Premating absolute and relative food consumption in females was significantly reduced in the 6 mg/kg/day + DPH (↓7.5%) and 20 mg/kg/day + DPH (↓13%) groups, as compared to the DPH alone group. Similar reductions in relative food consumption occurred in the same dose groups on GDs 0 to 7. These changes are considered as related to BMN 110 treatment. The paternal NOAEL for general toxicity was 1 mg/kg/day IV, based on

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decreased body weight and body weight gain at 6 and 20 mg/kg/day. BMN 110 did not produce any significant inhibition of weight gain during pregnancy, compared to the DPH alone control group. Therefore, the maternal NOAEL during pregnancy was 20 mg/kg/day IV.

Fertility parameters in female and male rats were not adversely affected by BMN 110 treatment. BMN 110 had no effects on rat embryo-fetal developmental parameters, including corpora lutea, implantations, resorptions (early and late), number of dams with resorptions, the percent dead or resorbed conceptuses, litter sizes, number of live fetuses, percent live male and female fetuses, fetal body weights, and soft tissue and skeletal alterations. BMN 110 had no effects on sperm motility, count, or density. Thus, the NOAEL for rat fertility and embryo-fetal development was 20 mg/kg/day IV.

In a segment 2 study, time-mated rabbits were administered BMN 110 via intravenous infusion daily at doses of 1, 3 and 10 mg/kg during gestation days 7 to 20. There were no drug-related effects on mortality, clinical signs, changes in body weight, or food consumption. All dose levels of BMN 110 produced red, tan, mottled, raised and/or pitted areas on the liver. Thus, the maternal NOAEL was < 1 mg/kg/day IV. BMN 110 had no effects on embryo-fetal developmental parameters, including corpora lutea, implantations, resorptions (early and late), number of does with resorptions, the percent dead or resorbed conceptuses, litter sizes, live fetuses, percent live male and female fetuses, and fetal body weights. BMN 110 did not increase the incidence of gross external, soft tissue, or skeletal alterations. Thus, the NOAEL for embryo-fetal development in rabbits was 10 mg/kg/day IV. In the dose-range finding study, low levels of BMN 110 (94 ng/ml) were detected in fetal rabbit plasma at the 20 mg/kg/day dose level, which confirmed that the drug crossed the placental barrier. CI-M6PR, the receptor which mediates BMN 110 cellular uptake, is expressed in the placenta.

In the pre-/postnatal study in rats, females were administered BMN 110 at daily doses of 1, 6 and 20 mg/kg intravenously (bolus) from gestation day 7 through lactation day 20. The study included a control group treated with the vehicle for BMN 110, and a control group treated with the vehicle and DPH (referred to as DPH alone). DPH was administered at 10 mg/kg/day via intraperitoneal injection to all rats in the BMN 110 treatment groups to prevent anaphylactic reactions. The effects of BMN 110 for all study parameters were evaluated based on comparison to the DPH alone control group. A small number of deaths occurred during pregnancy in the 1, 6, and 20 mg/kg/day + DPH groups. However, mortality was not dose dependent, and 3/7 deaths were preceded by signs of anaphylaxis or DPH toxicity. Given the small number and dubious nature of these deaths, the interpretation of the offspring observations is not substantially impacted, although the possible involvement of maternal toxicity in the adverse effects in offspring cannot be excluded. BMN 110 at 20 mg/kg/day produced signs of maternal toxicity including significant reductions in body weight, body weight gain, and food consumption. The 6 and 20 mg/kg/day BMN 110 + DPH groups had a statistically significant decrease in the percentage of liveborn fetuses and a statistically significant increase in the percentage of stillbirths. These effects were dose-dependent. The increase in percentage of stillbirths at 6 and 20 mg/kg/day exceeded the range of

