

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

208078Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 208-078

Submission date: March 28, 2018

Drug: amifampridine phosphate

Applicant: Catalyst Pharmaceuticals Inc.

Indication: Lambert Eaton Myasthenic Syndrome in adults

Reviewing Division: Division of Neurology Products

Discussion:

The pharmacology/toxicology reviewer and supervisor conducted a thorough review and found the nonclinical information submitted with this NDA sufficient to support approval for the indication listed above.

The carcinogenic potential of amifampridine was assessed in a 2-year carcinogenicity study in rats. The Executive Carcinogenicity Assessment Committee concluded that the study was adequate and positive for uterine tumors (endometrial carcinoma and combined endometrial adenoma/endometrial carcinoma/squamous cell carcinoma) at the mid and high doses. Other neoplasms were not considered clearly drug related. An additional study in mice is recommended by the division as a post-marketing requirement.

Conclusions: I agree that this NDA can be approved from a pharm/tox perspective and that the mouse carcinogenicity study can be a post-marketing requirement.

Comments on labeling were provided separately.

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/s/

PAUL C BROWN
11/28/2018

MEMORANDUM

**DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration**

**Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research**

Date: November 28, 2018

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 208-078 (3,4-diaminopyridine phosphate, amifampridine, Firdapse)

NDA 208-078, submitted by Catalyst Pharmaceuticals, proposes approval of 3,4-diaminopyridine (3,4-DAP) phosphate for the (b) (4) treatment of Lambert Eaton Myasthenic Syndrome in adults. The nonclinical sections of the NDA were submitted on July 22, 2015 as Part 1 of a rolling submission. The final portion (CMC) was submitted on December 15, 2016. Following initial review, a Refusal-to-File letter was issued (February 12, 2016), based on clinical and CSS deficiencies. The NDA was resubmitted on March 28, 2018, with a request for priority review, and filed on May 27, 2018 (Acknowledge Resubmission After Refuse-to-File letter, April 25, 2018). Clinical development was conducted under IND 106263, which was originally submitted by BioMarin Pharmaceuticals (December 14, 2010); sponsorship was transferred to Catalyst on March 8, 2013.

A full battery of GLP nonclinical studies was conducted for amifampridine phosphate. The data were reviewed by Dr. Banks-Muckenfuss (Pharmacology/Toxicology NDA Review and Evaluation, NDA 208078, Melissa Banks-Muckenfuss, Ph.D., November 28, 2018). Based on that review, Dr. Banks-Muckenfuss has concluded the nonclinical data are adequate and recommends approval. A brief summary of selected nonclinical data is provided in this memo; a detailed review and discussion of the nonclinical data are in Dr. Banks-Muckenfuss's review.

Pharmacology

The sponsor conducted only two studies to assess the primary pharmacology of amifampridine phosphate. In Study BMN125-10-111, amifampridine phosphate (1-3000 μ M), 3-N-acetyl metabolite (100-10000 μ M; N-(4-aminopyridin-3-yl) acetamide), and the positive control, 4-AP (1 mM), were applied to CHO cells transiently transfected with human Kv1.7 (hKv1.7). The IC₅₀ values for amifampridine phosphate and 3-N-acetyl amifampridine were 338.4 and >3000 μ M, respectively. 4-AP inhibited the hKv1.7 channel by 79.3% at 1 mM. In Study BMN125-10-112, amifampridine phosphate (1-3000 μ M), 3-N-acetyl amifampridine (1-3000 μ M), and the positive control, 4-AP (1-2 mM) were applied to HEK293 or CHO cells stably transfected with cDNA encoding human Kv1.1, 1.2, 1.3, 1.4, or 1.5. The IC₅₀ values for amifampridine phosphate were 767.5, 1278.8, 524.8, 1860.3, and 490.8 μ M for hKv 1.1, 1.2, 1.3, 1.4, and 1.5, respectively. The

3-N-acetyl metabolite exhibited <50% inhibition at the highest concentration tested. 4-AP inhibited hKv 1.1, 1.2, 1.3, and 1.4 by 73.8, 67, 76.8, and 61%, respectively, at 1 mM and hKv 1.5 by 90.2% at 2 mM. The IC₅₀ values for 4-AP are fairly consistent with those reported in published literature (Judge SIV, Bever CT. Pharmacol Therap 111:224-259, 2006).

The secondary pharmacology of amifampridine phosphate or the 3-N-acetyl metabolite was tested against a comprehensive panel of in vitro targets (receptors/binding sites/enzymes) in several studies (100034669, BMN125-10-084, BMN125-10-084, 100014186). No off-target effects were identified; IC₅₀ values for both compounds were greater than the highest concentrations of each tested (10-30 μM) for all targets evaluated.

Amifampridine phosphate was tested in a core battery of safety pharmacology studies. CNS effects were evaluated in male Wistar rat at single oral doses of 5 to 40 mg/kg (Study 20070139PGR). The only drug-related effect was a decrease in locomotor activity (“no motor activity”) in all dosed animals, up to 4 hrs post dose. Effects on respiratory function (tidal volume, minutes volume, respiratory rate) were assessed in male Sprague-Dawley rat following single doses of 1-10 mg/kg (Study BMN125-10-058); no drug-related effects were observed.

Effects of amifampridine phosphate on the cardiovascular system were assessed in in vitro and in vivo studies. In a hERG assay in CHO-KI cells, the IC₅₀ was >30 μM (Study 1711-AGE-03). In an in vitro female New Zealand White rabbit Purkinje cell assay (1710/AGE/03), there were concentration-dependent (30 and 100 μM; none at ≤10 μM) increases in APD₉₀ (1 Hz: 11-12 and 15-18%, respectively; 0.2 Hz: 18 and 40%, respectively) and APD₅₀ (1 Hz: 15-16 and 14-21%, respectively; 0.2 Hz: 26 and 46%, respectively). The in vivo assay was conducted in 8 male beagle dogs; single oral doses of 0.05-0.50 mg/kg were administered to each animal according to a crossover design. The only effects observed was an 8% decrease in PR interval at 0.5 mg/kg and a 7% decrease in mean arterial pulse pressure at 0.15 and 0.5 mg/kg.

PK/ADME

The PK/ADME of amifampridine and amifampridine phosphate was assessed in Sprague-Dawley rat, beagle dog, and human (and, to a lesser extent, in monkey, rabbit, and minipig). Following oral administration in rat, amifampridine was rapidly absorbed (T_{max} < 1hr) and widely distributed to tissues, with highest levels in organs of excretion (kidney, liver, and GI tract) and lowest levels in eye (lens), fat, and the CNS. The t_{1/2} following oral administration in rat and dog was ~0.4-0.6 and ~1.7-2.5 hrs, respectively. The major route of elimination in rat and dog was renal, with urinary excretion accounting for ~88% of dose after oral administration. Serum protein binding (measured in vitro) was low (<15%) in all species tested (rat, dog, monkey, and human). The major metabolic pathway, in vitro and in vivo, was N-acetylation of amifampridine, resulting in N-(4-aminopyridin-3-yl) acetamide (3-N-acetyl metabolite), in rat and human. The 3-N-acetyl metabolite is not formed in dog because of the absence of the N-acetylation pathway.

Toxicology (doses are expressed as mg/kg amifampridine phosphate unless otherwise specified; conversion factor of 52.7% to calculate free base from salt and 1.9 to calculate salt from free base)

Rat (Sprague-Dawley)

Single-dose: In single-dose studies, amifampridine or amifampridine phosphate was administered by intravenous (IV) injection or oral gavage. In the IV study (12.5, 25, or 50 mg/kg; 0, 2.5, 10, or 25 mg/kg amifampridine phosphate), the first animal (male) dosed at 50 mg/kg convulsed and died; no additional animals received that dose. At 25 mg/kg, death, convulsions, and other clinical signs were observed; clinical signs (including irregular breathing and forelimb agitation) were observed at 10 mg/kg. The NOAEL was 2.5 mg/kg.

In the oral (gavage) study of amifampridine phosphate (0, 2.5, 10, 25, 50, or 100 mg/kg), deaths, accompanied by convulsions, occurred at 50 and 100 mg/kg. Clinical signs (e.g., hypersalivation, excessive grooming, unsteady gait, or agitated forelimb movements) were observed at doses ≥ 25 mg/kg. Doses of 2.5 and 10 mg/kg were NOAELs. In the oral (gavage) study of amifampridine (0, 2.5, 10, 25, or 50 mg/kg), similar findings were observed; convulsions and death occurred in one HD female.

No drug-related effects on body weight or upon macroscopic examination were observed in the IV or oral studies. No toxicokinetic (TK) data were collected in any of the studies.

Repeat-dose: amifampridine phosphate was administered by oral gavage for 7 days (0, 1, 10, 30, or 60 mg/kg/day, given TID), 21 days (0, 75, 150, or 300 mg/kg/day, given TID), 4 weeks (+2-week recovery; 0, 7.5, 24, or 75 mg/kg/day, given TID), 13 weeks (+4-week recovery; 0, 7.5, 22.5, or 75 mg/kg/day, given TID), and 26 weeks (+4-week recovery; 0, 7.5, 22.5, or 75 mg/kg/day, given TID). One additional 4-week study was conducted to collect toxicokinetic data to support the 4-week (+2-week recovery) study. Drug-related death and severe clinical signs (e.g., paddling, twitching/jumping/shaking) were observed at the mid and high doses in the 21-day study; the study was terminated after Day 2.

The primary drug-related findings in the other subchronic and chronic studies were clinical signs (e.g., hyperactivity, excessive grooming, hypersalivation, tremors) and salivary gland hypertrophy. (Convulsions were not reported in studies conducted with TID dosing.) In the 4-week study, clinical signs were evident at all but the low dose. The Week 4 (amifampridine) toxicokinetic data (conducted in the 4-week TK study) are summarized in the following table (highest daily C_{max} ; for AUC, "t" was 12.75-15.00 hr)

DOSE (mg/kg/day)	MALES		FEMALES	
	C_{max} (ng/mL)	AUC _t (ng*hr/mL)	C_{max} (ng/mL)	AUC _t (ng*hr/mL)
7.5	16.9	43.3	13.2	46.4
24	51.9	211	82.9	302
75	556	2411	285	3565

In the 13-week study, one HD TK female, exhibiting severe clinical signs (whole body myoclonic jerks, limb paddling), was sacrificed moribund on Day 92; this death was considered drug-related. Plasma amifampridine exposure in this animal was similar to those in other HD animals. Notable clinical signs in several other HD TK animals (limb paddling, myoclonic jerking, sensitivity to touch) were associated with higher plasma amifampridine levels, thought

to be the result of being dosed in the fasted state. (Plasma levels at 0.5 hr post dose [$\sim T_{max}$] were 2700-4240 ng/mL in these TK animals.) Clinical signs were not evident in main-study animals. Mandibular salivary gland hypertrophy was detected in males and females at the high dose at the end of the dosing, but not recovery, period. The Day 92 TK data are summarized in the following table (highest daily C_{max} ; AUC values are $AUC_{(D1+D2+D3)}$; $t_{1/2}$ is range; NC = not calculable).

DOSE (mg/kg/day)	MALES			FEMALES		
	C_{max} (ng/mL)	AUC (ng*hr/mL)	$t_{1/2}$ (hr)	C_{max} (ng/mL)	AUC (ng*hr/mL)	$t_{1/2}$ (hr)
amifampridine						
7.5	19.0	70.4	1.02-1.48	32.5	94.2	0.407-1.15
22.5	88.5	314	0.748-1.73	223	577	0.723-1.00
75	938	3166	0.957-1.26	1204	4275	0.840-1.10
3-N-acetyl amifampridine						
7.5	587	4635	1.95-2.28	615	4685	1.61-2.13*
22.5	1757	12572	1.65-3.05	1587	13026	1.63 [#]
75	3627	40907	NC	3400	35984	NC

*not calculable in one animal, [#]not calculable in two animals

In the 26-week study, clinical signs observed at the mid and high doses consisted of CNS (e.g., paddling, twitching, and tremors) and respiratory (dyspnea, tachypnea, labored breathing, panting) effects. Submandibular salivary gland hypertrophy, “characterized microscopically by diffuse enlargement of acinar cells with slightly basophilic, stippled cytoplasm,” was observed at the high dose in males and females. Salivary gland hypertrophy was not observed in recovery animals. The Day 182 TK data (collected in nonfasted animals) are summarized in the following table (C_{max} is highest daily value; AUC values are $AUC_{(D1+D2+D3)}$; $t_{1/2}$ for dose 1 only).

DOSE (mg/kg/day)	MALES			FEMALES		
	C_{max} (ng/mL)	AUC (ng*hr/mL)	$t_{1/2}$ (hr)	C_{max} (ng/mL)	AUC (ng*hr/mL)	$t_{1/2}$ (hr)
amifampridine						
7.5	25.2	70.2	2.1	25.3	91.1	1.6
22.5	109	279	1.4	208	470	1.3
75	779	1994	1.3	801	3480	1.0
3-N-acetyl amifampridine						
7.5	714	5230	2.2	633	4860	1.4
22.5	3180	15380	2.1	1690	14550	1.6
75	4050	52100	2.7	3690	47600	2.7

Dog (Beagle)

Single dose: In the single-dose study, amifampridine phosphate was administered to dogs (4/sex) as an oral capsule at doses of 1.9, 3.8, 5.7, or 6.25 mg/kg/day, given TID. Two planned higher doses were removed from the study because of clinical signs observed at the lower doses. Hypersalivation and diarrhea were observed at all doses; shaking, tremors, panting, and coughing were observed at the mid and high doses. One female was particularly affected at the high dose, exhibiting ataxia, hypersalivation, tremors, and unresponsiveness. The low dose was considered

an NOAEL. Plasma amifampridine exposures at the low, mid, and high doses were: C_{max} (highest daily value) of 156, 438, and 446 ng/mL, respectively; plasma $AUC_{(0-\infty)}$ of 457, 1420, and 1480 ng*hr/mL, respectively.

Repeat-dose: amifampridine phosphate was administered by oral gavage in a 14-day dose-ranging study (0.75-24 mg/kg/day, given TID), a 4-week study (0, 1.9, 5.7, or 7.5 mg/kg/day, given TID), a 4-week (+2-week recovery) study (0, 1, 2.5, or 6.25 mg/kg/day, given TID), and a 9-month (+4-week recovery) study (0, 1, 1.9, and 3.8 mg/kg/day, given TID).

In the dose-ranging study, one male exhibited prolonged convulsions at the high dose and was sacrificed. Clinical signs (e.g., hypersalivation, impaired locomotor activity, tremors, hindlimb stiffness, unresponsive) were observed at 7.5-12 mg/kg/day, although no convulsions were observed. In the 4-week study, two HD males died (one found dead, one sacrificed moribund); convulsions were observed in these two males and in HD survivors. Clinical signs (e.g., tremors, stiffness of hindlimbs, loss of balance, hypersalivation, cough) occurred at the mid and high doses. An increase in white filament in the posterior lens capsule and vitreous body were detected in MD and HD females upon ophthalmologic examination during Week 4. Myodegeneration of the tongue and skeletal muscles was observed at all doses. The low and mid doses were considered an NOAEL and an MTD, respectively. The TK analysis was limited (only one sampling time after the 2nd and 3rd doses).

In the 4-week (+2-week recovery) study, no NOAEL was identified because of CNS clinical signs (e.g., tremors, subdued behavior, decreased locomotor activity) at all doses; convulsions were observed at the high dose. The Week 4 TK data for amifampridine are summarized in the following table (C_{max} is highest daily).

DOSE (mg/kg/day)	MALES		FEMALES	
	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)
1	51	500	56	523
2.5	166	1414	146	1314
6.25	367	3146	324	2841

In the 9-month study, there were no premature deaths; however, convulsions were observed in MD and HD males. Clinical signs (e. g., tremors, lip licking hypersalivation) were observed at all doses. Corneal opacities were detected in two HD animals (1/sex); no drug-related findings were detected in recovery animals. Day 273 TK data for amifampridine are summarized in the following table; plasma samples were collected for analysis predose, 0.5, 1, 2, and 6 hrs after the first dose, 1 hr after the 2nd dose, and 1 hr after the 3rd dose ($AUC_{(0-24h)} = 3 \times AUC_{(0-6h)}$).

DOSE (mg/kg/day)	MALES			FEMALES		
	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	$t_{1/2}$ (hr)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	$t_{1/2}$ (hr)
1.0	68.3	688	2.5	81.4	653	2.1
1.9	151	1300	2.2	159	1220	2.0
3.8	283	2590	2.2	260	2310	1.9

The primary drug-related effects in both rat and dog were clinical signs, including convulsions in both species. Convulsions are a known risk for this class of compounds. In humans, at the maximum recommended single dose of 20 mg, the C_{max} for amifampridine in slow acetylators was 56.7 ng/mL ($AUC_{(0-\infty)}$ was 146 ng*hr/mL); the C_{max} for 3-N-acetyl amifampridine in fast acetylators was 268 ng/mL ($AUC_{(0-\infty)}$ was 1213 ng*hr/mL). In rat, TK data are not available for studies in which convulsions were observed; however, the C_{max} at the highest doses not associated with convulsions ranged from 285 to 1204 ng/mL for amifampridine and from 3400 to 4050 ng/mL for 3-N-actyl amifampridine. In dog, the amifampridine C_{max} at the NOAEL for convulsions was 146-166 ng/mL in the 4-week study but only 68.3-81.4 ng/mL in the 9-month study. At the low-effect dose for convulsions in the 9-month study, the C_{max} was 151-159 ng/mL. In neither species is there a 10-fold margin between the lowest amifampridine C_{max} at the NOAEL for convulsions in animals and that in humans at the 20 mg dose.

Reproductive and Developmental Toxicology

A standard battery of reproductive and developmental toxicology studies of orally (gavage) administered amifampridine phosphate was conducted in Sprague-Dawley rat and New Zealand White rabbit.

In a combined fertility and early embryonic (to implantation) and embryofetal development study in rat, amifampridine phosphate was administered to males and females at doses of 0, 7.5, 22.5, or 75 mg/kg/day, given TID, prior to (4 and 2 weeks, respectively) and throughout mating and continuing in females throughout gestation day (GD) 17; dams were sacrificed on GD 21 for evaluation of litter and fetal parameters. Satellite animals were dosed for a limited toxicokinetic analysis (TK females were dosed on GDs 6-17); TK parameters were calculated. Drug-related clinical signs (e.g., excessive grooming, twitching) were observed only in HD males, during the post-mating phase. No adverse effects were observed on mating or fertility indices, on litter parameters, or on fetal development, except for a slight, dose-related increase in mean fetal weight in males ($\leq 6\%$).

In the embryofetal development study in rabbit, amifampridine phosphate was administered at doses of 0, 9, 30, or 57 mg/kg/day, given TID on GDs 7-20. Satellite animals were dosed for toxicokinetic analysis; however, samples were not analyzed. Does were sacrificed on GD 29 for evaluation of litter and fetal parameters. Dose selection was based on data from a preliminary dose-ranging study in nonpregnant females (doses of 3, 9, 30, or 90 mg/kg/day, given TID) and a dose-ranging study in pregnant does (0, 9, 30, or 57 mg/kg/day, given TID on GDs 7-20). In the preliminary study, one death, preceded by convulsions, occurred at the high dose. In the dose-ranging study, two deaths (one MD and one HD [TK] does), preceded by convulsions (presumed in the HD doe), were attributed to drug; no drug-related developmental effects were observed. The GD 19 TK data are summarized in the following table (highest daily C_{max}).

DOSE (mg/kg/day)	C _{max} (ng/mL)	AUC _(0.25-6h) (ng*hr/mL)	
		1 st DOSE	3 rd DOSE
amifampridine			
9	83.2	85.7	89.2
30	520	107	463
90	477	733	825
3-N-acetyl amifampridine			
9	992	2230	2380
30	3740	9490	10200
90	5290	14600	15500

In the pivotal rabbit study, there were 8 deaths. The five deaths at the high dose, two accompanied by clinical signs (whole body tremors, violent circling, lateral recumbent position) and three by reduced body weight gain and food consumption, were considered drug-related. None of the three mid-dose deaths was considered drug-related, although a cause of death was not identified in one. In survivors, clinical signs (excessive licking, twitching) were observed at the high dose. Although absolute body weight was not notably affected, body weight loss was observed in mid and high dose groups during the first week of gestation (GD 7-11), which contributed to the reduced overall body weight gain at these doses. Food consumption was reduced at the mid and high doses. Reproductive performance and litter parameters were not affected. The sponsor reported two problems encountered in the evaluation of skeletal specimens: (1) “skeletal process malfunction resulting in damaged specimens” and (2) “After processing specimens in preparation for skeletal evaluation, a number of fetuses were found with dissociated identification tags.” Different sets of samples were affected, i.e., no sample was affected by both problems. According to the sponsor, neither problem affected the validity of the study results. No drug-related developmental effects were observed. TK analysis was conducted on samples collected at 0.5 and 2 hrs after each dose on GDs 7 and 20; levels were higher for both amifampridine and 3-N-actyl amifampridine at 0.5 hrs post dose. On GD 20, plasma amifampridine levels were 52.9-67.7, 143-176, 430-563 ng/mL at low, mid, and high dose, respectively; plasma 3-N-acetyl amifampridine levels 642-822, 2726-3080, and 3338-3988 ng/mL at low, mid, and high dose, respectively.

In the pre- and postnatal development study in rat, amifampridine phosphate was administered at doses of 0, 7.5, 22.5, or 75 mg/kg/day, given TID, from GD 6 through lactation day (LD) 20. Satellite groups were dosed from GD 6 through LD 14 for a limited toxicokinetic analysis. There were no drug-related deaths. Clinical signs were observed at the mid and high doses during gestation (excessive grooming/licking/scratching/chewing, twitching, eye squinting, convulsion [1 HD]) and at the high dose during lactation (excessive grooming/licking/chewing, twitching, limb paddling). No effects were observed on body weight or body weight gain. Selected findings are summarized in the following table (*p<0.05).

FINDING	DOSES (mg/kg/day)			
	0	7.5	22.5	75
F0 generation				
dams with stillborn pups	1/25	2/25	4/24	5/25
stillborn pups	4/281	2/287	18/275	11/287
F1 generation				
Day 4 viability index (%)	95	97	90	89
pup weight (g), LD 21				
males	55.47	54.98	52.53	51.19*
females	53.41	53.48	51.23	49.67
vaginal opening	32.3	32.0	33.3*	33.2*

The TK data are summarized in the following table; plasma and milk samples were collected 0.5 hrs post dose.

DOSE (mg/kg/day)	SAMPLING TIME	PLASMA		MILK
		1 st DOSE	3 rd DOSE	1 st DOSE
amifampridine				
7.5	GD 6	12.4	21.6	
22.5		39.4	113	
75		412	706	
7.5	LD 14	11.8	23.3	9.46
22.5		46.2	92.8	36.5
75		374	980	298
3-N-acetyl amifampridine				
7.5	GD 6	312	454	
22.5		672	1100	
75		1560	1990	
7.5	LD	323	666	264
22.5		743	1510	589
75		1640	2600	2440

Carcinogenicity

The carcinogenic potential of amifampridine phosphate was assessed in a 104-week dietary carcinogenicity study in Sprague-Dawley rat. The sponsor initially proposed (b) (4) however, the Executive CAC recommended the dietary route. No Executive CAC review was conducted for the dietary study protocol because an SPA was not submitted. (During clinical development, the Division informed the sponsor that a carcinogenicity study in mouse may be conducted post-approval, if the NDA is approved.)

In the 104-week study, amifampridine phosphate was administered in the diet to Sprague-Dawley rats at doses of 0, 15, 48, 152/105 mg/kg in males and 0, 15, 48, 114/105 mg/kg in females. The high dose was initially higher in females because of higher plasma exposures compared to males; however, after Week 16, it was determined that 105 mg/kg was a maximum feasible dose for both males and females. Toxicokinetic analysis was conducted in satellite animals dosed for up to 52 weeks. Animals were group housed (“2 animals/sex/cage”; presumed

to mean 2 males or females/cage), which is not optimal for dietary studies because of the inability to ensure adequate dosing of individual animals.

According to the sponsor’s and FDA’s statistical analysis (Statistical Review and Evaluation, Carcinogenicity Study, NDA 208078, Mbodj Malick, Ph.D., September 20, 2018), there was a dose-dependent, statistically significant increase in survival (all dose groups) in males (47, 67, 73, and 72% at low, mid, and high dose, respectively) and a statistically significant increase in survival at the high dose in females (73% vs 48% in control). Neoplastic findings consisted of a significant increase in uterine tumors at the mid and high doses, as summarized in the following table.

UTERINE TUMORS*	DOSES (mg/kg/day)			
	0	15	48	105
benign adenoma, endometrial	0/60	0/59	0/60	1/60
malignant carcinoma, endometrial	0/60	3/59	13/60	9/60
malignant squamous cell carcinoma	0/60	1/59	0/60	0/60
total number of affected animals	0/60	4/59	13/60	10/60

*significant increase in malignant carcinoma, mid (p<0.001) and high (p=0.0025) doses; significant increase in combined benign adenoma/malignant carcinoma/malignant carcinoma, squamous cell, mid (p<0.001) and high (p=0.0012) doses

The study was reviewed by the Executive CAC. The Committee concluded:

“...the study was adequate and positive for uterine tumors (endometrial carcinoma and combined endometrial adenoma/endometrial carcinoma/squamous cell carcinoma) at the mid and high doses tested.”

According to the sponsor’s evaluation, there was also a statistically significant increase in schwannomas, as summarized in the following table (doses expressed in mg/kg).

TISSUE	SCHWANNOMA TYPE	MALES				FEMALES			
		0	15	48	105	0	15	48	105
heart	malignant endocardial	0/60	0/60	1/60	0/59	0/60	0/60	0/60	2/60
Harderian gland	malignant	0/60	0/60	0/60	1/60	0/60	0/60	0/60	1/60
skin/subcutis	malignant	0/60	0/60	1/60	1/60	0/60	0/60	0/60	0/60
	benign	0/60	0/60	1/60	0/60	0/60	0/60	0/60	0/60
cervix	malignant					0/60	1/59	1/60	3/60
uterus	malignant					0/60	0/59	1/60	0/60
clitoral gland [#]	malignant					0/0	1/3	0/1	0/1
mandibular salivary gland	malignant	0/60	0/60	0/60	0/60	0/60	0/60	0/60	1/60
total number of primary tumors		0/60	0/60	3/60	2/60	0/60	2/60	2/60	7/60
total number of affected animals		0/60	0/60	3/60	2/60	0/60	2/60	2/60	4/60

[#]not included in the sponsor’s statistical analysis because clitoral gland was not examined in all females

According to the sponsor, “Although morphologically distinct, endocardial Schwannomas in the heart were observed in one male given 25 mg/kg/day and two females given 55 mg/kg/day; therefore, they were also included in the inter-group statistical comparisons” (mid and high doses expressed as free base). The sponsor considered the following findings statistically significant (at

the $p = 0.05$ level, not consistent with FDA's method analysis of rare tumors such as schwannomas):

- Combined schwannoma in Harderian gland, heart, and skin/subcutis in males, dose-response ($p = 0.0367$); p -values for pairwise comparisons at mid and high doses were >0.05
- Cervix schwannoma, dose-response ($p = 0.0394$); p -value for pairwise comparison at high dose >0.05
- Combined schwannoma in cervix, Harderian gland, heart, mandibular salivary gland, and uterus, dose-response ($p = 0.0280$); p -values for pairwise comparisons at mid and high doses were >0.05

The incidences of schwannoma were reported as not significantly increased in either males or females in the FDA statistician's review. In females, a p -value of 0.0251 was obtained by trend test for schwannoma combined across tissues (statistical significance at 0.025 for rare tumors, i.e., spontaneous rate $<1\%$), which is borderline; however, pair-wise comparisons were not significant ($p=0.5253$, 0.2784, and 0.0803 at low, mid, and high doses in females; statistical significance at $p=0.05$ for rare tumors).

The 52-week TK data are summarized in the following table.

DOSE (mg/kg/day)	MALES		FEMALES	
	C_{max} (ng/mL)	AUC _(0-24h) (ng*hr/mL)	C_{max} (ng/mL)	AUC _(0-24h) (ng*hr/mL)
amifampridine				
15	9.55	127	13.9	149
48	73.5	762	116	1120
105	392	4820	565	7000
3-N-acetyl amifampridine				
15	578	8990	617	8850
48	2250	31900	1880	27800
105	3760	63800	3360	53800

Genetic Toxicology

The sponsor conducted a standard battery of genetic toxicology assays for amifampridine phosphate; all assays were conducted consistent with the relevant OECD and ICH guidances. Amifampridine phosphate was negative in the Ames and the in vivo rat (Sprague-Dawley) micronucleus assays but positive (significant trend test; increase in small colonies) in the in vitro mouse lymphoma *tk* assay, in the absence of metabolic activation (24-hr incubation).

Conclusions and Recommendations

The nonclinical studies conducted by the sponsor support approval of the NDA, with appropriate labeling and a post-marketing requirement for a carcinogenicity study in mouse.

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/s/

LOIS M FREED
11/28/2018

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 208078
Supporting document/s: 1, 4, 9 (eCTD0000, 0002, 0007)
Applicant's letter date: 7/22/15, 12/16/15, 3/28/18
CDER stamp date: 7/22/15, 12/16/15, 3/28/18
Product: Firdapse®, amifampridine phosphate
(3,4-diaminopyridine, 3,4-DAP, BMN125)
Indication: Lambert-Eaton myasthenic syndrome (LEMS)
Applicant: Catalyst Pharmaceuticals, Inc.
Review Division: DNP
Reviewer: Melissa Banks-Muckenfuss, PhD
Supervisor: Lois Freed, PhD
Division Director: Billy Dunn, MD
Project Manager: Heather Bullock, RN, BSN, MSHS

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208078 are owned by Catalyst Pharmaceuticals or are data for which Catalyst Pharmaceuticals has obtained a written right of reference. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208078.

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1 Executive Summary

1.1 Introduction

Amifampridine (3,4-diaminopyridine, to be marketed as Firdapse®) has been developed by Catalyst Pharmaceuticals for the treatment of Lambert Eaton Myasthenic Syndrome (LEMS).

1.2 Brief Discussion of Nonclinical Findings

Commonly accepted as a potassium channel blocker for decades (cf., Kirsch & Narahashi, 1978), the sponsor's data demonstrate that amifampridine shows a relatively weak concentration-dependent block of several Kv_1 voltage-gated potassium channels (IC_{50} s of approximately 300 to 1900 μ M). Related compound 4-aminopyridine is also known to show broad-spectrum voltage-gated potassium channel blockade at millimolar concentrations (e.g., Dunn and Blight, 2011); however, there has been some debate about the precise mechanism in recent literature (e.g., Wu et al, 2009). One major human metabolite was identified for 3,4-diaminopyridine, the 3-N-acetyl metabolite; the metabolite was not pharmacologically active at the potassium channels tested in the sponsor's assays. In vitro assays of amifampridine and the 3-N-acetyl metabolite demonstrated a lack of clear secondary pharmacological activity but were only tested up to a maximum concentration of 10 μ M.

In oral acute and subchronic toxicity studies, mortality and CNS toxicity (e.g., convulsions) were observed. CNS and respiratory clinical signs included: tremors, convulsions, non-responsiveness to stimuli, stiffness of limbs, outstretched limbs, hyperesthesia, and labored breathing. The NOAELs were 13 mg/kg in mice and 5.3 mg/kg in rat, with mortality and/or convulsions occurring at doses \geq 26 mg/kg in mice and \geq 40 mg/kg/day (given TID dose) in rats. In dogs, 2 mg/kg/day (given TID) was identified as a maximum tolerated dose, with tremors, emesis, and convulsions occurring at doses \geq 3 mg/kg/day (given TID), and deaths occurring at 4 mg/kg/day (given TID).

In the chronic toxicity studies in rat and dog, adverse CNS and respiratory clinical signs occurred. Systemic exposures at the NOAELs were in a clinically relevant range.

The 26-week toxicity study of amifampridine (0, 3.9, 11.8, and 39.5 mg/kg/day, given TID) by oral gavage in rats showed dose-related CNS and respiratory clinical signs at MD and HD. Enlarged salivary glands with increased organ weights, correlating histologically with acinar cell hypertrophy, were observed at the HD; this effect showed reversibility after the recovery period. Amifampridine exposure increased with increasing dose, with females generally showing slightly higher exposures than males. Systemic exposure to the 3-N-acetyl metabolite increased with increasing dose, but in a less-than-dose-proportional manner. At the NOAEL, C_{max} and AUC exposures to amifampridine were less than or similar to those at the MRHD and exposures to the 3-N-acetyl metabolite were approximately 3 times that at the MRHD.

The 9-month toxicity study of BMN-125 (0, 0.53, 1.0, and 2.0 mg/kg/day, given TID) by oral capsule in dogs showed drug-related CNS and respiratory clinical signs. Dose-related clinical signs included: hypersalivation, panting, tremors, aggressive behavior, and/or convulsions (\geq MD). Reduced body weights were observed. Clearly drug-related histopathological alterations were not observed. Increased amifampridine exposures were observed with increasing doses, without clear sex differences; dogs did not show exposures to the 3-N-acetyl metabolite. At the NOAEL (LD), the C_{max} and AUC exposures were similar to exposures at the MRHD (80 mg/day).

Amifampridine was negative in an in vitro bacterial reverse mutation (Ames) assay, and in the in vivo rat micronucleus and rat hepatocyte unscheduled DNA synthesis (UDS) assays; amifampridine was positive in an in vitro mouse lymphoma assay in the absence of metabolic activation.

A 2-year carcinogenicity bioassay in rats was conducted using dietary administration (to maximize exposures without TID dosing) at doses of approximately 0, 8, 25, and 55 mg amifampridine/kg/day. Drug-related uterine endometrial adenocarcinomas and carcinomas were observed at the MD and HD. In addition to exceeding the incidence observed in concurrent controls and reaching statistical significance, the incidences at the MD and HD (7%, 22%, and 17% in the LD, MD, and HD, respectively) clearly exceeded the background incidence of endometrial carcinomas in Harlan Sprague Dawley rats (i.e., approximately 1 to 4%; Dinse et al., 2010; National Toxicology Program, 2013, 2017). A seemingly dose-related but low incidence of Schwannomas in females did not reach statistical significance. Additionally, drug-related non-neoplastic findings were observed, to include retinal atrophy in HDF and other dose-related alterations in female reproductive tissues (e.g., uterine dilatation, ovarian cysts). The C_{max} was reduced and the AUC was increased with dietary exposures. The no-effect level for tumorigenicity was 55 mg/kg in males and 8 mg/kg in females; at those doses, AUC exposures to amifampridine and the 3-N-acetyl metabolite in males were approximately 8 and 13 times (respectively) those at the MRHD, but the AUC exposures in females were less than and approximately 2 times (respectively) those at the MRHD.

Reproductive and developmental toxicity was evaluated in a combination fertility and developmental toxicity study in rats, an embryofetal development study in rabbits, and a pre- and postnatal development study in rats. In rats, litter loss, stillbirth, and reduced pup viability occurred, slight reductions were observed in male mating and fertility indices (without a clear effect on sperm parameters), and a slight delay in sexual maturation was observed in females. In rabbits, mortality and reduced body weight gains were observed at \geq MD; clearly drug-related adverse embryofetal effects were not observed. Significant maternal toxicity was observed in both rats and rabbits.

Four potentially genotoxic impurities were referred for nonclinical evaluation. Of these, two (b) (4) were of potential toxicologic concern. The sponsor stated that (b) (4) is being controlled at NMT (b) (4)%, which would not exceed the TTC of (b) (4) μ g/day at the MRHD of 80 mg/kg. (b) (4) was not detected in the batches of

amifampridine phosphate used in the 2-year dietary carcinogenicity assay. (b) (4) was shown to be negative in an in vitro Ames assay, as conducted by the sponsor.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, taking into consideration the population and the relative lack of other available treatments, the application is recommended for approval. It is recommended that important observed toxicities (e.g., endometrial tumors) be addressed in labeling.

1.3.2 Additional Nonclinical Recommendations

PMRs/PMCs

- 1) As previously agreed upon with the Division (correspondence dated 9/1/10), the sponsor will conduct a mouse carcinogenicity assay in Phase 4.

1.3.3 Suggested Labeling

Labeling is currently under discussion; the selections below are suggested wording.

-----INDICATIONS AND USAGE-----

FIRDAPSE is a potassium channel blocker indicated for the (b) (4) treatment of Lambert-Eaton Myasthenic Syndrome (LEMS) in adults. (1)

Use in Special Populations

Pregnancy: Based on animal data, may cause fetal harm (8.1)

8.1 Pregnancy (second paragraph)

(b) (4)

(b) (4)

12.1 Mechanism of Action

The mechanism by which amifampridine exerts its therapeutic effect in LEMS (b) (4)

(b) (4)

NONCLINICAL TOXICOLOGY

(b) (4)

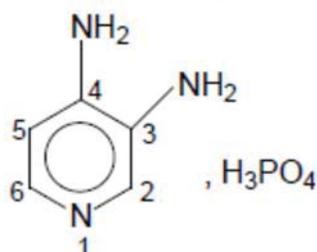
2 Drug Information

2.1 Drug

CAS Registry Number	446254-47-3
Generic Name	amifampridine phosphate
Code Name	DAPP, 3,4-DAP Phosphate, BMN-125
Chemical Name	3,4-diamonopyridine phosphate Pyridine-3,4-diamine phosphate 3,4-Pyridinediamine, phosphate (1:1)
Molecular Formula/Molecular Weight	$C_5H_{10}N_3O_4P$ or $C_5H_7N_3 \cdot H_3PO_4$; 207.1 g/mol free base = 109.1 g/mol

Structure

(below, from the sponsor's submission)



Pharmacologic Class

potassium channel blocker

2.2 Relevant INDs & NDAs

IND 106263

3,4-DAP

LEMS

Catalyst

2.3 Drug Formulation

The amifampridine phosphate 10 mg tablet composition is shown in sponsor's Table 3.2.P.1-1, below.

Table 3.2.P.1-1. Amifampridine Tablet Drug Product Composition

Component	Quantity per Tablet (mg)	Percentage per Tablet (%)	Function
Amifampridine Phosphate	18.98 ^a	(b) (4)	Active
Microcrystalline Cellulose, NF, Ph. Eur.			(b) (4)
Colloidal Silicon Dioxide, NF, Ph. Eur.			
Calcium Stearate, NF, Ph. Eur.			
Total per Tablet	(b) (4)	100	

Ph. Eur. = European Pharmacopeia; NF = National Formulary

^a18.98 mg of amifampridine phosphate drug substance corresponds to 10.00 mg of amifampridine base form.

2.4 Comments on Novel Excipients

None.

2.5 Comments on Impurities/Degradants of Concern

CMC identified four potential impurities of amifampridine for nonclinical assessment: (b) (4)

The sponsor conducted (Q)SAR analyses for (b) (4) these assessments were not ICH M7-compliant. Based on these (Q)SAR analyses, the sponsor reported that (b) (4) and (b) (4) did not show alerting structures. (b) (4) is

structurally-related to 3,4-diaminopyridine. The lack of structural alerts for (b) (4) was verified in an internal ICH M7-compliant assessment.

The sponsor reported that (b) (4) showed a structural alert (in a non-ICH M7 compliant DEREK for Windows assessment), and conducted an Ames assay, which was negative (for review, see **Special Toxicology**). In the Ames assay, the concentrations present in the formulations were not verified, but cytotoxicity was observed.

The sponsor stated that (b) (4) is not isolated because it is immediately processed to (b) (4). Additionally, the sponsor plans to control (b) (4) at NMT (b) (4) % (in agreement with FDA), which is below the TTC of (b) (4) µg/day at a MRHD of 80 mg/day.

2.6 Proposed Clinical Population and Dosing Regimen

The sponsor is seeking approval of amifampridine phosphate 10 mg tablets for the treatment of Lambert-Eaton myasthenic syndrome (LEMS). The MRHD is 80 mg/day, in 3 to 4 divided doses per day (maximum individual dose= 20 mg).

2.7 Regulatory Background

IND 106263 was submitted on January 13, 2011, by BioMarin Pharmaceuticals, Inc., and was later purchased by Catalyst Pharmaceuticals, Inc. Catalyst was granted Orphan Designation (09-2953) on November 12, 2009, and Breakthrough Therapy Designation for LEMS in adults on August 22, 2013. NDA 208078 for Firdapse was originally submitted on December 16, 2015, and received a refuse-to-file (RTF) letter dated February 12, 2016, citing clinical, clinical pharmacology, and CSS deficiencies.

Outside the US, Firdapse was approved to treat LEMS in the EU on December 23, 2009 (EMA/793638/2009).

3 Studies Submitted

3.1 Studies Reviewed

Several Pharmacology studies, including studies BMN125-10-112 (101124.DPW), BMN125-10-111 101123.DPW), 100034669, 100014186, BMN125-10-084, BMN125-10085

Safety Pharmacology studies, including studies: 20070139PGR, BMN125-10-058, BMN125-10-059, 1710-AGE-03, 1711-AGE-03

Several ADME studies

Acute and Subchronic Oral Administration Toxicity studies in rodents and dogs, including studies: (b) (4) 266/004, (b) (4) 266/005, (b) (4) 266/012, (b) (4) 266/014, aa40847

Study BMN125-10-055 (13-week rat toxicity)

Study S12033 (26-week rat toxicity study)

Study S12032 (9-Month dog toxicity study)

Study (b) (4)-010310 (Ames assay)

Study (b) (4)-010515 (MLA)

Study (b) (4)-010417 (Micronucleus assay)

Study (b) (4)-020404 (UDS assay)

Study 8264515 (2-year rat carcinogenicity assay)

Study (b) (4)-080408 (Ames assay of (b) (4))

Study BMN125-13-012 (8283616; Combination Rat Fertility and EFD)

Study BMN125-13-015 (Rabbit EFD), and supporting studies

Study BMN125-13-025 (PPND)

3.2 Studies Briefly/Not Reviewed

Selected ADME studies

Studies by IV or IP route of administration

Several non-pivotal studies

3.3 Previous Reviews Referenced

None.

4 Pharmacology

4.1 Primary Pharmacology

In vitro, amifampridine phosphate showed weak concentration-dependent inhibition of human Kv1.1, 1.2, 1.3, 1.4, and 1.5 potassium channels in HEK293- or CHO-transfected cells (non-GLP; see sponsor's summary table, below).

Channel	Test Article	Concentration (µM)	% Inhibition			IC ₅₀ (µM)
			Mean	SD	N	
hKv1.1	BMN125	1	1.4 %	5.0 %	2	767.5
		10	4.6 %	1.6 %	2	
		30	8.9 %	4.9 %	2	
		100	22.1 %	3.2 %	2	
		300	37.6 %	1.4 %	2	
		1000	53.6 %	1.8 %	2	
	3-N-acetyl BMN125	100	1.6 %	0.8 %	2	> 3000
		1000	0.5 %	2.0 %	2	
		3000	4.0 %	3.6 %	2	
hKv1.2	BMN125	1	0.8 %	1.1 %	2	1278.8
		10	-0.1 %	1.5 %	2	
		30	-3.4 %	5.1 %	2	
		100	6.4 %	7.4 %	2	
		300	24.3 %	1.8 %	2	
		1000	43.3 %	0.7 %	2	
	3-N-acetyl BMN125	100	-2.2 %	2.7 %	2	> 3000
		1000	5.0 %	5.7 %	2	
		3000	2.2 %	0.2 %	2	
hKv1.3	BMN125	1	0.1 %	1.6 %	2	524.8
		10	7.4 %	3.2 %	2	
		30	3.8 %	1.7 %	2	
		100	19.0 %	0.5 %	2	
		300	43.2 %	6.9 %	2	
		1000	61.2 %	10.0 %	2	
	3-N-acetyl BMN125	100	-2.0 %	1.8 %	2	> 3000
		1000	-0.1 %	3.2 %	2	
		3000	19.5 %	3.9 %	2	
hKv1.4	BMN125	1	-1.8 %	2.0 %	2	1860.3
		10	3.8 %	0.6 %	2	
		30	-0.5 %	4.6 %	2	
		100	0.0 %	1.8 %	2	
		300	12.1 %	2.6 %	2	
		1000	24.4 %	4.7 %	2	
	3-N-acetyl BMN125	100	7.1 %	4.8 %	2	> 3000
		1000	13.6 %	0.3 %	2	
		3000	21.5 %	3.6 %	2	
hKv1.5	BMN125	1	0.8 %	2.7 %	2	490.8
		10	6.2 %	2.0 %	2	
		30	8.7 %	5.2 %	2	
		100	30.4 %	3.5 %	2	
		300	39.3 %	2.7 %	2	
		1000	59.8 %	8.6 %	2	
	3-N-acetyl BMN125	100	-0.3 %	0.2 %	2	> 3000
		1000	4.5 %	4.3 %	2	
		3000	2.7 %	4.7 %	2	

In a separate assay, amifampridine phosphate showed weak inhibition (IC₅₀ ≥ 338.4 µM) of human Kv1.7 potassium channels in transiently transfected CHO cells (non-GLP).

In the in vitro assays, the 3-N-acetyl metabolite did not inhibit the potassium channels tested ($IC_{50} > 3000 \mu\text{M}$). 4-aminopyridine (4-AP) was used as a positive control in the in vitro assays. The data showed that 1 mM 4-aminopyridine inhibited hKv1.1 current by 73.8%, hKv1.2 current by 67.0%, hKv1.3 current by 76.8%, and hKv1.4 by 61.0%; at 2 mM, 4-aminopyridine inhibited hKv1.5 currents by 90.2%. The concurrent results were similar to the historical reference data for 4-AP.

4.2 Secondary Pharmacology

In vitro binding assays of 29, 40, and 44 receptors, channels, or enzymes showed no activity (i.e., >50% inhibition or stimulation of ligand binding) at 10 μM amifampridine phosphate or its 3-N-acetyl metabolite. Notably, this is several-fold below the concentration used to demonstrate the primary pharmacology (i.e., voltage-gated potassium channel blocking activity). L-type calcium channels were included in the binding assay, but other calcium channels were not tested. In Study BMN125-10-085, nearly 50% (47%) inhibition of ligand binding was observed for protein serine/threonine phosphatase, PPP3Ca (calcineurin, PP2B); this was not investigated further. Additionally, the 3-N-acetyl metabolite (10 μM) showed a 40% increase in control specific binding of the agonist radioligand at ETA (endothelin 1) receptors, which may suggest an allosteric effect (Study 100014186); this was not further investigated.

4.3 Safety Pharmacology

A battery of GLP-compliant assays was performed for amifampridine phosphate. An Irwin test was conducted in male Wistar rats, testing a single oral (gavage) dose of amifampridine phosphate at 0, 5, 10, 20, and 40 mg/kg; a few clinical signs were observed but were not considered adverse (i.e., NOAEL= 40 mg/kg). Respiratory safety was evaluated by plethysmography following a single dose of amifampridine phosphate (0, 0.58, 1.58, and 5.27 mg free base/kg) administered to male SD rats (GLP exceptions were noted for test article characterization and exposure analysis); no drug-related changes in tidal volume, respiration rate, or minute volume were reported. The sponsor conducted a cardiovascular study in telemetered dogs in a Latin square design, administering single amifampridine phosphate doses (0, 0.03, 0.08, and 0.26 mg free base/kg) by oral gavage to male beagle dogs (GLP exceptions were noted for test article characterization and exposure analysis). Amifampridine phosphate was shown to shorten the PR interval (up to 8%, lasting up to 10-13 hr postdose) and increase arterial pulse pressure (up to 7%).

An action potential duration assay (Study 1710-AGE-03) testing amifampridine phosphate (0.1, 1, 10, 30, and 100 μM) showed increases (approximately 18% and 40%, respectively) in the duration of the action potential at 90% and 50% repolarization at 30 and 100 μM during bradycardia (0.2 Hz; with a lesser but still significant effect at the 1 Hz stimulation frequency). The effect was attributed to blockade of potassium channels (class III antiarrhythmic effect).

A hERG assay (Study 1711-AGE-03) testing amifampridine phosphate (0.3, 3, and 30 μM) in transfected CHO-K1 cells demonstrated little to no effect on hERG currents; an IC_{50} was not determined. In Study 100014186 (non-GLP), the 3-N-acetyl metabolite was tested by an automatic patch clamp procedure in a hERG-transfected CHO-K1 cell line and showed low potential to inhibit the hERG channel ($\text{IC}_{50} = 39 \mu\text{M}$).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods for the determination of amifampridine phosphate and the 3-N-acetyl metabolite were validated for rat plasma (e.g., TRTPR13-027; a summary was provided) and dog plasma (Study TRTPS13-053). In rat and dog plasma, amifampridine phosphate plasma concentrations could be measured over the concentration range of 0.5 to 500 ng/mL, and the 3-N-acetyl metabolite plasma concentrations could be measured over the concentration range of 1 to 1000 ng/mL.