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historical control values from the testing laboratory. The percent survival of pups during postpartum days 1-4 (viability index) was significantly reduced in the 20 mg/kg/day BMN 110 + DPH group (94.4% compared to 98.7% in the DPH alone group). The reduction in viability index was correlated with a statistically significant increase in pup mortality during postpartum days 2-4 with 20 mg/kg/day + DPH (3.8% compared to 0.6% in the DPH alone group). BMN 110 was detected in the milk of F0 dams treated with BMN 110 at doses of 6 and 20 mg/kg/day. The maternal NOAEL was 6 mg/kg/day IV based on significantly reduced body weight gain and food consumption at 20 mg/kg/day. The NOAEL for pre- and post-postnatal development was 1 mg/kg/day IV, based on the increase in percentage of stillbirths at 6 and 20 mg/kg/day, and the increase in postnatal deaths at 20 mg/kg/day. Given the incidence of increased stillbirths in the 6 and 20 mg/kg/day groups, which is indicative of developmental toxicity, the increase in pup mortality during postpartum days 2-4 was most likely related to the adverse effects from *in utero* exposure to BMN 110, rather than oral exposure to BMN 110 in milk. It is noted that the mean maternal plasma drug concentration in the high-dose group at 1 minute post-dose was more than 1000 times the drug concentration in milk.

Body weight, body weight gain, food consumption, sexual maturity, and learning and memory functions were not adversely affected by the treatment with BMN 110. The reproductive parameters for the F1 generation (mating and fertility, C-section and litter parameters, F2 generation survival, and external evaluation) were not affected by BMN 110 treatment. Although not statistically significant, a reduction in pregnancy rate was observed in the offspring of the maternal 20 mg/kg/day BMN 110 + DPH group. Several developmental tests to evaluate behavior, locomotor activity, sensory functions, and reflex development (e.g. static righting reflex, cliff aversion, startle response) in the F1 generation were not conducted. Since the omitted tests are routinely included in pre- and postnatal developmental studies, the evaluation of F1 generation development was suboptimal in this study, and this should be conveyed in the Pregnancy section of the labeling.

In the repeated IV dose toxicity studies, the major findings were related to anaphylactic reactions in rats, likely due to the administration of an antigenic heterologous protein. These reactions were generally mitigated by pretreatment with DPH. No target organs of toxicity were identified in the chronic studies in rats or cynomolgus monkeys. However, most of the animals in both of these studies developed neutralizing antibodies to BMN 110. Thus, the utility of these studies in characterizing the toxicity of BMN 110 is very limited. Since the potential target- or pharmacology-related toxicity may have been eliminated by the neutralizing antibodies, the interpretation of results from both chronic toxicity studies (i.e. absence of target organ toxicity or any major adverse effects aside from anaphylactic reactions) is confounded.

The steady state AUC_{0-t} at the tolerated dose of 20 mg/kg/week in the 26-week rat IV toxicity study and at the NOAEL of 20 mg/kg/week in the 52-week monkey IV toxicity study was 42 times the human steady state AUC_{0-t} (mean of 577 mcg•min/mL from all patients on week 22 of dosing) at the recommended dose of 2 mg/kg IV weekly. Given the confounding factor related to neutralizing antibody formation in the toxicology

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studies, it is difficult to evaluate the animal to human exposure margins in the context of safety assessment. However, it is reasonable to conclude that the toxicology studies have provided the best possible investigation of the potential toxicity of elosulfase alfa, given the large exposure margins that were achieved.

12 Appendix/Attachments

Amendment 0007: Responses to FDA Information Request (June 13, 2013)

Pharmacology and Toxicology (non-clinical):

17. Provide justification for not performing evaluations of behavior, locomotor activity, sensory functions, and reflex development in the F1 generation in the study entitled "A Developmental and Perinatal/Postnatal Reproduction Study of BMN 110 by Intravenous Injection in Rats, Including a Postnatal Behavioral/Functional Evaluation". These parameters are routinely included in pre- and postnatal developmental studies (see ICH guidance S5(R2)).

BioMarin Response:

In the study entitled "A Developmental and Perinatal/Postnatal Reproduction Study of BMN 110 by Intravenous Injection in Rats, Including a Postnatal Behavioral/Functional Evaluation (Study No. BMN110-12-013)", evaluation of behavior, memory and learning, locomotor activity, overt coordination, and sensory function were conducted in F1 generation animals as per the ICH S5 (R2) guidelines. Low fetal exposure of the F1 generation during pregnancy is presumed based on the fetal exposure data from the rabbit embryo-fetal study (81-371 ng/mL; Study No. 110-10-008). Additionally, BMN 110 was detected in the milk of some lactating F0 rats (102- 212 ng/mL); however, F1 exposure to BMN 110 via milk is not expected, as BMN 110 is a protein that would be degraded in the digestive tract of the weaning rat.