In vitro, ^{14}C -amifampridine phosphate (0.3, 1, 3, and 10 μM) was not highly bound (showing no concentration dependence) to plasma proteins from rat (5-10%), dog (9-15%), monkey (10-11%), or human plasma (9-12%). Amifampridine phosphate partitioned approximately equally into plasma and blood cells. In hepatocytes, the rate of formation of the 3-N-acetyl metabolite was slightly higher in rat than human and monkey; rabbit hepatocytes formed low levels of the 3-N-acetyl metabolite, and it was not observed in dog hepatocytes. In human hepatic S9 fraction with acetyl coenzyme A co-factor, formation of the 3-N-acetyl metabolite was observed; metabolism did not occur with CYP450 mono-oxygenase or flavin mono-oxygenase enzymes. Intrinsic metabolic clearance of amifampridine phosphate in rat, monkey, and human hepatocytes generally decreased with increasing concentration, which was attributed to saturation of the responsible enzyme system.

^{14}C -amifampridine phosphate was reported to show low permeability in Caco-2 cell monolayers and did not appear to be a substrate or inhibitor of P-gp. In vitro, no effects on human uptake or efflux transporters were reported for amifampridine phosphate (30 μM) and the 3-N-acetyl metabolite (40 μM), except a slight inhibition (~17%) of OATP1B3. Amifampridine phosphate was reported to not inhibit CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A4; amifampridine phosphate (up to 30 μM ; but not the 3-N-acetyl metabolite) reportedly showed a potential to induce CYP1A2, CYP2B6, and CYP3A4.

In vivo, rodent (rat) and nonrodent (dog) species show extensive oral absorption and renal excretion; however, the primary circulating component in rat is the 3-N-acetyl metabolite while the drug is reportedly excreted unchanged (or is "metabolize[d]... into a number of apparently chromatographically less polar metabolites") in dog.

The sponsor conducted bioavailability studies comparing the amifampridine free base and the phosphate salt. In rats (12/sex/group), no clinical signs were reported after 1

mg/kg of each form; the resulting TK were similar. In dogs (N=1/sex, crossover), 1 mg/kg of each form elicited clinical signs (e.g., emesis, salivation, spasms, tremors, red buccal membranes, transient locomotor difficulty, head shaking); while exposures were similar (i.e., “in the same order of magnitude”), the sponsor noted that clinical signs were more sustained when the females were given the salt form (which the sponsor posited may have resulted from slower elimination of the drug or its metabolites).

In albino rats, administration of amifampridine phosphate (0.422 mg/kg IV and 3, 13, or 40 mg/kg/day PO, given TID) resulted in clinical signs at 40 mg/kg/day. The PK data suggested saturable first pass and food effects, and sex differences were generally < 2-fold. The sponsor stated that the absolute bioavailability after the first PO dose ranged from 12% to 156%, increasing with dose level. Amifampridine phosphate was shown to be extensively converted to the 3-N-acetyl metabolite, a major human metabolite, in rats. Exposure to the 3-N-acetyl metabolite increased with increasing doses but was less-than-dose-proportional (which was attributed to a saturation of the N-acetylation metabolic process).

In albino rats (N= 3-9/sex), a single radiolabeled dose of amifampridine phosphate was administered IV (2.5 mg/kg, as the salt, 5 mL/kg) and PO (25 mg/kg, as the salt; by gastric intubation, 5 mL/kg) in a crossover design with a 28-day washout period between doses, and blood and excreta samples were obtained. Oral bioavailability was estimated to be 93% in males and 75% in females. Maximal plasma radioactivity was achieved by 2 hours postdose. Oral terminal half-life was 1.4-1.8 hr. Based on tissue distribution, oral absorption was rapid and extensive (present in almost all tissues by 0.25 hr postdose) and tissue concentrations were highest at 2 hours postdose. The highest tissue concentrations of radioactivity were observed in organs of excretion (i.e., liver, kidney, and GI tract) and glandular tissues (i.e., lacrimal, salivary, pharyngeal mucous, preputial, pituitary, and thyroid). The lowest concentrations were observed in eye, fat, and CNS tissues. After approximately 72 hr, few tissues showed measurable radioactivity (i.e., liver, nasal mucosa, esophagus, small intestine, and thyroid); by 168 hr, measurable activity was only observed in esophagus and thyroid in the female animal. The sponsor stated that there was a “notable persistence of radioactivity in the thyroid gland in female,” but not male, rats. Excretion was primarily urinary (90% of the recovered dose, by 168 hr postdose), with fecal recovery accounting for only 6-7% of recovery.

In dogs (N=3/sex), a single radiolabeled dose of amifampridine phosphate was administered IV (0.05 mg/kg, as the salt) and PO (0.5 mg/kg, as the salt; by gastric intubation, 5 mL/kg) in a crossover design with a 28-day washout period between doses, and blood and excreta samples were obtained. Oral absorption was rapid (C_{max} = 0.75 to 2 hr), and plasma concentrations were BLOQ by 168 hr (in most animals by 144 hr). Radioactivity was distributed equally between plasma and blood cell fractions. No clear sex difference was observed. The terminal half-life of plasma radioactivity was approximately 39 to 167 hours. The primary route of elimination was urinary (~90%), and 96% of the urinary recovery was obtained by 24 hr postdose. Fecal elimination accounted for approximately 5% of recovery.

6 General Toxicology

6.1 Single-Dose Toxicity

The sponsor conducted single dose toxicity studies by several routes; however, this review focused on studies that used the clinical route of administration (i.e., oral). Intravenous studies with amifampridine phosphate in mice and rats showed convulsions and death within 2 hours; the IV single dose NOEL in mice was 10 mg/kg and in rats was 2.5 mg/kg.

In mice, single oral doses caused death (preceded by clonic convulsions, lacrimation, salivation, and rapid breathing) within an hour postdose at 52.7 mg/kg, rapid breathing and clonic convulsions within an hour postdose at 26.4 mg/kg, and slightly subdued behavior at 13.2 mg/kg.

In rats, single oral doses caused death at 52.7 to 26.4 mg/kg within 1 to 4 hours postdose (preceded by rapid breathing and/or pedaling movements, excessive grooming, lacrimation, hypersalivation, pale limb, agitated forelimb movements, jumping, and/or clonic convulsions), and “agitated movements of the forelimb” (F), hyperactivity, and/or excessive grooming for 1 hour and occasionally up to 4 hours postdose at 13.2 mg/kg.

In an MTD study in beagle dogs (non-GLP), doses of approximately 1, 2, and 3 mg free base/kg/day (given TID) amifampridine phosphate were tested for 1 day (with a 2-day washout between doses), followed by 3.29 mg/kg/day for 7 days. The MTD was determined to be 2 mg/kg/day. Animals showed clinical signs following all doses, including: hypersalivation and diarrhea, as well as coughing, shaking/tremor, emesis, squinty eyes, and rubbing face at ≥ 3 mg/kg/day. On the first day of the 7-day dosing portion of the study, one HDF showed ataxia, hypersalivation, mild tremors, and was non-responsive to stimuli at approximately 6 hr after the second daily dose. A short time later, the animal showed “pronounced muscle tremors” for which IV midazolam was administered. The animal recovered approximately an hour later but was not administered the 3rd daily dose. The animal was reportedly normal for the remainder of the study. Effects on body weight were variable; the HDF showing severe clinical signs had a body weight loss of 9%. TK analysis showed that C_{max} occurred at approximately 45 minutes postdose, and clear sex differences were not observed. See the sponsor’s summary Table 9, below.

Table 9 Toxicokinetic Parameters of Amifampridine

Dose (mg/kg/day)	Animal ID	C _{max} (ng/mL)	T _{max} (hr)	T _{1/2} (hr)	AUC ₀₋₆ (hr*ng/mL)	AUC _{0-∞} (hr*ng/mL)	AUC%Extrap_obs (%)
1.0	1F1	168	0.50	1.82	464	518	10.5
	1F2	152	0.50	1.82	369	410	10.1
	1M1	167	0.50	1.63	421	457	7.9
	1M2	136	1.00	1.67	398	441	9.8
	Mean	156	0.63	1.7	413	457	9.6
	SD	15.1	0.25	0.097	40.2	45.4	1.1
3.0	1F1	457	1.00	1.81	1500	1680	10.7
	1F2	471	0.50	1.79	1210	1340	9.8
	1M1	413	0.50	1.91	1130	1280	11.5
	1M2	410	1.00	1.63	1260	1380	8.5
	Mean	438	0.75	1.8	1280	1420	10.1
	SD	30.9	0.29	0.115	157	176	1.3
3.29	1F1	501	0.50	2.16	1420	1670	14.7
	1F2	414	0.50	1.88	1160	1310	11.3
	1M1	449	0.50	1.65	1310	1430	8.3
	1M2	418	1.00	1.85	1350	1520	11.2
	Mean	446	0.63	1.9	1310	1480	11.4
	SD	40.2	0.25	0.209	109	151	2.6

6.2 Repeat-Dose Toxicity

RAT

Study (b) (4)/266/011: 3,4-Diaminopyridine phosphate salt – 7-day preliminary study by multiple oral (gavage) administration in the rat.

Conducted by (b) (4) Initiated 10/4/2002
GLP, US FDA (no separate pathology report, but a signed authentication statement)

Species: Sprague-Dawley rats: Ico: OFA.SD. (IOPS Caw), 5/sex/gp

(b) (4)
6 weeks old; 138- 217 g

Doses: 0, 1, 10, 30, and 60 mg/kg/day (as 0, 0.53, 5.27, 15.81, and 31.62 mg free base/kg/day, given TID)

Drug: amifampridine phosphate, Batch 010086, (b) (4)
(b) (4) 5 mL/kg

Route: PO (gavage)

No mortality occurred. Dose-related clinical signs included excessive grooming at ≥LMD, hypersalivation at ≥HMD, and hyperactivity at the HD. One HDF showed tremors after the second daily dose on D1. No drug-related effects on body weight and food consumption were reported. A slight increase in serum urea was observed in HDF (~30%, [ss]). Slightly reduced heart weight was reported in females and HDM. Gross examination showed dark or pale areas in the stomach sporadically in treated animals (2, 2, 1, 3 of 10 animals in the LD, LMD, HMD and HD groups compared to controls); the sponsor considered the macroscopic changes potentially drug-related. Microscopic examination was not conducted.

Study (b) (4) 266/013: 3,4-Diaminopyridine phosphate salt - 4 week toxicity study by multiple oral (gavage) administration in the rat followed by a 2 week treatment-free period.

Conducted by (b) (4), Initiated 10/24/2002, GLP (FDA), except drug characterization (No separate pathology report, but a signed authentication statement)

Species: Sprague-Dawley rats: Ico: OFA.SD. (IOPS Caw), (b) (4)
Main: 10/sex/gp, Rec: 5/sex Con & HD, TK 6/sex/drug group
6 weeks old; 138- 217 g

Doses: 0, 4, 13, and 40 mg free base/kg/day, given as TID

Drug: 3,4-diaminopyridine phosphate, Batch 010086, (b) (4)
5 mL/kg

Route: PO (gavage)

There were no early mortalities. Hypersalivation was observed at the MD and HD. Excessive grooming (during the first week) and hyperactivity were observed sporadically at the HD. Slightly increased body weight (6%) was observed in HDF; this was not observed after recovery. Food consumption was not altered. A slight increase in urea (15% and 19 in M and F, respectively, [ss]) was observed at the HD. Slightly reduced sodium (2%, [ss]) was observed at the HD. Dose-related increases in urine volume (up to 50%, [ss]) with associated reductions in urine specific gravity were observed in HDF. Increased liver and kidney weights were observed in HDF, but were without clear histological correlates, and showed recovery. C_{max} ranged from 12 to 16 $\mu\text{g/L}$ at LD, 17 to 33 $\mu\text{g/L}$ at MD, and 135 to 161 $\mu\text{g/L}$ at HD; T_{max} was 30 to 60 minutes postdose.

Study BMN125-10-055: 13-Week Three Times Daily Oral Gavage Toxicity and Toxicokinetic Study with BMN125 in Rats with a 4-Week Recovery Phase

Conducted by (b) (4) Initiated 4/28/10
GLP (FDA), except drug characterization

Species: Sprague Dawley rats, Hsd:Sprague Dawley[®]SD[®] rats (b) (4)
; 8 weeks old; 143-331 g

Drug: BMN125 phosphate salt, Lot 050132, (b) (4)

See the sponsor's summary design table, below.

Group ^{a,b}	No. of Animals		Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)	Free Base Equivalents Dose Level (mg/kg/day)	Dose Concentration ^{c,d} (mg/mL)
	Male	Female				
Toxicity Animals^e						
1 (Control)	25	25	0	0	0	0
2 (Low)	15	15	2.5	7.5	4.0	0.5
3 (Mid)	15	15	7.5	22.5	11.9	1.5
4 (High)	25	25	25	75	39.5	5
Toxicokinetic Animals^f						
5 (Control)	4	4	0	0	0	0
6 (Low)	19	19	2.5	7.5	4.0	0.5
7 (Mid)	19	19	7.5	22.5	11.9	1.5
8 (High)	19	19	25	75	39.5	5

a Groups 1 and 5 received vehicle control article only.

b Animals were dosed three times daily (approximately 6 hours between doses).

c Dose concentrations were based on the test article as supplied (phosphate salt). A correction factor was not used.

d Animals were dosed at a volume of 5 mL/kg/dose.

e Following dose administration for at least 13 weeks, toxicity animals designated for recovery euthanasia (last 10 animals/sex in Groups 1 and 4) underwent 4 weeks of recovery.

f One toxicokinetic animal/sex in Groups 5 through 8 served as replacement for possible mortality.

One TK HDF died on D92; this animal showed adverse severe clinical signs 1 hr after the second dose, including hunched posture, limb paddling (front legs), myoclonic jerking (entire body), clear oral and eye discharge, and irregular respiration. Drug-related clinical signs (mostly reported in TK animals after fasting), included: clear oral discharge, limb paddling of front legs, and sensitivity to touch. One TK MDF (no necropsy) and one HDF (carcinoma of the mammary gland) showed masses on D92 and D64, respectively.

Drug-related changes in body weight and body weight gain were not observed, although food consumption tended to be slightly increased at the HD. Increased liver (~10-15%), adrenal gland (~30%, F), and mandibular salivary gland weights (~10% in M, ~35% in F) were observed at the HD. Minimal to slight mandibular salivary gland hypertrophy was observed at the HD and was not observed in recovery animals. Clear histological correlates were not observed for adrenal or liver.

The sponsor provided the following summary data for exposures to amifampridine and the 3-N-acetyl metabolite (see sponsor's Tables 1-3 and 5-7, respectively, below).

Table 1
Toxicokinetic Parameters for BMN125 in Rat Plasma: Day 1

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/(mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC _{0-∞} (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)	
6	2.5	DA-1 Low	M	11.9	4.75	0.50	16.5	6.62	16.5	16.8	1.04	NA	
			F	15.5	6.19	0.33	21.1	8.44	21.1	21.4	1.09		
		DA-2 Low	M	9.70	3.88	0.50	14.4	5.77	11.9	NC	NC		NA
			F	13.6	5.44	0.50	15.3	6.14	12.8	NC	NC		
		DA-3 Low	M	11.4	4.55	0.50	12.1	4.82	10.7	NC	NC		NA
			F	15.2	6.09	0.25	15.5	6.20	15.5	NC	NC		
7	7.5	DA-1 Mid	M	59.5	7.94	0.50	74.9	10.0	74.9	77.8	1.37	NA	
			F	88.3	11.8	0.33	104	13.9	104	105	0.859		
		DA-2 Mid	M	52.2	6.96	0.50	65.3	8.71	50.7	NC	NC		NA
			F	79.1	10.5	0.50	73.7	9.83	73.7	74.2	0.891		
		DA-3 Mid	M	31.4	4.19	0.50	44.5	5.93	44.5	NC	NC		NA
			F	76.1	10.1	0.50	79.5	10.6	79.5	81.2	1.18		
8	25	DA-1 High	M	278	11.1	1.0	530	21.2	530	556	1.34	NA	
			F	342	13.7	0.50	543	21.7	543	554	1.09		
		DA-2 High	M	320	12.8	0.50	458	18.3	458	459	0.683		NA
			F	266	10.6	0.50	443	17.7	443	453	1.06		
		DA-3 High	M	168	6.73	0.50	222	8.86	222	242	1.71		NA
			F	184	7.36	0.50	280	11.2	280	297	1.48		

DA Dose administration

DN Dose normalized

NA Not applicable

C Not calculated; due to lack of quantifiable concentrations in the elimination phase, t_{1/2} and AUC_{0-∞} could not be determined.

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Table 2
Toxicokinetic Parameters for BMN125 in Rat Plasma: Day 44

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/(mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC _{0-∞} (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)	AR AUC _{0-6h}		
6	2.5	DA-1 Low	M	19.0	7.59	0.50	22.1	8.84	22.1	23.5	1.79	NA	1.34		
			F	33.3	13.3	0.50	34.6	13.8	34.6	35.3	1.39		1.64		
		DA-2 Low	M	16.8	6.73	0.25	22.3	8.90	17.6	19.4	0.556		NA	1.54	
			F	21.1	8.45	0.25	27.7	11.1	23.4	24.8	0.452			1.81	
		DA-3 Low	M	10.1	4.05	0.50	12.6	5.04	12.6	NC	NC		NA	57.0	1.05
			F	33.8	13.5	0.25	25.9	10.4	25.9	26.0	0.814			88.2	1.67
7	7.5	DA-1 Mid	M	62.0	8.27	0.50	83.3	11.1	83.3	88.0	1.68	NA	1.11		
			F	144	19.3	0.33	160	21.3	160	161	1.02		1.54		
		DA-2 Mid	M	82.4	11.0	0.25	119	15.8	119	120	0.940		NA	1.82	
			F	104	13.9	0.25	128	17.1	128	129	0.825			1.74	
		DA-3 Mid	M	50.1	6.68	0.50	60.5	8.07	60.5	62.1	1.16		NA	263	1.36
			F	264	35.2	0.25	162	21.7	162	164	1.10			451	2.05
8	25	DA-1 High	M	403	16.1	1.0	644	25.8	644	658	1.14	NA	1.22		
			F	902	36.1	0.50	1154	46.1	1154	1194	1.28		2.12		
		DA-2 High	M	592	23.7	0.25	874	34.9	874	877	0.767		NA	1.91	
			F	1240	49.6	0.25	1381	55.2	1381	1397	0.973			3.12	
		DA-3 High	M	326	13.0	0.50	644	25.8	644	655	1.04		NA	2161	2.91
			F	645	25.8	0.25	955	38.2	955	992	1.34			3489	3.41

AR Accumulation ratio (AUC_{0-6h}) versus Day 1.

DA Dose administration

DN Dose normalized

NA Not applicable

C Not calculated; due to lack of quantifiable concentrations in the elimination phase, t_{1/2} and AUC_{0-∞} could not be determined.

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Table 3
Toxicokinetic Parameters for BMN125 in Rat Plasma: Day 92

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/(mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC ₀₋₆ (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)	AR AUC _{0-6h}
6	2.5	DA-1 Low	M	18.3	7.31	0.50	24.3	9.71	24.3	25.2	1.48	1.47	
			F	32.5	13.0	0.50	30.4	12.2	30.4	30.8	1.15	1.44	
	DA-2 Low	M	19.0	7.60	0.50	26.9	10.8	26.9	27.3	1.02	1.86		
		F	30.4	12.2	0.25	34.9	14.0	30.3	31.7	0.407	2.28		
	DA-3 Low	M	18.0	7.20	0.50	19.2	7.70	19.2	19.8	1.30	70.4	1.60	
		F	30.3	12.1	0.25	28.8	11.5	28.8	29.1	1.02	94.2	1.86	
7	7.5	DA-1 Mid	M	88.1	11.7	0.50	110	14.7	110	116	1.73	1.47	
			F	196	26.2	0.33	182	24.2	182	183	0.975	1.74	
	DA-2 Mid	M	74.8	10.0	0.50	111	14.7	111	111	0.748	1.69		
		F	191	25.5	0.50	184	24.6	184	185	0.723	2.50		
	DA-3 Mid	M	88.5	11.8	0.50	93.7	12.5	93.7	96.1	1.19	314	2.11	
		F	223	29.7	0.50	211	28.1	211	213	1.00	577	2.66	
8	25	DA-1 High	M	427	17.1	1.0	826	33.0	826	855	1.26	1.56	
			F	924	37.0	0.50	1346	53.8	1346	1370	1.10	2.48	
	DA-2 High	M	938	37.5	0.25	1423	56.9	1423	1443	1.00	3.11		
		F	1204	48.2	0.25	1451	58.0	1451	1462	0.840	3.28		
	DA-3 High	M	690	27.6	0.50	918	36.7	918	928	0.957	3166	4.14	
		F	941	37.6	0.25	1478	59.1	1478	1501	1.01	4275	5.27	

AR Accumulation ratio (AUC_{0-6h}) versus Day 1.

DA Dose administration

DN Dose normalized

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Table 5
Toxicokinetic Parameters for the 3-N-acetyl BMN125 Metabolite in Rat Plasma: Day 1

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/(mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC ₀₋₆ (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)
6	2.5	DA-1 Low	M	444	177	1.5	1319	528	1319	NC	NC	
			F	428	171	1.0	1373	549	1373	1582	2.03	
	DA-2 Low	M	536	214	1.0	1486	595	1486	NC	NC		
		F	464	185	0.50	1383	553	1383	1465	1.44		
	DA-3 Low	M	501	201	0.50	1214	485	1214	1317	1.59	4122	
		F	488	195	1.0	1351	540	1351	NC	NC	NA	
7	7.5	DA-1 Mid	M	1300	173	1.0	4003	534	4003	5104	2.61	
			F	1130	151	1.0	3836	511	3836	NC	NC	
	DA-2 Mid	M	1390	185	1.0	4229	564	4229	NC	NC		
		F	1333	178	0.50	3999	533	3999	4462	1.85		
	DA-3 Mid	M	1230	164	0.50	3440	459	3440	NC	NC	NA	
		F	1247	166	0.50	3710	495	3710	4315	2.06	NA	
8	25	DA-1 High	M	3243	130	1.0	13210	528	13210	17788	2.86	
			F	2500	100	2.0	10386	415	10386	NC	NC	
	DA-2 High	M	4320	173	1.0	13594	544	13594	NC	NC		
		F	3233	129	1.0	12088	484	12088	NC	NC		
	DA-3 High	M	2617	105	0.50	10503	420	10503	NC	NC	NA	
		F	2477	99.1	1.0	10836	433	10836	NC	NC	NA	

DA Dose administration

DN Dose normalized

NA Not applicable

C Not calculated; due to lack of quantifiable concentrations in the elimination phase, t_{1/2} and AUC₀₋₆ could not be determined.

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Table 6
Toxicokinetic Parameters for the 3-N-acetyl BMN125 Metabolite in Rat Plasma: Day 44

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/ (mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC _{0-∞} (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)	AR AUC _{0-6h}
6	2.5	DA-1 Low	M	515	206	0.50	1411	564	1411	1652	2.04		1.07
			F	588	235	0.50	1581	633	1581	1875	2.31		1.15
	DA-2 Low	M	521	208	1.0	1721	688	1721	NC	NC		4364	1.16
		F	538	215	1.0	1577	631	1577	NC	NC		4645	1.14
	DA-3 Low	M	446	178	0.50	1232	493	1232	NC	NC		12478	1.02
		F	606	242	0.50	1487	595	1487	NC	NC		12860	1.10
7	7.5	DA-1 Mid	M	1129	150	0.50	4021	536	4021	5199	2.81		1.00
			F	1273	170	1.0	4188	558	4188	4920	2.15		1.09
	DA-2 Mid	M	1730	231	1.0	4861	648	4861	NC	NC		12478	1.15
		F	1520	203	1.0	4568	609	4568	NC	NC		12860	1.14
	DA-3 Mid	M	1324	176	0.50	3595	479	3595	NC	NC		12478	1.05
		F	1333	178	1.0	4104	547	4104	NC	NC		12860	1.11
8	25	DA-1 High	M	3253	130	1.0	12455	498	12455	16143	2.74		0.943
			F	2883	115	1.0	12174	487	12174	NC	NC		12478
	DA-2 High	M	3840	154	1.0	14453	578	14453	NC	NC		12478	1.06
		F	3510	140	1.0	12928	517	12928	NC	NC		12478	1.07
	DA-3 High	M	3327	133	1.0	11868	475	11868	NC	NC		38776	1.13
		F	2830	113	1.0	11696	468	11696	NC	NC		36798	1.08

AR: Accumulation ratio (AUC_{0-6h}) versus Day 1.

DA: Dose administration

DN: Dose normalized

C: Not calculated; due to lack of quantifiable concentrations in the elimination phase, t_{1/2} and AUC_{0-∞} could not be determined.

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Table 7
Toxicokinetic Parameters for the 3-N-acetyl BMN125 Metabolite in Rat Plasma: Day 92

Group	BMN125 Dose Level (mg/kg/dose)	Treatment	Gender	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC _{0-6h} (ng•hr/mL)	DN AUC _{0-6h} [(ng•hr/mL)/ (mg/kg/dose)]	AUC ₀₋₄ (ng•hr/mL)	AUC _{0-∞} (ng•hr/mL)	t _{1/2} (hr)	Total AUC* (ng•hr/mL)	AR AUC _{0-6h}	
6	2.5	DA-1 Low	M	472	189	0.50	1464	586	1464	1749	2.20		1.11	
			F	509	204	0.50	1475	590	1475	1736	2.13		1.07	
	DA-2 Low	M	528	211	0.50	1827	731	1827	2148	2.28		1.23		
		F	597	239	1.0	1759	703	1759	NC	NC		13026	1.27	
	DA-3 Low	M	587	235	0.50	1344	538	1344	1541	1.95		4635	1.11	
		F	615	246	0.50	1452	581	1452	1576	1.61		4685	1.07	
7	7.5	DA-1 Mid	M	1230	164	0.50	4143	552	4143	5597	3.05		1.03	
			F	1260	168	1.0	4057	541	4057	4794	2.20		1.06	
	DA-2 Mid	M	1217	162	0.50	4359	581	4359	4722	1.65		13026	1.03	
		F	1587	212	0.50	4707	628	4707	5121	1.63		13026	1.18	
	DA-3 Mid	M	1757	234	0.50	4070	543	4070	4603	1.87		12572	1.18	
		F	1500	200	1.0	4262	568	4262	NC	NC		13026	1.15	
8	25	DA-1 High	M	3360	134	1.5	12511	500	12511	NC	NC		40907	0.947
			F	2520	101	1.5	11123	445	11123	NC	NC		35984	1.07
	DA-2 High	M	3577	143	2.0	15750	630	15750	NC	NC		40907	1.16	
		F	3400	136	1.0	12732	509	12732	NC	NC		35984	1.05	
	DA-3 High	M	3627	145	1.0	12647	506	12647	NC	NC		40907	1.20	
		F	3073	123	1.0	12129	485	12129	NC	NC		35984	1.12	

AR: Accumulation ratio (AUC_{0-6h}) versus Day 1.

DA: Dose administration

DN: Dose normalized

C: Not calculated; due to lack of quantifiable concentrations in the elimination phase, t_{1/2} and AUC_{0-∞} could not be determined.

* Total AUC calculated as AUC_{0-6h} DA-1 + AUC_{0-6h} DA-2 + AUC_{0-6h} DA-3.

Study title: 26-Week Repeat Dose Toxicology and Toxicokinetic Study of Amifampridine Administered as the Phosphate Salt (BMN 125) in Sprague-Dawley Rats with a 4-Week Recovery

Study no.: S12033
 Study report location: EDR, SDN1 (7/22/15)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 2/27/14
 GLP compliance: Yes (FDA), except:
 ▪ Manufacture and characterization of drug (GMP)
 ▪ Dose concentration verification analysis (GMP)
 QA statement: Yes
 Drug, lot #, and % purity: BMN125, Lots DAPP-021; DAPP-022; 14-11315-S, (b) (4) % pure

Methods (See the sponsor's Table 1, below, for study design summary)

Route of administration: PO, gavage
 Species/Strain: Sprague Dawley rat; Hsd: Sprague Dawley[®]TM SD[®]TM
 (b) (4)
 Age: 6.4 weeks old (Main and Rec)
 8.9 weeks old (TK)
 Weight: 99-198 g

Table 1 Study Design

Group ^{a,b}	No. of Animals Main/(Recovery ^c)		Day 1 and 182 TK Animals ^d		Dose Concentration (mg/mL) ^{e,f}	Dose Level (mg/kg/dose) ^{b,e,f}	Dose Level (mg/kg/day) ^{e,f}
	Male	Female	Male	Female			
1 (Control)	20 (5)	20 (5)	2	2	0	0	0
2 (Low)	20 (5)	20 (5)	12	12	0.26	1.3	3.9
3 (Mid)	20 (5)	20 (5)	12	12	0.79	3.9	11.8
4 (High)	20 (5)	20 (5)	12	12	2.63	13.2	39.5

^a Group 1 received vehicle control article (Water for injection, WFI) only.
^b Animals were dosed three times daily, approximately 6 ± 2 hours between doses.
^c Following dose administration for 26 weeks, toxicity animals designated as recovery animals underwent 4 weeks of recovery without treatment.
^d Number for each cohort. The Day 182 TK groups had 2 extra animals per gender per group available as backup in case of mortality.
^e Dose levels and concentrations expressed as amifampridine free base. A correction factor of 1.9 should be applied to correct for the phosphate salt content of the test article.
^f All animals were dosed at a volume of 5 mL/kg/dose.

Observations and Results

Mortality [Twice daily]

There were no clearly drug-related deaths.

Clinical Signs [Pre-dose, daily for 7 days, then weekly & prior to necropsy]

One HDF (#903) was inadvertently dosed twice at the third dosing on D15; this animal showed clear oral and nasal discharge and limb paddling (which was resolved at 2 hr postdose).

Drug-related CNS (e.g., limb paddling, twitching, and tremors) and respiratory findings (e.g., dyspnea, tachypnea, panting, and/or labored breathing) were observed at the HD, and in a few MD animals (see the sponsor's Table 6, below). Red nasal and/or ocular discharge was observed in all groups, but was clearly increased at the HD; red discharge around the eye was often identified as porphyrin.

Table 6 Incidence of Selected Clinical Observation Signs ^a

Gender	Male				Female			
	1	2	3	4	1	2	3	4
Group	1	2	3	4	1	2	3	4
Dose Level (mg fb/kg/day)	Vehicle (0.0)	Low (3.9)	Mid (11.8)	High (39.5)	Vehicle (0.0)	Low (3.9)	Mid (11.8)	High (39.5)
N	25	25	25	25	25	25	25	25
Red Nasal Discharge	2/2	8/6	5/5	18/10	1/1	1/1	1/1	5/3
Red Ocular Discharge	20/9	14/5	16/10	36/13	4/3	6/3	12/6	4/4
Neurologic ^b	0	0	0	8/7	0	0	1/1	5/5
Respiratory ^c	0	0	2/2	13/7	0	0	0	2/2

^a Expressed as number of findings/number of animals affected during the treatment phase of the study.

^b Includes "paddling", "twitching", tremors.

^c Includes dyspnea, tachypnea, panting, "labored breathing", etc.

The "neurologic" signs at the HD included abnormal posture, "small jumps," "head bobs" (reportedly associated with breathing), "jumping," "twitching and jumping," and "shaking." No overt convulsions were reported, but the detailed description of a CNS sign in a HDF on D11 was suggestive:

"Jumping upwards in cage multiple times. Large amount of clear discharge from mouth. Very disoriented and would hunch up and fall backwards while arm and leg muscles were very tense. After jumping, animal began to limb paddle and also had a small amount of red discharge from her left eye and a small amount of clear discharge from her right eye. Limb paddling was observed until 10:22pm" [i.e., approximately 1 hour after the episode started].

Body Weights [Pre-dose, daily for 7 days, then weekly & prior to necropsy]

At W26, mean body weight was slightly reduced (approximately 5%) in HDM.

Food Consumption [Weekly]

No consistent drug-related effect was observed.

Ophthalmoscopy [Pre-dose, Week 26 or 30]

The ophthalmological evaluation was conducted by a board-certified veterinary ophthalmologist [REDACTED] (b) (6). Although eye discharge was reported in many animals throughout the study, no drug-related changes were reported. Red discharge around the eyes was observed in many animals. The pathologist attributed changes observed at necropsy (e.g., ruptured or smaller eye) to study procedures.

Clinical Pathology [Week 26 or 30]

Hematology [parameters assessed, from the sponsor below]

White Blood Cell Count	Neutrophils (% and absolute)
Red Blood Cell Count	Eosinophils (% and absolute)
Hemoglobin	Basophils (% and absolute)
Hematocrit	Lymphocytes (% and absolute)
Mean Cell Volume	Monocytes (% and absolute)
Mean Cell Hemoglobin	Platelet Count
Mean Cell Hemoglobin Concentration	Reticulocytes
Differential White Blood Cell Count	Abnormalities of blood smear
Activated Partial Thromboplastin Time (aPTT)	Prothrombin Time (PT)

Fibrinogen was not assessed. No clearly drug-related changes were observed.

Clinical Chemistry [parameters assessed, from the sponsor below]

Alanine aminotransferase (ALT)	Gamma Glutamyl Transferase
Albumin	Globulin
Albumin/ Globulin Ratio	Glucose
Alkaline Phosphatase (ALP)	Inorganic Phosphorus
Aspartate Aminotransferase (AST)	Potassium
Blood Urea Nitrogen (BUN)	Sodium
Calcium	Total Bilirubin
Chloride	Total Protein
Cholesterol	Triglycerides
Creatinine	

Slightly increased blood sodium and chloride (<5%) in treated animals were observed; the sponsor did not consider these clinically relevant. Blood phosphorus was slightly increased (11%, [ss]) in HDF. BUN was increased in MDF and HDF (~40%, [ss]) but was slightly reduced in HDM (16%, [ss]). ALT and AST were increased (40-50%, [ss]) and triglycerides were slightly reduced (25%, [ss]) in HDM. No statistically significant alterations were observed after recovery.

Urinalysis [parameters assessed, from the sponsor below]

Volume (~16 hr)	Protein
Bilirubin	Specific Gravity
Blood	Urobilinogen
Color and Clarity	Urine microscopic examination
Glucose	including the presence of WBC, RBC,
Ketones	crystals and bacteria)
pH	

No drug-related changes were reported.

Gross Pathology

Enlarged salivary glands were observed (2 HDF) and were considered drug-related; these observations correlated with acinar cell hypertrophy histologically. Enlarged uterus was observed in all groups, but with increased incidence at HD.

Organ Weights

Increased submandibular salivary gland weights [ss] were reported in HDM (~12%, [ss]) and HDF (~80%, [ss]); clear changes were not observed after recovery. Increased liver weights were reported at the MD and HD, in the main (approximately 5% to 20%, most [ss]) and recovery animals (approximately 10-15%, [nss]). Adrenal weight was increased (25%, [ss]) in HDF; a slight increase (13%, [nss]) was observed at recovery. Ovary weight was increased (50%, [ss]) in the main study HDF and was slightly increased in recovery HDF (~40% absolute, ~25% relative to BW [nss]).

Histopathology

Adequate Battery	Yes, (see Histopathology Inventory) Con and HD only; Target tissues for LD & MD
Separate, Signed Report Peer Review	Yes, (b) (6) No

Histological Findings

The only drug-related finding reported by the pathologist was acinar cell hypertrophy in the submandibular salivary gland at the HD; this finding was reversible at recovery. The pathologist described the observations as follows,

“Hypertrophy was characterized microscopically by diffuse enlargement of acinar cells with slightly basophilic, stippled cytoplasm. Intensity of this change ranged from slight to moderate with some increased intensity noted for females versus males in Group 4.”

Congestion was observed in the liver of few MDM and HDM and correlated with the observed liver enzyme changes but was not clearly dose-related. The estrous cycling

pattern appeared slightly different between control and HDF; enlarged uterus was reported at necropsy. See selections from the sponsor's summary table, below.

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX STATUS AT NECROPSY: K0, INCL. DEATHS										
	DOSE GROUP:		1		2		3		4	
	SEX :	M	F	M	F	M	F	M	F	
	NO.ANIMALS:	20	20	20	20	20	20	20	20	
SALIVARY GLANDS	:	20	20	20	20	20	20	20	20	
- Hypertrophy:acinar	:	-	-	-	-	-	-	17	19	
Grade 2:	:	-	-	-	-	-	-	14	10	
Grade 3:	:	-	-	-	-	-	-	3	9	
- Atrophy:acinar cell	:	-	1	-	-	-	-	-	1	
Grade 3:	:	-	1	-	-	-	-	-	1	
- Concretions:ducts	:	-	-	-	-	-	-	-	1	
Grade 3:	:	-	-	-	-	-	-	-	1	
LIVER	:	20	20	1	-	2	-	20	20	
- Congestion	:	-	-	-	-	2	-	1	-	
Grade 3:	:	-	-	-	-	2	-	1	-	
VAGINA	:	-	20	-	-	-	-	-	20	
- Cycle:Diestrus	:	-	3	-	-	-	-	-	4	
- Cycle:Proestrus	:	-	3	-	-	-	-	-	8	
- Cycle:Estrus	:	-	3	-	-	-	-	-	4	
- Cycle:Metestrus	:	-	11	-	-	-	-	-	4	

Toxicokinetics

Blood sampling for TK was performed as below, from the sponsor.

Table 2 Toxicokinetic Profile Plasma Sampling Strategy

Group	Rat	Time Point (hr.)																		
		Day 1 and Day 182 Dose 1 (0 hr.)						Day 1 and Day 182 Dose 2 (6 hr.)					Day 1 and Day 182 Dose 3 (12 hr.)							
		Pre-Dose (0)	0.167	0.333	0.5	1	1.5	2	6	6.25	6.5	7	8	12	12.25	12.5	13	14	24	
TK1 ^a	1				X ^b															
	2															X ^b				
TK2, TK3, & TK4 ^a	1	X						X					X ^b							
	2	X						X					X ^b							
	3		X					X						X ^b						
	4		X					X						X ^b						
	5			X					X						X ^b					
	6			X					X						X ^b					
	7				X					X							X ^b			
	8				X					X							X ^b			
	9					X					X								X ^b	
	10					X						X								X ^b
	11						X						X							X ^b
	12							X						X						X ^b

^a The Day 182 TK groups had 2 extra animals/gender/group available as back-up. These animals were included in the TK collection and analysis if a regular TK animal was removed from study early (i.e. un-scheduled sacrifice, found dead, etc.).

^b Terminal blood collection.

Amifampridine exposure increased (generally greater than dose-proportionally) with increasing dose; accumulation was observed with repeated dosing. Females generally showed slightly greater exposures than males (1.6 – 1.8-fold). See the sponsor’s Table 3, below.

Table 3: Day 1 and 182 Amifampridine Mean Toxicokinetic Parameters

Day	Group (Dose) mg/kg/dose	Gender	C _{max} (ng/mL)			t _{max} (hr)			t _{1/2} (hr)	AUC _{last} (hr*ng/mL)			Total AUC (hr*ng/mL)
			Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 1	Dose 2	Dose 3	
1	2 (1.3 mg/kg/dose)	Male	11.8	10.8	8.74	0.50	0.50	0.50	2.0	18.3	13.2	8.70	40.2
		Female	22.7	21.4	12.5	0.33	0.50	0.50	0.6	19.0	21.1	12.5	52.6
		Combined	17.2	16.1	10.6	0.42	0.50	0.50	1.3	18.6	17.1	10.6	46.3
	3 (3.9 mg/kg/dose)	Male	23.0	38.9	38.2	1.00	0.25	0.50	1.6	50.2	45.9	34.8	131
		Female	83.1	41.8	109	0.50	0.50	0.25	1.2	81.9	80.5	56.1	219
		Combined	53.1	40.3	73.8	0.75	0.38	0.38	1.4	66.1	63.2	45.4	175
	4 (13.2 mg/kg/dose)	Male	159	155	363	1.00	1.00	0.50	1.4	294	313	411	1018
		Female	349	150	497	0.50	0.25	0.50	1.6	596	378	673	1647
		Combined	254	152	430	0.75	0.63	0.50	1.5	445	346	542	1333
182	2 (1.3 mg/kg/dose)	Male	25.2	14.5	14.1	0.50	0.25	0.25	2.1	37.0	22.8	10.4	70.2
		Female	25.3	21.3	23.2	0.50	0.50	0.50	1.6	33.6	38.6	18.9	91.1
		Combined	25.2	17.9	18.6	0.50	0.38	0.38	1.8	35.3	30.7	14.6	80.6
	3 (3.9 mg/kg/dose)	Male	84.1	92.3	109	0.50	0.50	0.50	1.4	108	105	66.4	279
		Female	174	142	208	0.33	0.50	0.50	1.3	164	152	154	470
		Combined	129	117	158	0.42	0.5	0.5	1.3	136	129	110	375
	4 (13.2 mg/kg/dose)	Male	464	779	327	0.33	0.50	0.50	1.3	624	749	621	1994
		Female	801	603	752	0.50	0.25	1.00	1.0	1240	1000	1600	3840
		Combined	633	691	539	0.42	0.38	0.75	1.2	931	877	1110	2918

Systemic exposure to the 3-N-acetyl metabolite increased with increasing dose, but the less than dose-proportional increase suggested saturation of the NAT metabolic pathway. See the sponsor’s Table 4, below. Accumulation was not shown for the 3-N-acetyl metabolite.

Table 4: Day 1 and 182 3-N-acetyl amifampridine Mean Toxicokinetic Parameters

Day	Group (Dose) mg/kg/dose	Gender	C _{max} (ng/mL)			t _{max} (hr)			t _{1/2} (hr)	AUC _{last} (hr*ng/mL)			Total AUC (hr*ng/mL)
			Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 1	Dose 2	Dose 3	
1	2 (1.3 mg/kg/dose)	Male	355	511	634	1.50	1.00	1.00	1.9	1250	1420	2090	4760
		Female	452	520	766	1.00	0.50	0.50	1.5	1320	1990	2410	5720
		Combined	403	515	700	1.25	0.75	0.75	1.7	1290	1700	2250	5240
	3 (3.9 mg/kg/dose)	Male	1090	1460	1640	1.00	1.00	1.00	2.3	3160	4160	6040	13360
		Female	1270	1360	1500	1.00	1.00	0.25	1.6	3430	3850	6180	13460
		Combined	1180	1410	1570	1.00	1.00	0.63	1.9	3290	4010	6110	13410
	4 (13.2 mg/kg/dose)	Male	3090	3440	3840	1.00	1.00	1.00	2.9	10800	11600	20300	42700
		Female	3060	2870	3880	1.00	1.00	1.00	4.1	11200	12300	17000	40500
		Combined	3070	3150	3860	1.00	1.00	1.00	3.5	11000	12000	18700	41700
182	2 (1.3 mg/kg/dose)	Male	491	610	714	1.00	1.00	0.50	2.2	1520	1690	2020	5230
		Female	540	592	633	0.50	1.00	0.50	1.4	1320	1440	2100	4860
		Combined	515	601	673	0.75	1.00	0.50	1.8	1420	1560	2060	5040
	3 (3.9 mg/kg/dose)	Male	1210	1400	3180	1.50	1.00	0.50	2.1	4160	4300	6920	15380
		Female	1480	1690	1540	0.50	1.00	0.50	1.6	3750	4620	6180	14550
		Combined	1340	1540	2360	1.00	1.00	0.50	1.9	3950	4460	6550	14960
	4 (13.2 mg/kg/dose)	Male	3410	3620	4050	1.00	1.00	2.00	2.7	11000	13000	28100	52100
		Female	2590	3350	3690	1.00	2.00	1.00	2.7	10900	13400	23300	47600
		Combined	3000	3490	3870	1.00	1.50	1.50	2.7	11000	13200	25700	49900

Dosing Solution Analysis [D1, 3M, & last dosing day]

Formulation analyses showed that amifampridine concentrations were within 5.4% of nominal concentrations.

DOG

Study 266-014: To determine the toxicity of the test item in the beagle dog following multiple daily oral (gavage) administration for 4 weeks.

Conducted by (b) (4) Initiated 4/11/07, GLP (FDA [1978]); no separate pathology report, but a signed authentication)

Species: Beagle dogs, (b) (4) 5 months old; 6 to 10 kg; 4/sex/group
Drug: 0, 1.9, 5.7, and 7.5* mg/kg/day (0, 1, 3, and 4 mg free base/kg/day) amifampridine phosphate, given as a TID dose
Batch 010086, (b) (4) by PO (gastric intubation), 5 mL/kg

* One male on days 0 and 2 and one female on day 5 did not receive the third daily administration due to clinical signs judged too severe, or aggressiveness, therefore, on these days, these animals received only 5 mg/kg.

There were two HDM early deaths (#175 was sacrificed moribund and #176 was found dead) on the first day of treatment (D0) after the 2nd daily dose. Prior to sacrifice/death, panting was observed after the 1st daily dose and increased muscular tension and convulsions were observed after the 2nd daily dose. Cause of death was presumed to be convulsions. At necropsy, these animals both had a GI tract filled with fluid, with dark areas in the intestines, lung, liver, pancreas, salivary glands, cervical lymph node, and/or kidney. Several organs showed alterations, including congestion. Moderate multifocal myodegeneration was observed in skeletal muscle and tongue of these animals, and was accompanied by minimal to moderate hemorrhage and/or macrophage infiltration. Alterations were also observed in liver (slight sinusoidal lymphoid infiltrate, moderate hepatic cell vacuolation, minimal centrilobular hepatocyte necrosis, and/or moderate multifocal congestion), kidney (moderate, diffuse corticomedullary congestion), and lung (slight pneumonia, [multifocal] congestion, slight alveolar edema, or interstitial pneumonitis). Slight depletion of the red pulp of the spleen and acinar vacuolation of the mandibular salivary glands were observed in #176. Early mortality #175 showed vacuolation of the germinal layer in testes.

Clinical signs were most pronounced over the first few days of dosing. At the HD, CNS and respiratory clinical signs observed included convulsions (3 of 4 males on D0), stiffness of limbs, changes in muscular tone (increased/decreased), extended outstretched limbs, locomotor difficulties, labored breathing with blue oral mucous membranes, hyperesthesia, tremors, aggressiveness, anxious behavior, coughing, and

red ears and/or ocular and/or buccal membranes. Clinical signs at the MD included: hyperesthesia and tremors (most males and 1F, after the 2nd or 3rd daily administration on D0 and D1), aggressive behavior (in 2M, after the 3rd daily dose on D0), increased muscular tension or poor muscular tone, stiffness of hindlimbs, locomotor difficulties, emesis (in 1F, after the 1st daily dose on D0), subdued (1F on D1), hypersalivation, sneezing, coughing, and/or red ears and/or mucous membranes. Clinical signs at the LD included liquid feces, salivation, stiffness of hindlimbs, locomotor difficulties, and single instances of panting (M) and anxious behavior (F).

An increased incidence of white filaments in the posterior lens capsule and within the vitreous body was reported in all MDF and HDF at W4; the sponsor indicated that this “may correspond to a transient higher density of the vitreous body.”

In males, body weight losses occurred at the MD (3.5%) and HD (8%) over the first week. Body weight recovered in MDM, but a loss of 2% remained in HDM on W4. In females, body weight losses occurred at the LD (1.5%), MD (2.9%), and HD (6%) over the first week; body weight recovered by W4. Overall, body weight gains were reduced at MD and HD. Generally, food consumption was transiently (D0 through D7) and slightly (up to 25%) reduced at the MD and HD; HDF showed a very slight reduction (5%) through W4.

No drug-related organ weight alterations were reported. Dark and pale areas in the GI tract were observed in a few treated females and two HDM; the changes were reported to correlate with mucosal congestion in the GI tract and liver. In addition to the two early mortalities, dose-related minimal to slight (F) or moderate (in M) myodegeneration and/or myoregeneration in larynx, tongue, and/or skeletal muscle was observed in 1LDF, 1 LDM, all MD animals, all HDM and 3 HDF. Minimal to moderate, focal/multifocal alveolar macrophages were observed in the lungs in 2LDF, 2MDF, and 1HDF (with dose-related increasing severity). Congestion was observed in the heart (2HDM), adrenal (2HDM and 3HDF), and kidney (minimal corticomedullar congestion; 2MDF, 3HDF, as well as moderate in the 2 HDM early mortalities). Nearly all animals were sexually immature. Epididymal intratubular cellular debris was observed in all groups, but with dose-related increased incidence in treated males.

No clearly drug-related changes in blood pressure, heart rate, or ECG were reported. Two HDF showed reduced red blood cell parameters (up to 10%) on D28. Urine volume was reduced (65%) in HDF and in one HDM (#178).

The sponsor provided the following summary TK data, below.

Tableau 3 - 3,4-Diaminopyridine mean pharmacokinetic parameters in males and females beagle dogs (n=4 for each dose).

Sex	Category of dose	Animal number	Period	Dose (mg)	T _{1/2} (min)	T _{max} (min)	C _{max} (µg/L)	AUC _{last} (min.µg/L)	AUC _{inf} (min.µg/L)	AUC _{extra} (%)	Vd/F (L)	Cl/F (mL/min)
Females	Low	F163-166	D0	6.8	162.3	60	72.3	14372	19570	26.6	81.1	346
			W4	7.1	172.3	30	52.9	11159	15609	28.5	113.5	456
	Intermediate	F171-174	D0	20.7	163.5	60	198.6	40185	55042	27.0	88.7	376
			W4	21.2	167.9	60	178.6	35957	48428	25.8	105.8	437
	High	F179-182	D0	26.5	128.0	60	319.7	57692	71432	19.2	68.5	371
			W4	27.3	114.3	60	321.7	57282	67937	15.7	66.3	402
Males	Low	M159-162	D0	8.5	186.0	60	74.9	14288	20432	30.1	111.0	414
			W4	9.2	165.3	60	76.9	16548	23536	29.7	92.7	389
	Intermediate	M167-170	D0	25.7	136.0	60	204.2	41445	51098	18.9	98.5	502
			W4	26.0	129.3	60	239.1	43856	54450	19.5	88.9	477
	High	M175-178	D0	34.9	92.2	60	310.8	58923	64310	8.4	72.2	543
			W4	33.8	154.9	60	311	59840	80396	25.6	93.9	420

Study aa40847: 3,4-Diaminopyridine phosphate salt - 4-week oral (gavage) toxicity study in the beagle dog followed by a 2-week treatment-free period.