Regarding evaluations of behavior, locomotor activity, sensory functions, and reflex development in the F1 generation in study BMN 110-12-013, one male and one female per litter were evaluated using the Passive Avoidance and Water Maze (M-maze) tests on Day 24, post partum and Day 70, post partum, respectively. The Passive Avoidance evaluation assesses learning, short term retention and long term retention memory. M-Maze evaluation assesses overt coordination, swimming ability, learning and memory. Reflex and development parameters (preweaning) were not conducted in this study because the parameters have been shown to be highly correlated with body weights and/or physical development (ICH S5 (R2) -Note 21 (4.1.2); Lochry et al, 1984) since body weights in the male and female F1 generation, preweaning and postweaning, were not affected by maternal administration of BMN 110.

In this pre- and postnatal development study, no CNS-related observations were recorded in dams administered up to 20 mg/kg BMN 110. Body weights in the male and

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female F1 offspring, preweaning and postweaning, were not affected by maternal administration of BMN 110 (refer to BMN110-12-013, Section 14.1.3 and 14.2.3 for males and females, respectively). Memory and learning as measured by the passive avoidance and water maze assessments were not affected by maternal doses of BMN 110 at dose levels up to 20 mg/kg (refer to BMN110-12-013, Section 14.1.6 and 14.2.6 for males and females, respectively). Overall, maternal administration up to 20 mg/kg/BMN 110 did not affect the behavior, memory and learning, locomotor activity, overt coordination, and sensory function parameters or reflex development based on body weight change of the F1 generation.

Additionally, in the CNS safety pharmacology study (Study No. 0110-08-021), where the rats (6 to 7 weeks of age) were given a single administration of up to 20 mg/kg BMN 110, IV, no CNS effect was observed with a corresponding Cmax concentration of 354,417 ng/mL.

Safety Evaluation and Regulation of Chemicals, Homburger (ed), 3rd Int. Conf., Zurich 1984, pp 49-61, Standardization and Application of Behavioral Teratology Screens, Lochry, A Hoberman and M Christian.

Reference ID: 3396720

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/s/

FANG CAI
10/25/2013

DAVID B JOSEPH
10/25/2013
I concur with Dr. Cai's recommendations. See my Team Leader memo for additional comments.

BLA 125460A.doc

Comments on BLA 125460: N-acetylgalactosamine-6-sulfate sulfatase (elosulfase alpha; Vimizim)

From A. Jacobs, AD

Date Oct 24, 2013

Overall, the studies in animals are confounded by neutralizing antibodies. The studies in rats are also confounded by the administration of i.p. diphenhydramine. Furthermore, the animals are given an excess of the enzyme, whereas patients would not have a large excess. For the rat studies, the pharm/tox review compared effects for diphenhydramine with *N*-acetylgalactosamine-6-sulfate sulfatase to diphenhydramine alone, which is the best that can be done, given that this is how the studies were conducted. Studies that utilized a diphenhydramine alone control group (i.e., all rat studies) also included a control group treated with the vehicle for *N*-acetylgalactosamine-6-sulfate sulfatase. The neutralizing antibodies interfere with knowing the amount of free N-acetylgalactosamine-6-sulfate sulfatase. The neutralizing antibodies confound knowing the exposure multiples of free enzyme in animals in humans.

1. I concur that there are no pharm/tox approval issues and that there is no need for a PMR for any nonclinical studies

2. I concur that Pregnancy category C is appropriate.

3. It appears that in normal humans there is some N-acetylgalactosamine-6-sulfate sulfatase in the human placenta and in human milk.

4. I have conveyed some other comments to the reviewer and team leader, and they have addressed them as appropriate.

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/s/

ABIGAIL C JACOBS
10/24/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125,460 **Applicant: Biomarin** **Stamp Date: 3/29/2013**

Drug Name: BMN110 **BLA Type: Original**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		Carcinogenicity study was not conducted, but carcinogenicity assessment was submitted.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	√		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		√	
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			N/A
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Fang Cai 4/25/2013

Reviewing Pharmacologist Date

David Joseph 4/25/2013

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

FANG CAI
04/25/2013

DAVID B JOSEPH
04/25/2013