Conducted by (b) (4) Initiated 4/11/07, GLP (OECD; no separate pathology report, but signed authentication)

Species: Beagle dogs, (b) (4) 6-8 months old; 6 to 9 kg; 3/sex/group main study and 2/sex recovery Con & HD

Drug: 0, 1, 2.5, and 6.25 mg/kg/day (0, 0.5, 1.3 and 3.3 mg free base/kg/day) amifampridine phosphate, given as a TID dose

Batch 050129, (b) (4) 5 mg/mL

There were no deaths in the study. No NOAEL was determined, based on CNS clinical signs at all doses. At the LD and MD, CNS clinical signs observed included: tremors, subdued behavior, paresis, decreased activity, stiff hindlimb and/or labored breathing. At the HD, increased clinical sign incidence and severity (particularly on D1, and less severe on following days) were observed, including: liquid feces, hypersalivation, increased muscle tone, anxious behavior, aggressiveness, prostrate, hyperesthesia, poor motor coordination, loss of balance and tonic convulsion. Body weight losses ($\leq 4\%$ in HDM, $\leq 6\%$ in HDF) in were observed during the first week and were associated with reduced food consumption; mean body weight, compared to control, was reduced in HDF on D28 (approximately 5%). Body weight gains (from D0 to D28) were reduced in treated females (although not clearly dose-related).

Dose-related reductions in RBC parameters were observed in treated males and HDF (up to 10% in HDM, [ss]; 5% in HDF); alterations were not observed after recovery. Few clearly drug-related changes were observed. Minimal myopathy was observed sporadically (in skeletal, psoas, or diaphragm muscle) in treated animals, but was considered incidental by the pathologist.

The sponsor provided the following TK summary, below.

Occasion	Dose (mg/kg/day)	Sex	T1			T2			T3			AUC _{0-24h} (ng.h/mL)
			C _{max} (ng/mL)	T _{max} ^a (h)	AUC _{0-6h} (ng.h/mL)	C _{max} (ng/mL)	T _{max} ^a (h)	AUC _{0-6h} (ng.h/mL)	C _{max} (ng/mL)	T _{max} ^a (h)	AUC _{0-6h} (ng.h/mL)	
day 0	1	Male	46	0.75	136	37	2.00	158	57	0.75	186	513
		Female	59	0.25	143	31	0.25	120	57	0.75	151	438
	2.5	Male	145	0.75	391	106	2.00	442	176	0.75	473	1375
		Female	188	0.75	421	96	2.00	369	120	0.75	389	1243
	6.25	Male	352	0.75	918	271	2.00	1072	329	0.75	1047	3211
		Female	345	0.75	912	326	0.25	1074	377	0.75	956	3059
week 4	1	Male	51	0.25	136	37	2.00	154	46	0.75	170	500
		Female	56	0.25	139	36	2.00	156	54	0.75	185	523
	2.5	Male	95	0.75	303	112	2.00	480	166	0.75	514	1414
		Female	146	0.75	396	96	2.00	399	136	0.75	441	1314
	6.25	Male	287	0.75	833	241	2.00	997	367	0.75	1094	3146
		Female	261	0.75	741	221	2.00	922	324	0.75	984	2841

^a Median values were calculated instead of the mean

Study title: 9 Month Repeat Dose Oral Toxicology Study of Amifampridine (BMN125) Phosphate in Beagle Dogs

Study no.: S12032
Study report location: EDR, SDN1
Conducting laboratory and location: (b) (4)
Date of study initiation: 10/31/13
GLP compliance: Yes (FDA), except

- Drug characterization (GMP)
- Formulation analysis

QA statement: Yes
Drug, lot #, and % purity: Amifampridine phosphate, Lots DAPP-020; DAPP-021; DAPP-022, (b) (4)
% pure

Methods (see the sponsor's summary design Table 1, below)

Frequency of dosing: TID
Route of administration: PO (capsule)
Species/Strain: Beagle dog (Canis familiaris)
Number/Sex/Group: Main: 4/sex/gp
Recovery 2/sex/group
Age: 5.6-6.6. months
Weight: 6.2 to 10.15 kg

Table 1 Study Design

Group	Free Base Dose Level ^b		Dose Conc. ^c	Dose Volume ^c	Main		Recovery	
	mg/kg/Day	mg/kg/Dose	mg/mL	mL/kg/Dose	♀	♂	♀	♂
1 (Control ^a)	0	0	0	0.052	4	4	2	2
2 (Low)	0.53 (1.0)	0.18 (0.34)	13	0.014	4	4	2	2
3 (Mid)	1.0 (1.9)	0.33 (0.63)	13	0.025	4	4	2	2
4 (High)	2.0 (3.8)	0.67 (1.27)	13	0.052	4	4	2	2

^a Control animals received water (Water for Injection grade) in capsule.

^b Amounts in parentheses represent amifampridine phosphate equivalent. Dose levels and dose concentration were expressed as free base. To correct for salt content, a correction factor of 1.9 is used.

^c Amifampridine phosphate was administered orally in capsule.

Note: Day 1 constituted the first day of dose administration.

Observations and Results

Mortality [Twice daily]

One HDM (#4M3) was removed from the study on D213 based on behavioral issues (i.e., the sponsor cited “intractably fractious” behavior). On D197, the animal became “very aggressive even with intense socialization.” Seizures were reported on D200 and D209; the event on D200 documented that the animal was “laying rigid on the floor, unresponsive. Animal remained unsteady....”. On D211, ulcerations on the lip mucosa were observed (the pathologist attributed these to self-trauma), with increased sensitivity to stimuli and increased aggression with handling. The ulcerations were described moderate acute inflammation and slight hemorrhage of the subcutis and underlying musculature.

Clinical Signs [3x/day for 2W, then weekly; 30 – 90 minutes postdose]

Dose-related CNS clinical signs were observed at all doses, including: sneezing, tremors, hypersalivation, lip-licking, “squinty eyes,” panting, and aggressive behavior; seizures were observed in MD and HD males. See the sponsor’s Table 6, below. Although no seizures were recorded in females, one HDF with “tremors” reported in the sponsor’s table was described as, “Possible seizure... with significant head tremors extending to muscles around the shoulders, but was ambulating normally” on D2. One MDM experienced a seizure on D111, described as “full body twitching, hypersalivation, and a short 30-45 second seizure... recovered within 3 minutes.”

Table 6 Clinical Observation Incidence Summary

Gender	Male				Female			
	1	2	3	4	1	2	3	4
Group	1	2	3	4	1	2	3	4
Dose Level (mg fb/kg/day)	Vehicle (0.0)	Low (0.53)	Mid (1.0)	High (2.0)	Vehicle (0.0)	Low (0.53)	Mid (1.0)	High (2.0)
N	6	6	6	6	6	6	6	6
Emesis ^a	17/6 (0)	20/5 (0)	28/6 (1)	29/6 (0)	15/6 (2)	29/6 (2)	19/6 (0)	26/6 (0)
Diarrhea ^a	41/6 (16)	28/6 (5)	66/6 (0)	26/6 (8)	36/6 (12)	48/6 (22)	16/3 (1)	63/6 (15)
Sneezing	0	7/2	61/6	99/6	0	2/2	12/6	86/6
Tremors	0	0	1/1	10/3	0	1/1	2/2	4/2
Seizures	0	0	1/1	3/2	0	0	0	0
Panting	3/2	4/2	11/4	19/5	8/2	8/3	8/2	15/6
Hypersalivation	0	1/1	1/1	6/2	0	0	0	7/3
Lip-Licking	0	0	12/3	38/6	0	2/1	5/3	27/6
Squinty Eyes	0	0	0	4/3	1/1	0	0	5/3

Note: The results were expressed as # of findings/# of animals with findings over the course of the study.

^a Numbers in parentheses indicate instances of emesis or diarrhea with redness, blood, etc.

Body Weights [D1, 2x/week for 2W, then weekly]

Body weight losses were reported in the first week, but were seen in all groups (dose-related in females). Although not strictly dose-related in males, mean body weights in the treated animals were approximately 10% lower than controls. At W39, slight body weight differences (i.e., reduced 3% to 7%, compared to controls) were observed in treated females, MDM and HDM.

Feed Consumption [daily, quantitative]

All animals were offered a daily ration of 250 to 400 g of food. No clear changes in food consumption were observed.

Ophthalmoscopy [pre-dose, D24, D268, D301]

Examination was performed by [REDACTED] ^{(b) (6)} The HDM sacrificed early showed bilateral moderate ocular congestion. No drug-related findings were reported.

ECG [pre-dose, D24, D268, D301]

Examination was performed by [REDACTED] ^{(b) (6)} No drug-related findings were reported.

Clinical Pathology [pre-dose, D27, D272, D300]

Hematology [parameters from the sponsor, below]

White Blood Cell Count	Neutrophils (% and absolute)
Red Blood Cell Count	Eosinophils (% and absolute)
Hemoglobin	Basophils (% and absolute)
Hematocrit	Lymphocytes (% and absolute)
Mean Cell Volume	Monocytes (% and absolute)
Mean Cell Hemoglobin	Platelet Count
Mean Cell Hemoglobin Concentration	Reticulocytes
Differential White Blood Cell Count	Abnormalities of blood smear

Activated Partial Thromboplastin Time (APTT)	Prothrombin Time (PT)
--	-----------------------

At W4, transient dose-related reductions (up to 17%, [ss]) in PT were observed in treated animals. Reticulocyte counts were increased (63%, [nss]) in HDM. At W39, reticulocyte counts were increased approximately 18% in HDM and HDF. After the recovery period, reticulocyte counts were increased 1.5- to 3-fold in HDM and HDF, respectively, compared to controls.

Clinical Chemistry [parameters from the sponsor, below]

Alanine aminotransferase (ALT)	Creatinine
Albumin	Globulin
Albumin/ Globulin Ratio	Glucose
Alkaline Phosphatase (ALP)	Inorganic Phosphorus
Aspartate Aminotransferase (AST)	Potassium
Blood Urea Nitrogen (BUN)	Sodium
Calcium	Total Bilirubin
Chloride	Total Protein
Cholesterol	Triglycerides

At W4, transient, dose-related, slight increases in blood sodium and chloride (and calcium in males) were observed. At W39, blood phosphorus was slightly reduced at HD (25-37%, [ss]).

Urinalysis [parameters from the sponsor, below]

Volume	Protein
Bilirubin	Specific Gravity
Blood	Urobilinogen
Color and Clarity	Urine microscopic examination including the presence of WBC, RBC, crystals and bacteria)
Glucose	
Ketones	
pH	

No drug-related changes were observed.

Gross Pathology

No drug-related macroscopic changes were observed.

Organ Weights

Salivary gland weights were increased (up to 36%) in MDF and HDF. Adrenal weight was reduced up to 19% at HD. Dose-related reductions in thyroid weight were observed in males (up to approximately 20%, [nss]). Uterus weights in treated females were approximately 2-fold that of controls [nss], and ovary weights were increased approximately 30% to 40% [nss]. After recovery, reduced prostate and uterus weights were observed in treated animals (up to ~50%, compared to controls).

Histopathology

Adequate Battery	Yes (see Histopathology Inventory) Main: all groups; Recovery: not done
Separate, Signed report Peer Review	Yes (b) (6) No

Histological Findings

Few potentially drug-related changes were observed. Slight corneal fibrosis was observed in one HDM. Moderate pyelonephritis was observed in the kidney in one HDF. No observations were considered drug-related by the pathologist. Because no drug-related findings were reported in the main study, histopathological assessment of tissues at recovery was not performed.

Toxicokinetics [see the sponsor's Table 2, below]

Table 2: Toxicokinetic Profile Plasma Sampling Strategy

TK Series	Animals	Reported Time Points
Day 1, 28 and 273	All animals in Group 1 ^a	1 hr post 3 rd dose (13 hr)
Day 1, 28 and 273	All animals in Groups 2, 3 and 4	pre-dose, 0.5, 1, 2, 6 hr post 1 st daily dose, 1 hr post 2 nd dose (7 hr after 1 st dose), 1 hr post 3 rd dose (13 hr after 1 st dose)

^a Group 1 animals had all 7 blood samples collected on Day 1, and 3 selected samples (1 hr post 1st, 2nd and 3rd dose) collected on Day 28 and 273. Only the sample of 1 hr post 3rd dose (13 hr) was analyzed per Protocol Amendment 1.

The 3-N-acetyl metabolite was not quantifiable in any group. Dose-proportional increases in exposure to amifampridine were observed. No clear sex difference was observed. The sponsor provided summary TK Table 3, below.

Table 3: Day 1, 28 and 273 Amifampridine Mean Toxicokinetic Parameters

Day	Group	Dose Level (mg fb/kg/dose) ^a	Gender	C _{max} (ng/mL)	t _{max} (hr)	t _{1/2} (hr)	AUC (hr*ng/mL)		
							AUC ₀₋₆	AUC ₀₋₂₄	AUC _{0-∞}
1	2	0.18 (0.53)	Male	81.4	0.58	1.8	215	645	241
			Female	74.0	1.00	1.9	211	634	240
			Mean	77.7	0.79	1.9	213	639	241
	3	0.33 (1.0)	Male	141	0.92	1.7	415	1250	459
			Female	149	0.67	1.7	391	1170	432
			Mean	145	0.80	1.7	403	1210	446
	4	0.67 (2.0)	Male	285	0.83	1.8	776	2330	897
			Female	281	0.67	1.6	793	2380	845
			Mean	283	0.75	1.7	785	2350	871
28	2	0.18 (0.53)	Male	72.8	0.92	2.1	231	693	278
			Female	78.4	0.92	1.9	221	663	249
			Mean	75.6	0.92	2.0	226	678	264
	3	0.33 (1.0)	Male	134	1.00	2.1	425	1280	508
			Female	145	0.75	1.9	388	1160	441
			Mean	140	0.88	2.0	407	1220	475
	4	0.67 (2.0)	Male	258	1.08	2.1	755	2260	918
			Female	253	0.75	2.0	761	2280	882
			Mean	256	0.92	2.1	758	2270	900
273	2	0.18 (0.53)	Male	68.3	1.00	2.5	230	688	290
			Female	81.4	0.58	2.1	218	653	253
			Mean	74.9	0.79	2.3	224	670	272
	3	0.33 (1.0)	Male	151	0.67	2.2	433	1300	517
			Female	159	0.58	2.0	406	1220	466
			Mean	155	0.63	2.1	420	1260	492
	4	0.67 (2.0)	Male	283	0.70	2.2	864	2590	1030
			Female	260	0.75	1.9	769	2310	876
			Mean	272	0.73	2.1	817	2440	953

Dosing Solution Analysis [D1, 3M, 6M, last day]

Dose formulation analysis showed that amifampridine concentrations were 5% to 20% (on D1) higher than nominal concentrations.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Mutagenicity Test on Salmonella Typhimurium His⁻ Using B.N. Ames's Technique with 3-4 Diamino Pyridine

Study no.: (b) (4) -010310
 Study report location: EDR, SDN1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 1/29/2001
 GLP compliance: Yes (FDA)
 QA statement: Yes
 Drug, lot #, and % purity: amifampridine phosphate, Batch 000077, (b) (4) % pure

Methods (see the sponsor's summary design table, below)

Strains: TA 1535, TA1537, TA98, TA100, TA102
 Concentrations in definitive study: See below from sponsor
 Basis of concentration selection: No Cytotoxicity/ Limit concentration (5000µg/plate)
 Negative control: distilled water for injectable preparations
 Positive control: See below from sponsor
 Formulation/Vehicle: distilled water for injectable preparations
 Incubation & sampling time: Incubated 48 hours at 37°C

Without S9 mix

Strain	TA1535		TA1537		TA98		TA100		TA102	
Assay	1	2	1	2	1	2	1	2	1	2
	0	0	0	0	0	0	0	0	0	0
Doses	50	50	50	50	50	50	50	50	50	50
µg/plate	150	150	150	150	150	150	150	150	150	150
	500	500	500	500	500	500	500	500	500	500
	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000

With S9 mix

Strain	TA1535		TA1537		TA98		TA100		TA102	
Assay	1	2*	1	2*	1	2*	1	2*	1	2*
	0	0	0	0	0	0	0	0	0	0
Doses	50	50	50	50	50	50	50	50	50	50
µg/plate	150	150	150	150	150	150	150	150	150	150
	500	500	500	500	500	500	500	500	500	500
	1500	1500	1500	1500	1500	1500	1500	1500	1500	1500
	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000

* with pre-incubation

Strains	Without metabolic activation		With metabolic activation		
	Product	Dose µg/plate	Product	without pre-incubation Dose µg/plate	with pre-incubation Dose µg/plate
TA1535	sodium azide	1	2-anthramine	2	1
TA1537	9-amino-acridine	50	2-anthramine	2	1
TA98	2-nitro fluorene	2	2-anthramine	2	1
TA100	sodium azide	1	2-anthramine	2	1
TA102	mitomycin C	0.125	benzo[a]pyrene	2	2

Study Validity

Standard methodology was used. Aroclor-1254-induced (500 mg/kg IP, single injection) S9 fraction from male Sprague Dawley rat (b) (4) was used as the metabolic activation system. The plate incorporation method was used.

Results- Negative

Clear concentration-dependent increases in revertant frequency with amifampridine treatment were not observed. Although the first assay showed revertant frequencies in TA1535 that were increased approximately 2-fold at all concentrations in the absence of metabolic activation, these observations did not meet the criteria for a positive response (i.e., 3x or concentration-dependent) and were not replicated in the confirmatory assay.

Study title: Mutagenicity Test on Bacteria (Salmonella Typhimurium His⁻) Using B.N. Ames Technique Strains TA1538 and TA98 With Metabolic Activation by Hamster S9

Study no.: (b) (4)-010506
 Study report location: EDR, SDN1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 1/29/2001
 GLP compliance: Yes (FDA)
 QA statement: Yes
 Drug, lot #, and % purity: amifampridine phosphate, Batch 000077, (b) (4) % pure

Methods (see the sponsor's summary design table, below)

Strains: TA 1538 and TA98 (because an aromatic amine is present in the structure)
Concentrations in definitive study: 0, 50, 150, 500, 1500, and 5000 µg/plate
Basis of concentration selection: No Cytotoxicity/ Limit concentration (5000µg/plate)
Negative control: distilled water for injectable preparations
Positive control: See below from sponsor
Formulation/Vehicle: distilled water for injectable preparations
Incubation & sampling time: Incubated approximately 48 hours at 37°C

Strains	Product	With metabolic activation	
		without pre-incubation Dose µg/plate	with pre-incubation Dose µg/plate
TA1538	2-anthramine	2	1
	Benzidine	100	50
TA98	2-anthramine	2	1
	Benzidine	100	50

* in the presence of S9 fraction of hamster liver

Study Validity

The two strains were selected on the basis of the presence of an aromatic amine in the structure. Aroclor-1254-induced (by IP injection) male hamster liver S9 fraction (Batch 99-A; from ^{(b) (4)}) was used as the metabolic activation system. Otherwise, standard methodology was used.

Results- Negative

Revertant frequencies were not increased after amifampridine treatment in the presence and absence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Mutation Assay at the TK Locus in L5178Y Mouse Lymphoma Cells using a Microtiter Cloning Technique (Trifluorothymidine Resistance) with 3,4-Diaminopyridine

Study no.: (b) (4)-010515
 Study report location: EDR, SDN1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 1/29/2001
 GLP compliance: Yes (FDA)
 QA statement: Yes
 Drug, lot #, and % purity: amifampridine phosphate, Batch 000077,
 (b) (4)% pure

Methods (see below from the sponsor)

CELL STRAIN : L5178Y mouse lymphoma cells
 VEHICLE : Fischer culture medium without serum (FM0)
 TYPE OF MUTATION STUDY : TK Locus (Trifluorothymidine Resistance)

TOXICITY TEST

- Treatment duration : Without S9-mix : short treatment : 3 hours
 : Without S9-mix : continuous treatment: 24 hours
 : With S9-mix : 3 hours

- Doses tested : Without S9-mix : 78.125- 156.3- 312.5- 625- 1250- 2500- 5000 µg/ml
 : With S9-mix : 78.125- 156.3- 312.5- 625- 1250- 2500- 5000 µg/ml

MUTAGENICITY TEST Carried out both without and with metabolic activation using hepatic microsomes from rat livers induced by Aroclor1254 (S9-mix)

- TREATMENT DURATION : Without S9-mix : short treatment : 3 hours
 : Without S9-mix : continuous treatment : 24 hours
 : With S9-mix : 3 hours

- Doses tested (µg/ml) : expressed as µg/ml
 • Without S9 mix : 700.3 - 910.3 - 1183.4 - 1538.5 - 2000 (assay 1: 3 hour treatment)
 592.6 - 888.9 - 1333.3 - 2000 - 3000 (assay 2: 24 hour treatment)
 • With S9 mix : 700.3 - 910.3 - 1183.4 - 1538.5 - 2000 assay 1)
 700.3 - 910.3 - 1183.4 - 1538.5 - 2000 (assay 2)

- POSITIVE CONTROLS : Without S9-mix :
 Methyl methanesulfonate (b) (4) 10 µg/ml (3 h treatment)
 Methyl methanesulfonate (b) (4) 2 µg/ml (24 h treatment)
 : With S9-mix :
 Cyclophosphamide (b) (4) 2 µg/ml

- EXPRESSION TIME : 2 days after treatment
 - NUMBER OF ASSAYS : 2
 - NUMBER OF REPLICATE CULTURES : 2 per dose
 - FACTOR LIMITING THE MAXIMUM DOSE : cytotoxicity

Study Validity

Standard methodology was used. In the preliminary cytotoxicity assay (3-hr, +/- S9), the RTG was 0.4-1.2% at 2500 µg/mL. The sponsor indicated that cytotoxicity was the basis for the maximum concentration tested, but the RTG at the maximum concentrations used in the pivotal assays did not reach the criterion of 10-20% in most cultures (i.e., 3-hr -S9 RTG was 26-38%; 3-hr +S9 RTG was 23-31%; 24-hr -S9 RTG was 10-87%). No explanation was provided for the difference in the cytotoxicity between Culture A and Culture B in the 24-hr continuous exposure (and it is noted that the RTG at second highest concentration, 2000 µg/mL, was 40%).

Results- Positive

No effects were observed in the 3-hr assays (see sponsor's Table 12, below); however, a weak clastogenic effect was demonstrated in the 24-hr without metabolic activation treatment assay (see sponsor's Table 14, below). Analysis compared to the accepted GEF of 126×10^{-6} also suggested a weak clastogenic effect, with increased small colony frequency. The mutation frequency was highest in Culture B, the culture in which the maximum concentration tested did not show the required level of cytotoxicity. While the study clearly showed methodological issues, the conservative interpretation is that a weak clastogenic effect was demonstrated in this assay as conducted.

TABLE 12

MUTATION ASSAY AT THE LOCUS TK IN L5178Y MOUSE LYMPHOMA CELLS

**RECAPITULATION OF 2 CULTURES IN THE ASSAY 1
WITHOUT METABOLIC ACTIVATION
(3 HOUR SHORT TREATMENT)**

ASSAY 1 without S9 3 H TREATMENT	RTG % (Relative total growth)						
	DOSE µg/ml						MMS 10 µg/ml
	0	700.3	910.3	1183.4	1538.5	2000	
Culture A	100	81.4	121.9	77.0	68.6	37.9	52.2
Culture B	100	73.5	70.1	64.5	56.1	25.8	89.3
MEAN	100.0	77.4	96.0	70.8	62.3	31.9	70.7
ASSAY 1 without S9 3 H TREATMENT	MUTATION FREQUENCY X 10^{-6} cells						
	DOSE µg/ml						MMS 10 µg/ml
	0	700.3	910.3	1183.4	1538.5	2000	
Culture A	84.7	69.2	89.1	93.3	73.2	99.3	582.4
Culture B	117.8	74.5	108	71.6	102.1	86	495.8
MEAN	101.3	71.9	98.6	82.5	87.7	92.7	539.1
Ratio		0.7	1.0	0.8	0.9	0.9	5.3

**RECAPITULATION OF 2 CULTURES IN THE ASSAY 1
WITH METABOLIC ACTIVATION**

ASSAY 1 with S9 3 H TREATMENT	RTG % (Relative total growth)						
	DOSE µg/ml						CPA 2 µg/ml
	0	700.3	910.3	1183.4	1539	2000	
Culture A	100	82.6	66.4	85.7	49.5	31.4	68.2
Culture B	100	77.7	49.8	61.8	34.3	23.1	75.3
MEAN	100.0	80.1	58.1	73.8	41.9	27.2	71.8
ASSAY 1 with S9 3 H TREATMENT	MUTATION FREQUENCY X 10^{-6} cells						
	DOSE µg/ml						CPA 2 µg/ml
	0	700.3	910.3	1183.4	1539	2000	
Culture A	222.8	127.1	152.9	161.3	124.7	96.1	946.5
Culture B	130	65.9	108.9	103.7	83.8	116.7	1045.1
MEAN	176.4	96.5	130.9	132.5	104.3	106.4	995.8
Ratio		0.5	0.7	0.8	0.6	0.6	5.6

Ratio= Mutation Frequency(treated)
Mutation frequency (control)

TABLE 14

MUTATION ASSAY AT THE TK LOCUS
IN L5178YMOUSE LYMPHOMA CELLS
WITHOUT METABOLIC ACTIVATION
(24 HOUR CONTINUOUS TREATMENT)

ASSAY 2

TIME 2 days after treatment

Starting date : 12/4/2001

Completion date: 24/4/2001

CULTURE A		MUTATION											VIABILITY at T2				Mutation Frequency x10 ⁻⁶ cells	RATIO	
COMPOUND	DOSE µg/ml	Number of positive wells										TOTAL Wells		Number of negative wells		PE %			
		SMALL COLONIES					LARGE COLONIES					+	-	Plate	TOTAL per 192				
		f	2	3	4	TOTAL per 384	f	2	3	4	TOTAL per 384								
Solvent Control	0	18	18	20	24	80	11	7	6	11	35	115	269	13	8	21	138.3	128.7	
TEST COMPOUND	592.6	19	20	18	21	78	13	12	11	13	49	127	257	11	6	17	151.5	132.5	1.0
	888.9	16	14	16	19	65	10	9	8	4	31	96	288	13	15	28	120.3	119.5	0.9
	1333.3	12	11	25	17	65	11	10	8	9	38	103	281	11	10	21	138.3	112.9	0.9
	2000	22	22	17	20	81	7	8	16	12	43	124	260	20	14	34	108.2	180.2	1.4
	3000	23	21	25	28	97	16	8	15	9	48	145	239	26	25	51	82.9	286.2	2.2
MMS	2	40	45	40	45	170	29	18	23	28	98	268	116	26	24	50	84.1	711.8	5.5

CULTURE B		MUTATION											VIABILITY at T2				Mutation Frequency x10 ⁻⁶	RATIO	
COMPOUND	DOSE µg/ml	Number of positive wells										TOTAL Wells		Number of negative wells		PE %			
		SMALL COLONIES					LARGE COLONIES					+	-	Plate	TOTAL per 192				
		f	2	3	4	TOTAL per 384	f	2	3	4	TOTAL per 384								
Solvent Control	0	9	6	3	9	27	10	9	13	9	41	68	316	13	12	25	127.4	76.5	
TEST COMPOUND	592.6	13	9	11	11	44	10	7	7	8	32	76	308	7	8	15	159.3	69.2	0.9
	888.9	9	18	14	15	56	8	12	10	8	38	94	290	11	12	23	132.6	105.8	1.4
	1333.3	14	12	20	21	67	12	13	14	12	51	118	266	13	12	25	127.4	144.1	1.9
	2000	20	19	20	17	76	13	8	16	16	53	129	255	16	18	34	108.2	189.2	2.5
	3000	23	25	23	16	87	14	15	15	14	58	145	239	27	26	53	80.5	294.7	3.9
MMS	2	41	49	35	37	162	26	25	23	18	92	254	130	19	19	38	101.2	534.9	7.0

PE: Plating efficiency

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mutagenicity Study using the Micronucleus Test in Rat with 3-4 Diamino Pyridine

Study no: (b) (4)-010417
 Study report location: EDR, SDN1
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 1/29/2001
 GLP compliance: Yes (FDA)
 QA statement: Yes
 Drug, lot #, and % purity: Amifampridine phosphate, Batch 000077, (b) (4)% pure

Methods

Doses in Definitive Study	0, 20, 40, and 80 mg/kg/day (given TID), PO
Formulation/Vehicle:	Distilled water for injectable preparations 10 mL/kg
Frequency of Dosing	Vehicle and Treated: 3 daily doses Pos Con: one dose
Species/Strain:	SD rats (b) (4) 5/sex/group mean body weights 159 g (M) and 152 g (F) 5-6 weeks old
Negative Control	Vehicle
Positive Control	Cyclophosphamide, 25 mg/kg IP

Study Validity

Animals were dosed once a day for 3 days and samples were collected 24 hr after the last dose. In a preliminary assay, the maximum tolerated dose based on convulsions and/or mortality was 125 mg/kg; however, in the confirmation assay two HD animals (satellite, M and F) were found dead at 24 hr after the first dose. Since mortality was demonstrated at 125 mg/kg, 80 mg/kg was selected as the maximum dose in the main assay. One male and one female (from the satellite group) at 80 mg/kg were found dead 48 hr after the first dose. It is noted that the males used in the main study had statistically significantly higher mean body weights than the other male groups; however, the differences did not exceed 20%.

The number of PCE assessed for each animal was 2000. The PCE:NCE ratio was determined by counting at least 1000 erythrocytes/animal. While the most recent (2016) OECD guideline 474 indicates that 4000 PCE/animal should be scored for MN, this assay was conducted in 2001 and complied with the 1997-version of OECD Guideline 474.

Results- Negative

Amifampridine did not show an effect on the PCE: NCE ratio, nor did it increase the frequency of MNPCE.

7.4 Other Genetic Toxicity Studies

Study title: Measurement of Unscheduled DNA Synthesis (UDS) in Rat Hepatocytes using an *In Vivo* Procedure with 3,4 Diaminopyridine

Study no: (b) (4)-020404
Study report location: EDR, SDN1
Conducting laboratory and location: (b) (4)
Date of study initiation: 1/29/2001
GLP compliance: Yes (FDA)
QA statement: Yes
Drug, lot #, and % purity: Amifampridine phosphate, Batch 010085, (b) (4)% pure

Methods

Doses in definitive study: 0, 20, 40 mg/kg [80 mg/kg not tolerated]
Frequency of dosing: Single PO dose (10 mL/kg)
Species/Strain: Male Fischer rats (b) (4)
3/group
Expression times: 2-4 hr and 12-16 hr postdose
Number of cells counted/animal: At least 150
Reference Substances: 2-4 hr: dimethylhydrazine, 10 mg/kg
12-16 hr: 2-acetamidofluorene
Vehicle: Distilled water

Study Validity

Standard methodology was used, using the OECD Guideline 486 (1997). A preliminary toxicity assay was conducted in 4 male Fischer rats; 80 mg/kg was selected as the MTD based on mortality (~90 minutes postdose) at ≥ 125 mg/kg. In the main assay, 80, 40 and 20 mg/kg were used; however, three of the four animals given 80 mg/kg were found dead at perfusion time. Therefore, animals from the 40 and 20 mg/kg groups only were assessed for the 12 to 16 hr expression assay as well as a second 2 to 4 hr expression assay.

Results- Negative

At 20 and 40 mg/kg amifampridine phosphate, genotoxic activity as not observed (i.e., the observed net nuclear grain counts did not exceed 0 and the percentage of cells in repair was not increased). The percentage of cells in S-phase was low (0% to 0.2%; the range was 0% to 0.1% in solvent control).

8 Carcinogenicity

Because the 2-year carcinogenicity study in rats was conducted by dietary administration, a palatability study was conducted to support dose selection.

Study title: 3-Week Palatability and Toxicokinetic Dietary Study for BMN 125 in Rats

Study no.: 8283015 (BMN125-13-011)
 Study report location: EDR (SDN1)
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 4/1/2013
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: Amifampridine phosphate, batches DAPP-018 and DAPP-019, (b) (4)% and (b) (4)%, respectively

Methods (see summary design table below, from the sponsor)

Route of administration: PO (dietary)
 Species/Strain: Hsd:Sprague Dawley®SD® rats
 Age: 8-10 weeks old
 Weight: M: 246-348 g; F: 189-230 g

Group	No. of Animals		BMN 125fb ^a	BMN 125fb ^b
	Male	Female	Target Diet Concentration (ppm)	Target Dose Level (mg/kg/day)
1 (Control) ^c	9	9	0	0
2 (Low)	9	9	36/55 ^d	4
3 (Low Mid)	9	9	110/170 ^d	12
4 (High Mid)	9	9	360/550 ^d	40
5 (High)	9	9	550/975 ^e	70 ^e

- a Concentrations were expressed as BMN 125fb (ppm, BMN 125fb/kg diet). A conversion factor of 1.9 was used to correct for phosphate salt content of the test article.
- b The approximate dose on a mg/kg/day basis was estimated based on expected body weight and food consumption values and the diet concentrations. The BMN 125fb dose levels of 4, 12, 40, and 70 mg/kg were approximately equivalent to 7.5, 22.5, 75, and 133 mg/kg/day of BMN 125 phosphate salt applying the correction factor of 1.9.
- c Group 1 received carrier (basal diet) only.
- d Diet concentrations for Groups 2, 3, and 4 were 36, 110, and 360 ppm, respectively, on Days 1 through 7 of the dosing phase and 55, 170, and 550 ppm, respectively, for Day 8 until the last day of dosing. The dose levels for Groups 2, 3, and 4 were the same dose levels used in a 13-week oral gavage toxicity study (Lee, 2011; (b) (4) Study No. 8227536, BioMarin Study No. BMN125-10-055).
- e Group 5 animals received 550 ppm (40 mg/kg/day) on Days 1 through 5 of the dosing phase and 975 ppm (70 mg/kg/day) on Days 6 until the end of the dosing phase.

Note from the sponsor: "Group 5 received test diets for 18 days because emerging data from Groups 1-4 were used to select the diet concentrations to use for Group 5. Initiation of Group 5 corresponded to Study Day 15 for Groups 1 through 4."

Observations and Results

Amifampridine concentrations were within $\pm 15\%$ of nominal in the diets. The “achieved” mean doses are provided in the sponsor’s table below.

Interval (day)	Group	Target Dose Level (mg/kg)	Sex	Achieved Mean Dose Level (mg/kg)	
4	2	4	M	2.3	
			F	2.5	
	3	12	M	6.9	
			F	7.2	
	4	40	M	23.9	
			F	19.9	
5	40	M	36.7		
		F	35.8		
9	5	70	M	55.2	
			F	55.2	
15	2	4	M	2.3	
			F	3.3	
	3	12	M	7.9	
			F	8.7	
	4	40	M	23.6	
			F	25.7	
	5	70	M	48.6 ^a	
			F	67.4 ^a	
	29	4	40	M	28.4 ^b
				F	32.9 ^b

a Achieved mean dose calculated over Days 15 through 17.
b Achieved mean dose calculated over Days 29 through 31.

There were no early mortalities. One HDF was reported as “thin” and 3 HDF showed “few feces.” Mean body weights showed slight reductions by D18 (3%, 4%, 3%, in males and 6%, 6%, 6% in females) at the LD, LMD, and HMD, respectively, compared to controls; these reductions partially resolved in males and remained similar in females by D32. Although there was no directly comparable control group (the HD was conducted separately), HDM animals showed slight body weight losses from D1 to D11 (M) or D15 (F). The sponsor reported “transient” reductions in food consumption and body weight gain; however, the sponsor did not consider them adverse because “adaptation occurred over time.” Food consumption data, while variable, usually showed reductions in the treated groups compared to controls. The sponsor stated that reductions in food consumption were apparent on days when blood samples were collected for toxicokinetic evaluations; these changes may have resulted from changes in eating “behavior associated with human activity in the animal room during portions of the night cycle when animals were consuming a large portion of their daily diet” and/or reduced palatability. No clearly drug-related adverse clinical pathology or anatomical pathology changes were observed.

Amifampridine exposures increased with increasing dose, generally greater than dose proportionally. 3-N-acetyl-amifampridine exposures increased with increasing dose, generally dose proportionally. Exposures in females were usually greater (by less than

2-fold) than in males. Exposures were generally higher on D15 or D29 compared to D4; steady state was reached at approximately D15. Amifampridine was extensively converted to 3-N-acetyl BMN 125 in rats. See the sponsor's summary TK Tables 1 and 3, below.

Table 1
Toxicokinetic Parameters for BMN 125fb in Rat Plasma

Interval (day)	Group	Target	Achieved Mean	Sex	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg)]	T _{max} (hr)	AUC ₀₋₆ ^a (ng hr/mL)	AUC ₀₋₁₈ ^a (ng hr/mL)	AUC ₀₋₂₄ (ng hr/mL)	DN AUC ₀₋₂₄ [(ng hr/mL)/(mg/kg)]
		Dose Level (mg/kg)	Dose Level (mg/kg)								
4	2	4	2.3	M	1.51	0.657	3.00	6.28	14.1	18.6	8.07
			2.5	F	2.31	0.923	3.00	9.47	22.8	26.8	10.7
	3	12	6.9	M	6.05	0.876	3.00	25.4	61.6	76.2	11.0
			7.2	F	6.66	0.925	10.0	31.0	81.3	102	14.1
	4	40	23.9	M	32.0	1.34	4.50	130	326	353	14.8
			19.9	F	35.2	1.77	10.0	145	419	475	23.9
15	2	4	2.3	M	2.95	1.28	4.50	12.1	28.9	34.5	15.0
			3.3	F	3.61	1.09	2.00	16.7	33.7	39.6	12.0
	3	12	7.9	M	9.02	1.14	6.00	42.8	98.5	114	14.5
			8.7	F	13.7	1.58	1.00	64.4	150	169	19.4
	4	40	23.6	M	41.2	1.75	6.00	111	437	470	19.9
			25.7	F	110	4.27	4.50	407	1140	1170	45.5
5 ^b	70	48.6 ^c	M	125	2.57	12.0	325	1210	1250	25.8	
		67.4 ^c	F	505	7.49	12.0	1100	5140	5620	83.3	
29	4 ^d	40	28.4 ^e	M	41.0	1.44	6.00	123	426	463	16.3
			32.9 ^e	F	135	4.11	6.00	336	1180	1250	38.1

Note: All TK parameters are calculated based on the start of the dark cycle (time 0 = approximately 17:00).
a AUC values from 0 to 6 hours and from 0 to 18 hours are reported to allow for comparison to (b) (4) Study No. 8227536 (BioMarin Reference No. BMN125-10-055) (2).
b The Day 15 parameters for Group 5 were calculated from a profile generated from staggered sample collections at various timepoints over Days 15 through 17.
c Achieved mean dose calculated over Days 15 through 17.
d The Day 29 parameters for Group 4 were calculated from a profile generated from staggered sample collections at various timepoints over Days 29 through 31.
e Achieved mean dose calculated over Days 29 through 31.

Table 3
Toxicokinetic Parameters for 3-N-Acetyl BMN 125 in Rat Plasma

Interval (day)	Group	Target	Achieved	Sex	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/(mg/kg)]	T _{max} (hr)	AUC ₀₋₆ ^a (ng hr/mL)	AUC ₀₋₁₈ ^a (ng hr/mL)	AUC ₀₋₂₄ (ng hr/mL)	DN AUC ₀₋₂₄ [(ng hr/mL)/(mg/kg)]	M:P Ratio AUC ₀₋₂₄
		BMN 125fb Dose Level (mg/kg)	Mean BMN 125fb Dose Level (mg/kg)									
4	2	4	2.3	M	140	60.7	3.00	686	1670	2070	902	152
			2.5	F	191	76.5	4.50	910	2190	2700	1080	135
	3	12	6.9	M	394	57.1	10.0	1890	5230	6450	935	84.6
			7.2	F	426	59.2	10.0	2120	6020	7380	1030	72.6
	4	40	23.9	M	1430	59.7	3.00	6690	18300	21100	881	59.7
			19.9	F	1410	70.7	10.0	5580	18200	21500	1080	45.3
15	2	4	2.3	M	186	80.7	4.50	958	2440	2970	1290	111
			3.3	F	257	77.9	4.50	1340	3210	3790	1150	95.8
	3	12	7.9	M	543	68.7	10.0	2480	6900	8140	1030	71.3
			8.7	F	625	71.9	4.50	3180	8820	10400	1190	61.6
	4	40	23.6	M	1700	72.2	10.0	4660	21400	24400	1030	51.8
			25.7	F	2150	83.5	12.0	7890	26800	30200	1180	25.9
5 ^b	70	48.6 ^c	M	3020	62.2	12.0	8590	32500	36400	749	29.1	
		67.4 ^c	F	3160	46.9	12.0	9610	39600	48200	715	8.57	
29	4 ^d	40	28.4 ^e	M	1680	59.0	12.0	5210	20600	24000	845	51.9
			32.9 ^e	F	2280	69.4	12.0	7110	25700	29600	901	23.7

Note: All TK parameters are calculated based on the start of the dark cycle (time 0 = approximately 17:00).
a AUC values from 0 to 6 hours and from 0 to 18 hours are reported to allow for comparison to (b) (4) Study No. 8227536 (BioMarin Reference No. BMN125-10-055) (2).
b The Day 15 parameters for Group 5 were calculated from a profile generated from staggered sample collections at various timepoints over Days 15 through 17.
c Achieved mean dose calculated over Days 15 through 17.
d The Day 29 parameters for Group 4 were calculated from a profile generated from staggered sample collections at various timepoints over Days 29 through 31.
e Achieved mean dose calculated over Days 29 through 31.

Study title: 104-Week Dietary Carcinogenicity Study with BMN125 in Rats

Study no.: 8264515
Study report location: EDR, SDN9
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/17/13
GLP compliance: Yes (FDA), Except:

- Drug characterization (GMP)
- Statistical analysis

QA statement: Yes
CAC concurrence: No
The sponsor originally proposed a (b) (4) however, the ExecCAC recommended a 2-year dietary study, supported by a 13-week dietary study for dose-selection (see minutes dated 12/14/11). The sponsor conducted a 3-week palatability study to support dose selection, which the Division indicated would not be adequate (Minutes dated 12/20/13). The protocol was not submitted for SPA prior to the initiation of the 2-year dietary study (June 2013). Justification for dose selection was provided in the NDA.

Drug information (below, from the sponsor)

Test Article	Storage	Batch No ^a	Retest Date ^b	Potency ^c
BMN 125 phosphate salt (also known as BMN125, 3,4-diaminopyridine phosphate, 3,4-DAP phosphate, 3,4-DAPP DS, amifampridine phosphate, Firdapse®)	(b) (4)	DAPP-022 DAPP-010 DAPP-012 DAPP-023 9103808	(b) (4)	(b) (4)

a Corresponds to Lot No.

b All batches were used before indicated retest dates

c Corresponds to purity

Adequacy of Carcinogenicity Study

Yes, the study was adequate.

- Drug concentrations in the dietary formulations were gradually increased over the first 3 weeks, with target dose reached by Week 4.
 - The originally proposed high doses (80 mg/kg in males and 60 mg/kg in females) were not feasible; the sponsor estimated that 55 mg/kg was the maximum dose achieved throughout the study.

- The sponsor calculated that the doses achieved with dietary administration (based on diet concentration, food consumption, and body weight assessed during Weeks 5, 26, and 52) were relatively consistent, ranging from 84 to 109%, 85 to 103%, and 94 to 120% of nominal for the 8-, 25-, and 55-mg/kg/day BMN125fb dose levels, respectively.
- The sponsor noted that plasma AUCs in the 55 mg/kg group were higher and the C_{max} values were lower than those observed at 75 mg/kg TID in the 13-week oral gavage toxicity study in rats ((b) (4) Study No. 8227536; BioMarin Study No. BMN125-10-055); see the sponsor's Text Table 5.1 (below).

Text Table 5.1: Comparative Toxicokinetic Parameters between the 13-Week Oral Gavage Toxicity Study and the Current Dietary Carcinogenicity Study

13-week TID Rat Oral Gavage Toxicity Study (b) (4) Study No. 8227275; BioMarin Study No. BMN125-10-055				
Dose Paradigm (highest dose used in study)	Interval	Sex	BMN 125 C _{max} (ng/mL)	BMN 125 Total AUC ^a (ng•hr/mL)
25 mg/kg/dose,	Day 92	Male	427 to 938	3166
75 mg/kg/day		Female	924 to 1204	4275
104-Week Dietary Carcinogenicity Study with BMN 125 in Rats (b) (4) Study No. 8264515; BioMarin Study No. BMN125-13-026				
Dose Paradigm (highest dose used in study)	Interval	Sex	BMN 125 C _{max} (ng/mL)	BMN 125 AUC _{0-24h} (ng•hr/mL)
55 mg/kg/day via diet	Week 52	Male	392	4820
		Female	565	7000

a Total AUC calculated as AUC_{0-6h} Dose-1 + AUC_{0-6h} Dose-2 + AUC_{0-∞} Dose-3
TID = Three times daily, approximately 6 hours apart

Appropriateness of Test Models

Yes, rat is a standard model.

Evaluation of Tumor Findings

Increased tumor incidences were observed for uterine endometrial tumors at MD and HD [ss], while tumors commonly observed in a 2-year bioassay were often reduced in incidence. An apparent increased incidence of schwannomas (benign and/or malignant) was observed (particularly in HDF) but was not statistically significant.

The sponsor attributed the dose-dependent increase in endometrial carcinomas to “an epigenetic (non-genotoxic) mechanism, and because the endometrium is responsive to estrogenic activity, dose-related changes in pituitary/endocrine axis.” The sponsor hypothesized that prolonged survival and “reduced pituitary prolactin release (e.g., by restricted feeding) favor[ed] continued cyclicity, delayed reproductive senescence (continued estrogenic drive) and persistent stimulation of the endometrium.” The tumors were usually observed late (i.e., 4 of 27 from Week 86 to Week 99, and the remaining 23 at or after Week 100), generally described as poorly-differentiated and highly invasive (and often the cause of unscheduled death). Notably, an effect on survival was less pronounced at the MD, but was clearly associated with increased endometrial tumors. The sponsor stated that the background incidence of endometrial carcinomas in Harlan Sprague Dawley rats is up to approximately 2% (see Dinse et al., 2010 and National Toxicology Program, 2013); this was clearly exceeded in the study.

Benign and/or malignant Schwannomas were observed in multiple tissues in 13 treated animals. Two animals developed the tumors early (Week 29 and Week 40), while the majority were observed in rats euthanized after Week 89 (including three observed only at terminal necropsy). Schwannomas were not observed in the concurrent controls, and the sponsor stated that, "No benign or malignant Schwannomas were observed among 170 male [sic] and 170 females from two historical studies conducted with Harlan SD rats at the test facility."

Reductions were observed for several other commonly observed tumors in rats (e.g., mammary gland fibroadenomas (F), pituitary adenomas/carcinomas (M & F), adrenal medullary pheochromocytoma (M), and pancreatic islet cell adenomas/carcinomas (M)), consistent with the reduced body weights observed during the study.

Methods (See the sponsor's summary methods table, below)

Route of administration: Dietary
Basis of dose selection: The doses were selected on the basis of a 3-week palatability study and a 13-week oral gavage toxicity study; however, maximum doses of 60-80 mg/kg were not achievable (see footnote f in the sponsor's table). The actual dose level achieved was approximately 55 mg/kg.

Species/Strain: Male and female Hsd:Sprague Dawley® SD® rats
Age: 5 weeks old
Weight: M: 143-178 g; F: 105-138 g
Animal housing: 2/sex/group
Deviation from study protocol: Many deviations were reported, but none were reported to have affected the integrity or interpretation of the study.

Group	Animals		Target Dose Level BMN 125fb ^{a,b,c} (mg/kg/day)	BMN 125fb Feed Concentration (ppm) ^{a,d}			
	Sex	Number		Week 1	Week 2	Week 3	Weeks 4-5
1 (Control) ^e	Male	60	0	0	0	0	0
	Female	60	0	0	0	0	0
2 (Low)	Male	60	8	115	115	115	115
	Female	60	8	115	115	115	115
3 (Mid)	Male	60	25	350	350	350	350
	Female	60	25	175	350	350	350
4 (High)	Male	60	80/55 ^f	350	700	1000	1300
	Female	60	60/55 ^f	175	350	700	1000

- a Concentrations were expressed as BMN 125fb. To express free base doses as the phosphate salt doses, multiply free base dose by the conversion factor of 1.9).
- b BMN 125fb dose levels of 8, 25, 55, 60, and 80 mg/kg/day were approximately equivalent to 15, 48, 105, 114, and 152 mg/kg/day BMN 125 phosphate salt, respectively.
- c The target dose on a mg/kg/day basis was estimated based on expected body weight and food consumption values and diet concentrations.
- d Due to gradual acclimation to diet containing test article (based on findings in ^{(b) (4)} Study No. 8283015; BioMarin Study No. BMN125-13-011), graded increases in diet drug concentration were implemented during the first 3 weeks of the study so as to achieve the target dose levels by Week 4 of the dosing phase. After Week 5 of the dosing phase, the feed concentration (mg BMN125fb/kg diet = ppm of BMN125 fb) was adjusted at least every 4 weeks to maintain animals at the targeted mg/kg/day dose level as animals gained body weight.
- e Group 1 received carrier (basal diet) only.
- f Initial female dose levels were lower than male dose levels due to increased BMN 125 exposure (AUC_{0-18hr}) in females compared with males at the same dose concentration in a previous study ^{(b) (4)} Study No. 8283015, BioMarin Study No. BMN125-13-011). However, based on food consumption and body weight data through Week 16, the dose levels of 80 (males) and 60 (females) mg/kg/day were not achievable. A target dose level of 55 mg/kg/day appeared feasible and was close to what was actually achieved throughout the study.

Observations and Results

Mortality

Survival was statistically significantly increased in treated male and female rats compared to controls. See the statistical analyses from Dr. Mbodj, below.

Table 2A: Intercurrent Mortality Comparison for Male Rats

Test Statistics	P-value for Control, Low, Med, high	P-value for Control vs Low	P-value for Control vs Med	P-value for Control vs High
Dose-Response (Likelihood Ratio)	0.0199*	0.0316*	0.0031*	0.0096*
Homogeneity (Log-Rank)	0.0075*	0.0308*	0.0030*	0.0095*

* = Significant at 5% level

Table 2B: Intercurrent Mortality Comparison for Female Rats

Test Statistics	P-value for Control, Low, Med, high	P-value for Control vs Low	P-value for Control vs Med	P-value for Control vs High
Dose-Response (Likelihood Ratio)	0.0192*	0.0662	0.1411	0.0072*
Homogeneity (Log-Rank)	0.0389*	0.0633	0.1347	0.0068*

* = Significant at 5% level

Survival at the end of the dosing phase was 47%, 68%, 75%, and 72% for males, and 48%, 65%, 60%, and 75% for females at doses of 0, 8, 25, and 55 mg/kg/day BMN125fb, respectively. The sponsor attributed the dose-dependent increase in survival to the reduced food consumption and BWG/BW observed in treated animals.

The incidences of endometrial adenocarcinomas and malignant Schwannomas identified as the cause of death were increased in BMN125-treated animals. Other common causes of death were apparently reduced with BMN125 treatment. See below from the sponsor's summary table.

Table
Summary of Severity of Microscopic Observations
All Animals

Test Article	(dosage)	Sex								
		M	1	2	3	4	5	6	7	
BMN 125	mg/kg/day	M	0	8	25	80/55				
		F	0	8	25	60/55				

Tissue/Observation	Group/Sex: Number of Animals	Group/Sex: 1/M 2/M 3/M 4/M 1/F 2/F 3/F 4/F							
		60	60	60	60	60	60	60	60
Death Comment	Number Examined:	32	20	16	17	31	21	24	16
	Unremarkable:	0	0	0	0	0	0	0	0
Endocardial Schwannoma	finding not present -	32	20	16	17	31	21	24	15
	Present	0	0	0	0	0	0	0	1
Endometrial adenocarcinoma	finding not present -	32	20	16	17	31	20	20	12
	Present	0	0	0	0	0	1	4	4
Malignant Schwannoma	finding not present -	32	20	14	16	31	21	22	14
	Present	0	0	2	1	0	0	2	2
Renal carcinoma	finding not present -	32	20	16	16	31	21	24	16
	Present	0	0	0	1	0	0	0	0
Chronic progressive nephropathy	finding not present -	25	17	13	17	31	21	24	16
	Present	7	3	3	0	0	0	0	0
Pituitary adenoma	finding not present -	30	17	16	15	27	16	22	16
	Present	2	3	0	2	4	5	2	0
Mammary adenocarcinoma	finding not present -	32	20	16	17	28	20	24	15
	Present	0	0	0	0	3	1	0	1
Mammary fibroadenoma	finding not present -	32	19	16	16	12	14	15	11
	Present	0	1	0	1	19	7	9	5

Clinical Signs

The sponsor reported few drug-related clinical signs during the study but included thin appearance (MDF) and discolored haircoat, brown and/or red (perioral in all treated males, and dorsal thorax in the HD and/or MD males). Evidence for drug-related convulsions and masses were also observed.

The sponsor indicated that late onset (generally after Day 379) clonic convulsions (mostly lasting <1 minute) occurred on study but were not dose-related (2, 2, 0, and 3 in males and 0,1,1, and 0 in females). In drug-treated animals, clonic convulsions/activity tended to occur earlier on study and euthanization was the outcome more often compared to the control males (that showed convulsions on 2 or 3 individual days between D680 and D719 and recovered after each without sequelae).

The sponsor stated that palpable masses were sporadically observed. Abdominal (17, 34, 28, and 37 in the Con, LD, MD, and HD, respectively) masses appeared increased in drug-treated males.

Body Weights

Body weight and mean body weight gains were dose-dependently (and statistically significantly) reduced in BMN125-treated animals. Over the entire dosing period, mean body weight gain was reduced at all doses (12%, 19%, and 28% in males and 15%, 36%, and 40% females at the LD, MD, and HD, respectively) compared to controls. Corresponding mean body weights were also dose-dependently and statistically significantly reduced (up to 9%, 14%, and 24% in males (occurring in weeks 64, 60, and 68) and 13%, 25%, and 30% in females (occurring in week 84 for all) at the LD, MD and HD, respectively, compared to controls); these differences remained similar at Week 105 (i.e., 9%, 14%, and 20% in males and 10%, 24%, and 27% in females at the LD, MD, and HD, respectively, compared to controls). See sponsor's Figures 8.1 and 8.2 below.

Figure 8.1: Mean Body Weight Data - Males

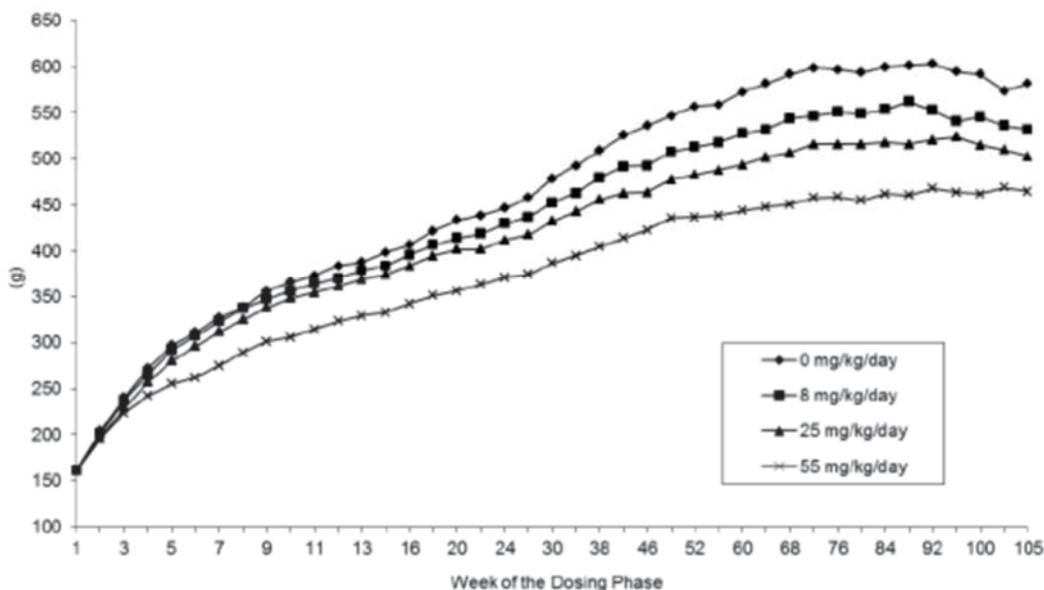
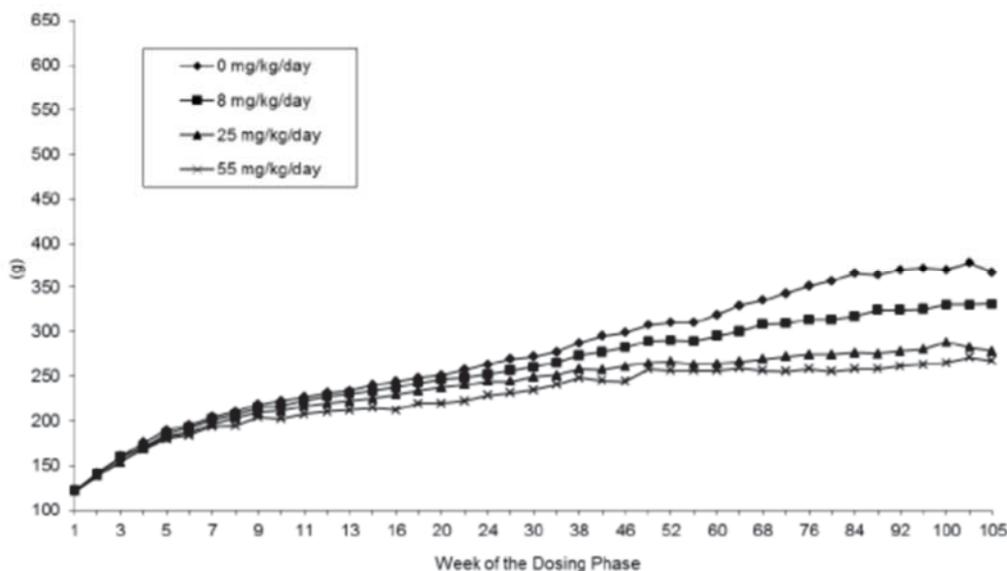


Figure 8.2: Mean Body Weight Data - Females



Food Consumption

Food consumption was generally dose-dependently reduced, which the sponsor indicated contributed to the observed reductions in BWG/BW. Maximal reductions in food consumption were 45% and 48% (Week 8 and Week 7 in males and females, respectively); observed reductions were generally 20-40% in males and 10-30% in females. The sponsor stated that although food consumption was reduced, it (and therefore achieved dose levels) were “consistent throughout the study.” It was noted that control males showed an increase in food consumption over Weeks 20 to 95 that

was not observed in drug-treated males. See the sponsor's Figures 8.3, 8.5, 8.4, and 8.6 for mean food consumption and calculated achieved dose, in males and females respectively, below.

Figure 8.3: Mean Food Consumption Data - Males

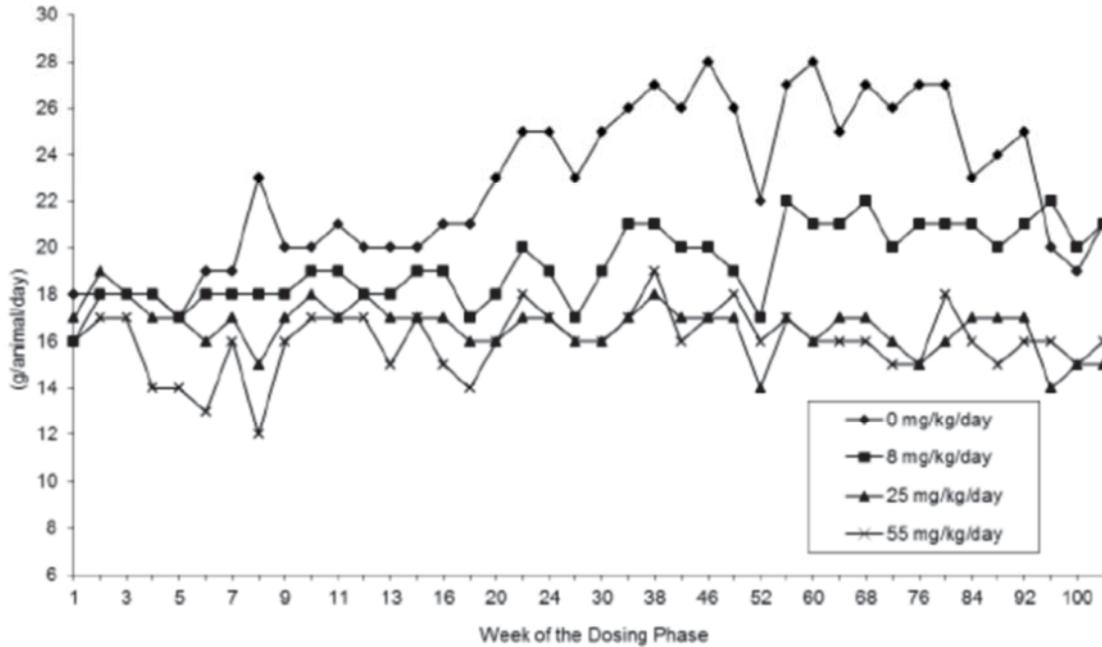


Figure 8.5: Achieved Dose Data - Males

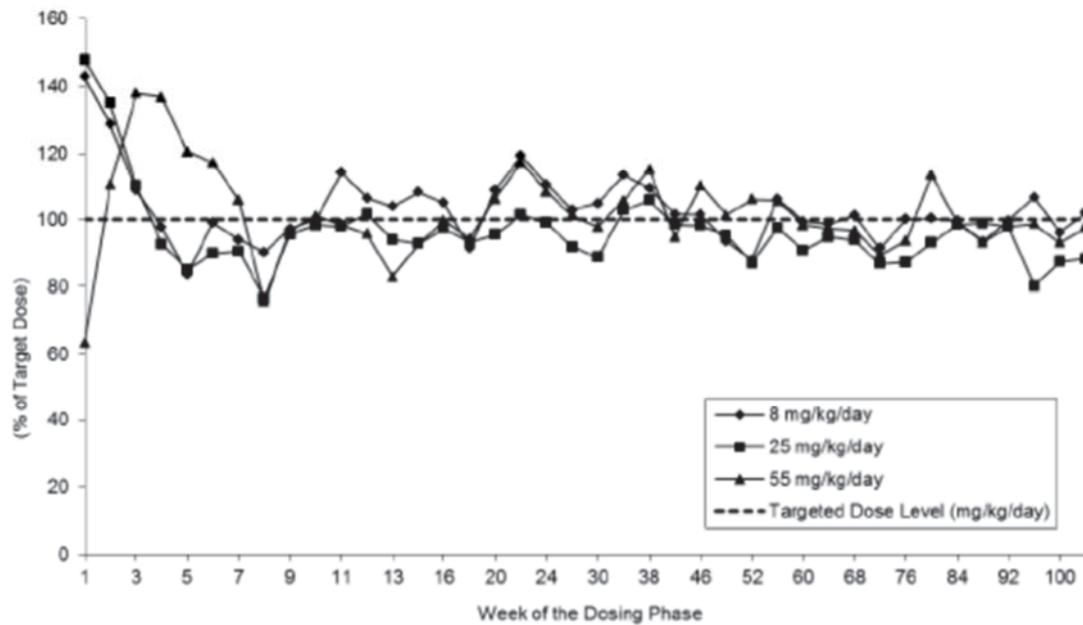


Figure 8.4: Mean Food Consumption Data - Females

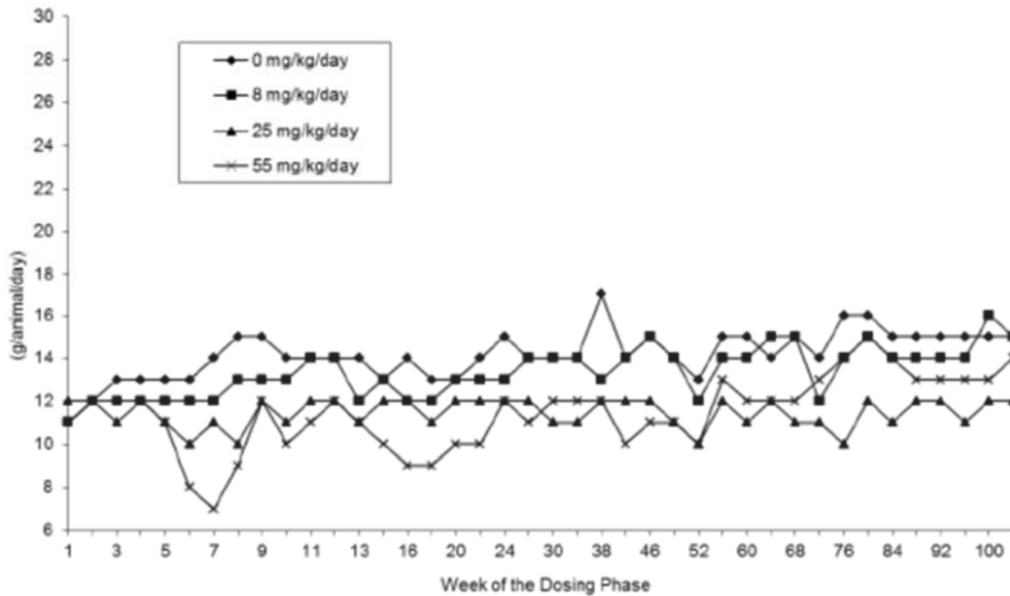
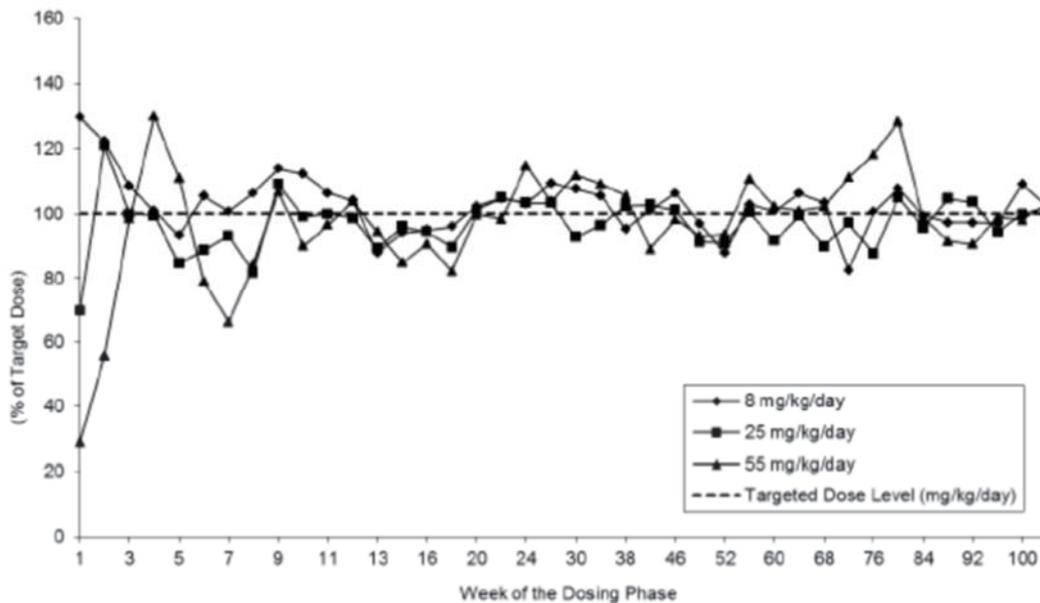


Figure 8.6: Achieved Dose Data - Females



The sponsor indicated that amifampridine exposure was relatively stable through the 104-week study based on food consumption and plasma AUC exposures. The targeted LD and MD of 8 and 25 mg/kg, respectively, were achieved; however, it was not possible to achieve the targeted HD of 60 or 80 mg/kg in females and males, respectively. The sponsor adjusted the HD (based on the observed “achieved dose”) beginning at approximately Week 19 to approximately 55 mg/kg/day; this dose was achieved for the majority of the dosing period in males and females.

Clinical Pathology [at necropsy]

Overall, mean WBC counts were slightly reduced in HDM (38%).

Gross Pathology

Drug-related findings were observed in the abdominal cavity, uterus, and cervix of females. Macroscopically observed masses in females usually represented uterine endometrial carcinomas or squamous cell carcinomas, and/or malignant Schwannomas of the cervix. In addition to masses, drug-related observations in the uterus included abnormal contents, cysts, or large. These observations correlated with microscopic findings of chronic inflammation, cystic hyperplasia, and/or dilation. Changes were also observed in the ovary. See selected from the sponsor's table, below.

Test Article	(dosage)	Sex	1	2	3	4				
BMN 125	mg/kg/day	M	0	8	25	80/55				
		F	0	8	25	60/55				
Tissue/ Observation	Group/Sex: Number of Animals:	1/M	2/M	3/M	4/M	1/F	2/F	3/F	4/F	
	Unremarkable:	17	15	21	35	6	8	8	12	
Abdominal Cavity	Number Examined:	0	0	0	1	0	0	3	1	
	Unremarkable:	0	0	0	0	0	0	0	0	
Abnormal contents		0	0	0	1	0	0	0	1	
Mass		0	0	0	0	0	0	3	1	
Cervix	Number Examined:	0	0	0	0	60	60	60	60	
	Unremarkable:	0	0	0	0	60	58	57	58	
Large Mass		0	0	0	0	0	0	1	0	
		0	0	0	0	0	1	2	2	
Ovary	Number Examined:	0	0	0	0	60	60	60	60	
	Unremarkable:	0	0	0	0	57	51	49	48	
Cyst		0	0	0	0	3	6	8	8	
Mass		0	0	0	0	0	3	1	4	
Not identified		0	0	0	0	0	0	2	1	
Not present		0	0	0	0	0	1	0	1	
Uterus	Number Examined:	0	0	0	0	60	60	60	60	
	Unremarkable:	0	0	0	0	51	40	34	40	
Abnormal contents		0	0	0	0	3	6	9	9	
Cyst		0	0	0	0	3	10	7	5	
Discolored		0	0	0	0	1	0	1	0	
Large Mass		0	0	0	0	2	4	8	10	
		0	0	0	0	1	2	7	5	
Not identified		0	0	0	0	0	0	1	0	
Not present		0	0	0	0	0	1	0	1	
Raised area		0	0	0	0	1	1	1	2	
Thickened wall		0	0	0	0	0	1	1	1	

Dose-dependent reductions in some findings occurred (see selected below, from the sponsor). A reduction in the incidence of mammary masses in females correlated with a decreased incidence of fibroadenomas in the mammary gland. Also, a reduced incidence of masses was noted in the pituitary gland of MDF and HDF, correlating with a decreased incidence of pituitary adenoma. In males, reduced incidences of small testes and/or soft and/or small seminal vesicles correlated with a decreased incidence of atrophy/degeneration of seminiferous tubules in the testes. A decreased incidence of rough surface occurred in the kidneys of HDM, which correlated with a reduced incidence and/or severity of chronic progressive nephropathy.

TABLE
Incidence of Macroscopic Observations
All Animals

Test Article	(dosage)	Sex								
		1	2	3	4	1/F	2/F	3/F	4/F	
BMN 125	mg/kg/day	M	0	8	25	80/55				
		F	0	8	25	60/55				

Tissue/Observation	Group/Sex: 1/M	2/M	3/M	4/M	1/F	2/F	3/F	4/F
	Number of Animals: 60	60	60	60	60	60	60	60
	Unremarkable: 17	15	21	35	6	8	8	12
Mammary Gland, Female	Number Examined: 0	0	0	0	60	60	60	60
	Unremarkable: 0	0	0	0	22	26	39	46
Cyst	0	0	0	0	0	2	0	0
Mass	0	0	0	0	37	32	21	14
Thickened	0	0	0	0	1	3	0	0
Mammary Gland, Male	Number Examined: 1	0	3	1	0	0	0	0
	Unremarkable: 0	0	0	0	0	0	0	0
Mass	1	0	3	1	0	0	0	0
Pituitary	Number Examined: 60	60	60	59	60	60	60	60
	Unremarkable: 57	55	60	56	45	43	47	58
Discolored	0	1	0	0	1	1	5	1
Large	1	0	0	0	0	3	2	0
Mass	2	4	0	3	13	14	6	1
Raised area	0	0	0	0	1	0	0	0
Testis	Number Examined: 60	60	60	60	0	0	0	0
	Unremarkable: 50	50	53	58	0	0	0	0
Large	1	1	0	0	0	0	0	0
Not present	0	0	1	0	0	0	0	0
Rough surface	1	0	1	0	0	0	0	0
Small	8	6	3	2	0	0	0	0
Soft	8	7	2	1	0	0	0	0
Seminal Vesicle	Number Examined: 60	60	60	60	0	0	0	0
	Unremarkable: 52	55	58	59	0	0	0	0
Small	8	5	2	1	0	0	0	0
Epididymis	Number Examined: 60	60	60	60	0	0	0	0
	Unremarkable: 56	59	59	59	0	0	0	0
Not present	0	0	1	0	0	0	0	0
Small	4	1	0	1	0	0	0	0
Kidney	Number Examined: 60	60	60	59	60	60	60	60
	Unremarkable: 48	44	45	57	60	57	56	55
Abnormal contents	0	0	0	0	0	0	1	0
Adhesion	0	0	0	0	0	0	1	0
Cyst	1	1	3	0	0	0	1	1
Discolored	5	4	4	0	0	1	0	1
Large	1	1	1	0	0	0	3	1
Mass	0	1	0	1	0	1	0	1
Raised area	0	0	0	1	0	0	0	1
Rough surface	12	15	13	1	0	1	0	0
Skin/Subcutis	Number Examined: 60	60	60	59	60	60	60	60
	Unremarkable: 43	47	48	48	55	60	56	59
Broken skin	0	0	0	1	0	0	0	0
Mass	14	11	10	9	5	0	3	1
Raised area	0	1	1	0	0	0	0	0
Scab	2	3	1	0	0	0	0	0

Histopathology

**Adequate Battery
Separate, Signed Report
Pathologist:**

Yes (see Histopathology Inventory)
Yes (see NDA 208078, SDN20, 6/13/18)

(b) (6)
[Redacted]

Peer Review:

(b) (6)
[Redacted]

Neoplastic

Several changes (both increases and decreases) in tumors were observed in the treated animals. Drug-related, statistically significant increases endometrial carcinomas were observed at MD and HD. A seemingly dose-related, particularly in females, increase in benign/malignant Schwannomas was also observed but was not statistically significant. Reductions in some commonly-observed tumors were also observed and were presumed related to the dose-related body weight reductions.

Dose-related (and statistically significant) increases in uterine endometrial adenocarcinoma/carcinoma occurred in females. The pathologist described the tumors as “generally poorly-differentiated and highly invasive, with extensive intraperitoneal or distant metastasis. Occasional tumors also exhibited regional squamous metaplasia.” The pathologist noted that the adenocarcinomas were often the cause of death. The sponsor reported that although a single squamous cell carcinoma in the uterus occurred in a LDF, it was included in the statistical evaluation of endometrial carcinomas. Endometrial carcinomas did not occur in the concurrent controls, but the sponsor noted that the background incidence of endometrial carcinomas in Harlan Sprague Dawley rats to be between 1.27 and 1.92% (Dinse et al., 2010 and National Toxicology Program, 2013). These tumors were observed beginning in Week 86, with the majority (23/27 tumors, inclusive of the benign adenoma) being observed after Week 100. The sponsor argued that the late onset of these tumors paralleled the extended survival, thereby prolonged estrogen exposure, in the drug-treated rats. According to the sponsor, the reduced food consumption (and reduced pituitary prolactin release) “favors continued cyclicity, delayed reproductive senescence (continued estrogenic drive) and persistent stimulation of the endometrium,” which would increase the risk of spontaneous proliferative uterine lesions. See the sponsor’s Text Table 4.8, below.

Text Table 4.8: Incidence of Endometrial Adenomas and Carcinomas

Sex	BMN 125fb			
	Females			
Dose Level (mg/kg/day)	0	8	25	55
Uterus				
Number Examined	60	59	60	60
Benign Adenoma, endometrial	0	0	0	1
Malignant Carcinoma, endometrial	0	3	13	9
Malignant Squamous Cell Carcinoma	0	1	0	0
Total Number of Primary Tumors	0	4	13	10
Total Number of Affected Animals	0	4	13	10

Benign and malignant Schwannomas were observed in various soft tissues (predominantly head and neck, heart, and female reproductive tract) in MDM, HDM, LDF, MDF, and HDF. See the sponsor’s Text Tables 4.6 and 4.7, below. A total of 13 rats (5 males, 8 females) showed Schwannomas in the study; most were observed after Week 89, with the exception one MDM at Week 29 (moribund sacrifice for mass; entire body pale) and one HDM at Week 40 (moribund sacrifice for general debilitation, no masses by palpation). Generally, Schwannomas occurred in single animals (of a sex

and group) in individual tissues, with exceptions being skin (Schwannomas observed in 2 MDM), heart (observed in 2 HDF) and cervix (observed in 3 HDF; i.e., 5%). The observation of benign and/or malignant Schwannoma did not reach statistical significance, when analyzed by tissue or with the tissues combined. The pathologist stated that “No benign or malignant Schwannomas were observed among 170 males and 170 females from two historical studies conducted with Harlan SD rats at the test facility.”

Text Table 4.6: Incidence of Benign and Malignant Schwannomas - Males

	Sex	BMN 125fb			
		Males			
Dose Level (mg/kg/day)		0	8	25	55
Heart					
	Number Examined	60	60	60	59
	Malignant Endocardial Schwannoma	0	0	1	0
Harderian Gland					
	Number Examined	60	60	60	60
	Malignant Schwannoma	0	0	0	1
Skin/Subcutis					
	Number Examined	60	60	60	60
	Malignant Schwannoma	0	0	1	1
	Benign Schwannoma	0	0	1	0
Total Number of Primary Tumors		0	0	3	2
Total Number of Affected Animals		0	0	3	2

Text Table 4.7: Incidence of Benign and Malignant Schwannomas - Females

	Sex	BMN 125fb			
		Females			
Dose Level (mg/kg/day)		0	8	25	55
Heart					
	Number Examined	60	60	60	60
	Malignant Endocardial Schwannoma	0	0	0	2
Harderian Gland					
	Number Examined	60	60	60	60
	Malignant Schwannoma	0	0	0	1
Cervix					
	Number Examined	60	59	60	60
	Malignant Schwannoma	0	1	1	3
Uterus					
	Number Examined	60	59	60	60
	Malignant Schwannoma	0	0	1	0
Clitoral Gland ^a					
	Number Examined	0	3	1	1
	Malignant Schwannoma	0	1	0	0
Mandibular Salivary Gland					
	Number Examined	60	60	60	60
	Malignant Schwannoma	0	0	0	1
Total Number of Primary Tumors		0	2	2	7
Total Number of Affected Animals		0	2	2	4

a The occurrence in clitoral gland was not included in statistical evaluation of neoplastic lesions because this tissue was not collected or examined in animals unless a macroscopic lesion was present.

The pathologist described the lesions as follows:

“Malignant Schwannomas were characterized by the presence of poorly-differentiated spindle-shaped cells, with indistinct cytoplasmic borders, and were located within a loose extracellular matrix often containing cyst-like spaces. These tumors were often highly locally invasive. Although morphologically distinct, endocardial Schwannomas in the heart were only observed in one male given 25 mg/kg/day and two females given 55 mg/kg/day; therefore, they were also included in the inter-group statistical comparisons. One female (Animal No. B70310) given 55 mg/kg/day had multiple incidental primary malignant Schwannomas, affecting the cervical region (mandibular salivary gland with local invasion), Harderian gland, and cervix, as well as a fatal endocardial Schwannoma.”

Tumors of the ovary were also reported in drug-treated females (note that “N” is a metastatic tumor, not the primary). This occurred in the presence of a roughly dose-dependent increase in ovarian cysts. See selection from the sponsor’s table, below.

Table
Summary of Severity of Microscopic Observations
All Animals

Test Article	(dosage)	Sex	1	2	3	4
BMN 125	mg/kg/day	M	0	8	25	80/55
		F	0	8	25	60/55

Tissue/ Observation	Group/Sex: Number of Animals:	1/M	2/M	3/M	4/M	1/F	2/F	3/F	4/F
		60	60	60	60	60	60	60	60
Ovary	Number Examined:	0	0	0	0	60	59	60	60
	Unremarkable:	0	0	0	0	51	42	41	41
B-Granulosa/theca cell tumor		0	0	0	0	0	2	3	1
N-Carcinoma		0	0	0	0	0	2	3	4
Cyst	finding not present -	0	0	0	0	52	47	48	45
	Present	0	0	0	0	8	12	12	15

In the pancreas, the incidences of acinar cell adenomas and carcinomas were slightly increased in treated males (i.e., 1 ConM, 2 LDM, 3 MDM, and 3 HDM; also 1 HDF). No clear hyperplasia or hypertrophy was observed.

Several tumors were noted to show reduced incidences, purportedly as a result of the reduced body weight/food consumption. Benign fibroadenoma of the mammary gland (38, 29, 20, and 11 in the Con, LD, MD, and HD groups, respectively) and adenoma/carcinoma of the pituitary gland (18, 26, 15, and 5 in the Con, LD, MD, and HD groups, respectively) were reduced in the MD and/or HD females. Benign and malignant pheochromocytoma of the adrenal medulla (13, 9, 6, and 1 in the Con, LD, MD, and HD groups, respectively), and adenoma and carcinoma of the islet cells of the pancreas (8, 2, 5, and 2 in the Con, LD, MD, and HD groups, respectively) were reduced in males. Fibroma and fibrosarcoma of the skin/subcutis was also reduced in males (5, 5, 3, and 1 in the Con, LD, MD, and HD, respectively).

Non-Neoplastic

Findings were observed in the eye and uterus in drug-treated females. An increased incidence of retinal atrophy was observed in HDF. The sponsor suggested that this

may be related to increased survival and “longer cumulative exposure to fluorescent light in surviving animals (De Vera Mudry et al., 2013).” Dose-dependent increases in uterine changes were observed in drug-treated females. The sponsor suggested that this finding may be secondary to reductions in food consumption and body weight gain. In the ovary, a roughly dose-dependent increase in ovarian cysts was observed. See selections from the sponsor’s table, below.

Test Article	(dosage)	Sex	1	2	3	4				
BMN 125	mg/kg/day	M	0	8	25	80/55				
		F	0	8	25	60/55				
Tissue/ Observation	Group/Sex: Number of Animals:	1/M	2/M	3/M	4/M	1/F	2/F	3/F	4/F	
Eye	Number Examined:	60	60	60	60	60	60	60	60	
	Unremarkable:	48	42	47	55	56	57	57	48	
Atrophy, retina	finding not present -	60	59	60	60	57	58	59	48	
	slight 2	0	0	0	0	0	1	1	2	
	moderate 3	0	0	0	0	3	1	0	8	
	marked 4	0	1	0	0	0	0	0	2	
	Total Incidence:	0	1	0	0	3	2	1	12	
Uterus	Number Examined:	0	0	0	0	60	59	60	60	
	Unremarkable:	0	0	0	0	22	10	11	17	
Dilatation	finding not present -	0	0	0	0	55	45	42	43	
	minimal 1	0	0	0	0	3	0	2	1	
	slight 2	0	0	0	0	1	6	3	4	
	moderate 3	0	0	0	0	0	2	6	4	
	marked 4	0	0	0	0	1	4	5	7	
	severe 5	0	0	0	0	0	2	2	1	
	Total Incidence:	0	0	0	0	5	14	18	17	
Cyst	finding not present -	0	0	0	0	60	59	60	59	
	Present	0	0	0	0	0	0	0	1	
Hyperplasia, cystic endometrial	finding not present -	0	0	0	0	43	31	39	44	
	minimal 1	0	0	0	0	6	14	10	10	
	slight 2	0	0	0	0	11	11	7	4	
	moderate 3	0	0	0	0	0	3	4	1	
	marked 4	0	0	0	0	0	0	0	1	
	Total Incidence:	0	0	0	0	17	28	21	16	
Inflammation, chronic	finding not present -	0	0	0	0	56	48	42	47	
	minimal 1	0	0	0	0	1	5	5	3	
	slight 2	0	0	0	0	0	5	7	3	
	moderate 3	0	0	0	0	2	1	6	5	
	marked 4	0	0	0	0	0	0	0	2	
	severe 5	0	0	0	0	1	0	0	0	
	Total Incidence:	0	0	0	0	4	11	18	13	
Metaplasia, squamous cell	finding not present -	0	0	0	0	34	26	46	48	
	minimal 1	0	0	0	0	13	27	7	5	
	slight 2	0	0	0	0	9	1	4	2	
	moderate 3	0	0	0	0	1	3	3	3	
	marked 4	0	0	0	0	2	1	0	2	
	severe 5	0	0	0	0	1	1	0	0	
	Total Incidence:	0	0	0	0	26	33	14	12	
Ovary	Number Examined:	0	0	0	0	60	59	60	60	
	Unremarkable:	0	0	0	0	51	42	41	41	
Cyst	finding not present -	0	0	0	0	52	47	48	45	
	Present	0	0	0	0	8	12	12	15	

Prostate	Number Examined:	60	60	60	60	0	0	0	0
	Unremarkable:	59	50	56	54	0	0	0	0
Inflammation, chronic	finding not present -	60	57	58	58	0	0	0	0
	minimal	1	1	1	0	0	0	0	0
	slight	2	1	0	1	0	0	0	0
	marked	4	1	1	1	0	0	0	0
	Total Incidence:	0	3	2	2	0	0	0	0
Inflammation, granulomatous	finding not present -	60	60	60	59	0	0	0	0
	moderate	3	0	0	1	0	0	0	0
	Total Incidence:	0	0	0	1	0	0	0	0

Drug-related reductions in common non-neoplastic alterations in rat, including cardiovascular and renal changes, were observed in the study. Dose-dependent reductions in the severity and/or incidence of cardiomyopathy and atrophy/degeneration of seminiferous tubular epithelium were observed in males. And, a decreased incidence of testicular changes occurred in the testes of the MD and HD males. In addition, MDF, HDF, and HDM exhibited a lower incidence and severity of chronic progressive nephropathy. These findings were also reported to be likely secondary to decreased food intake and reduced body weights (Keenan et al., 1995b).

Table
Summary of Severity of Microscopic Observations

Test Article	(dosage)	All Animals							
		Sex	1	2	3	4			
BMN 125	mg/kg/day	M	0	8	25	80/55			
		F	0	8	25	60/55			
Tissue/Observation	Group/Sex:	1/M	2/M	3/M	4/M	1/F	2/F	3/F	4/F
	Number of Animals:	60	60	60	60	60	60	60	60
Heart	Number Examined:	60	60	60	59	60	60	60	60
	Unremarkable:	43	48	49	56	58	59	56	56
Cardiomyopathy	finding not present -	50	53	55	59	60	59	60	59
	minimal	1	4	5	3	0	1	0	0
	slight	2	4	2	2	0	0	0	1
	moderate	3	2	0	0	0	0	0	0
	Total Incidence:	10	7	5	0	0	1	0	1
Kidney	Number Examined:	60	60	60	60	60	60	60	60
	Unremarkable:	7	5	8	26	39	34	43	48
Nephropathy, chronic progressive	finding not present -	11	9	13	36	41	41	50	54
	minimal	1	10	13	17	13	12	7	6
	slight	2	13	16	11	8	7	2	0
	moderate	3	11	9	7	1	0	1	0
	marked	4	10	11	8	0	0	0	0
	severe	5	5	2	4	0	0	0	0
	Total Incidence:	49	51	47	24	19	19	10	6
Dilatation, pelvis	finding not present -	60	60	60	60	60	59	57	59
	slight	2	0	0	0	0	0	1	0
	moderate	3	0	0	0	0	1	1	0
	marked	4	0	0	0	0	0	1	1
	Total Incidence:	0	0	0	0	0	1	3	1
Cyst	finding not present -	60	59	55	60	60	58	59	59
	Present	0	1	5	0	0	2	1	1
Epididymis	Number Examined:	60	60	60	60	0	0	0	0
	Unremarkable:	46	49	52	56	0	0	0	0

Hypospermia										
	finding	not present	-	47	52	53	57	0	0	0
		minimal	1	0	1	0	0	0	0	0
		slight	2	1	0	2	0	0	0	0
		moderate	3	2	0	0	1	0	0	0
		marked	4	6	4	2	1	0	0	0
		severe	5	4	3	3	1	0	0	0
	Total Incidence:			13	8	7	3	0	0	0
Testis										
	Number Examined:			60	60	60	60	0	0	0
	Unremarkable:			31	29	28	53	0	0	0
Atrophy/degeneration										
	finding	not present	-	42	50	53	54	0	0	0
		minimal	1	2	1	0	1	0	0	0
		slight	2	5	3	0	1	0	0	0
		moderate	3	1	0	2	2	0	0	0
		marked	4	5	3	2	1	0	0	0
		severe	5	5	3	3	1	0	0	0
	Total Incidence:			18	10	7	6	0	0	0
Polyarteritis										
	finding	not present	-	40	39	46	59	0	0	0
		minimal	1	7	0	2	0	0	0	0
		slight	2	9	12	5	1	0	0	0
		moderate	3	4	7	4	0	0	0	0
		marked	4	0	2	3	0	0	0	0
	Total Incidence:			20	21	14	1	0	0	0

Toxicokinetics

Plasma samples were analyzed for amifampridine and the 3-N-acetyl metabolite; the assessment was conducted by (b) (4) using a validated liquid chromatography/tandem mass spectrometry bioanalytical method (Method No. (b) (4) 13-027; BioMarin Study No. BMN125-13-009). The lower limits of quantitation were 0.500 ng/mL for BMN125 and 1.00 ng/mL for 3-N-acetyl BMN125 TK analysis was performed by (b) (4).

Group	Dosing Phase Week	Set No. (Six animals/sex/group)	Dosing Day of Study Week	Time Point	
				Hour ^{a,b}	Clock Time, Day ^c
1, 2, 3, 4	5, 26, 52	1st	Monday (1st TK day)	3	20:00, Monday
		2nd	Tuesday (2nd TK day)	4.5	21:30, Tuesday
		3rd	Tuesday (2nd TK day)	12	5:00, Wednesday
		4th	Tuesday (2nd TK day)	15	8:00, Wednesday
		5th	Tuesday (2nd TK day)	24	17:00, Wednesday
		6th	Wednesday (3rd TK day)	6	23:00, Wednesday
		7th	Thursday (4th TK day)	2	19:00, Thursday
		8th	Thursday (4th TK day)	10	3:00, Friday

TK = Toxicokinetic.

- Toxicokinetic time points during the dark cycle were staggered over several days so the disruption of blood sampling on dark cycle food consumption and associated exposure was minimized.
- Based on start of dark cycle (lights off), time 0 = approximately 17:00. Blood was collected ± 15 minutes of the target time.
- Column for reference only. Data and labels specified the time referenced to start of dark cycle.

Drug exposure was not observed in samples from the control group, except for one male and two females. The levels of amifampridine in these animals were just above the LLOD, were much lower than values in the treated animals, and were not

associated with exposures to the 3-N-acetyl metabolite. For these reasons, these outliers were considered artifact.

Generally, amifampridine and 3-N-acetyl amifampridine plasma exposures increased with increasing dose and exposures at 26 and 52 weeks were greater than those at week 5 (except HD was roughly similar). Exposures to the 3-N-acetyl metabolite were greater than to amifampridine. T_{max} ranged from 2 to 12 hr after the start of the dark cycle (later T_{max} with higher dose) for both parent and metabolite. Clear sex differences were not observed. Overall, the TK data showed that amifampridine was extensively converted to the 3-N-acetyl metabolite in rat; the reduced metabolite to parent ratio with increased doses likely indicated a saturable metabolic pathway.

The sponsor provided summary Text Tables 4.1 and 4.2 (below) for the mean toxicokinetic parameters of amifampridine and the 3-N-acetyl metabolite in rat plasma.

Text Table 4.1: Summary of the Mean Toxicokinetic Parameters for BMN 125fb in Rat Plasma

Week	Dose Group	Targeted BMN 125fb Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-12} (ng·hr/mL)	AUC_{0-24} (ng·hr/mL)	AR (AUC_{0-24})	
5	2	8	M	6.66	4.50	56.5	84.5	NA	
			F	9.48	4.50	69.0	105	NA	
	3	25	M	27.8	4.50	261	354	NA	
			F	46.0	6.00	360	472	NA	
	4	55	M	601	12.0	3,030	6,180	NA	
			F	364	6.00	3,020	4,740	NA	
	26	2	8	M	12.9	6.00	117	160	1.90
				F	15.6	6.00	127	163	1.55
3		25	M	61.1	4.50	455	609	1.72	
			F	99.8	6.00	847	1,190	2.51	
4		55	M	302	10.0	2,520	3,680	0.595	
			F	567	10.0	4,320	6,600	1.39	
52		2	8	M	9.55	2.00	92.3	127	1.50
				F	13.9	6.00	101	149	1.42
	3	25	M	73.5	6.00	578	762	2.15	
			F	116	4.50	829	1,120	2.36	
	4	55	M	392	10.00	2,860	4,820	0.779	
			F	565	12.0	4,530	7,000	1.48	

F = Female; M = Male; AR = Accumulation Ratio.

Text Table 4.2: Summary of the Mean Toxicokinetic Parameters for 3-N-acetyl BMN 125 in Rat Plasma

Week	Dose Group	Targeted BMN 125fb Dose Level (mg/kg/day)	Sex	C _{max} (ng/mL)	T _{max} (hr)	AUC ₀₋₁₂ (ng-hr/mL)	AUC ₀₋₂₄ (ng-hr/mL)	Ratio (AUC ₀₋₂₄)		
								M/P	AR	
5	2	8	M	442	4.50	4,260	6,580	77.9	NA	
			F	510	4.50	4,850	7,640	72.6	NA	
	3	25	M	1,420	10.0	13,100	20,100	56.7	NA	
			F	1,480	6.00	13,500	20,900	44.2	NA	
	4	55	M	4,140	12.0	32,400	70,000	11.3	NA	
			F	2,940	10.0	28,600	54,200	11.4	NA	
	26	2	8	M	678	6.00	6,250	9,880	61.6	1.50
				F	665	6.00	6,270	9,830	60.2	1.29
3		25	M	2,330	4.50	18,400	28,500	46.9	1.42	
			F	1,740	10.0	17,300	29,600	24.9	1.42	
4		55	M	3,580	6.00	35,100	64,400	17.5	0.919	
			F	4,090	10.0	34,700	59,600	9.03	1.10	
52		2	8	M	578	6.00	5,550	8,990	70.9	1.37
				F	617	6.00	5,550	8,850	59.3	1.16
	3	25	M	2,250	10.0	19,500	31,900	41.8	1.59	
			F	1,880	4.50	16,400	27,800	25.0	1.33	
	4	55	M	3,760	10.0	31,200	63,800	13.2	0.911	
			F	3,360	10.0	29,800	53,800	7.69	0.994	

F = Female; M = Male; M:P = Metabolite:parent; AR = Accumulation Ratio.

Formulation Analysis

Concentrations of amifampridine in the dietary admixture were assessed for all groups during Weeks 1, 5, 13, 26, 39, 52, 65, 78, 91, and 104. With few exceptions, the concentration of amifampridine in the dietary admixture was generally found to be within 15% of the targeted concentration. Amifampridine was not detected in the control diet.

The stability of the dietary admixture of amifampridine phosphate (i.e., 25 and 2000 ppm active free base) was assessed in (b) (4) Study No. 8281796 (BioMarin Study No. BMN125-13-006); the amifampridine dietary admixture was considered stable (i.e., within 15% of the targeted concentration) when stored for up to 8 and 15 days under room temperature, refrigerated (2 to 8°C), and frozen (-10 to -30 °C) conditions.

Other Carcinogenicity

A mouse carcinogenicity assay is planned for submission during Phase 4.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Combination Fertility and Developmental Toxicity Study of BMN 125 Administered to Rats by Oral Gavage

Study no.: BMN125-13-012 (8283616)
Study report location: EDR (SDN1)
Conducting laboratory and location: (b) (4)
Date of study initiation: 4/12/13
GLP compliance: Yes (FDA), except:

- Test article characterization
- Gestation weight and clinical signs for TK animals collected by non-GLP facility

QA statement: Yes
Drug, lot #, and % purity: Amifampridine phosphate, Lot DAPP-019, (b) (4) % pure

Methods (see sponsor's summary design table, below)

Route of administration: PO, gavage
Formulated in RO water
Species/Strain: Crl:CD(SD) rats
(b) (4)
M: 10 weeks old, 299-353 g
F: 12 weeks old, 245-312 g
Study design: Main study males were dosed beginning 4 weeks prior to mating until termination (total of >10 weeks prior to necropsy). Main study females were dosed beginning 2 weeks prior to mating, through GD17. Time-mated TK females were dosed from GD6-GD17.
Dose Selection Based on the 13W study in rats, in which 39.6 mg/kg/day amifampridine free base resulted in a single mortality and CNS clinical signs
Deviation from study protocol: Several deviations were reported, but none were reported to affect the integrity or interpretation of the study.

GROUP DESIGNATION AND DOSE LEVELS

Group ^a	No. of Animals		BMN 125fb Dose Level ^{b, c}	BMN 125fb Dose Level ^{b, c}	BMN 125fb Dose Concentration ^{c, d}
	Male	Female	(mg/kg/dose)	(mg/kg/day)	(mg/mL)
Main Study Animals					
1 (Control)	25	25	0	0	0
2 (Low)	25	25	1.3	3.9	0.3
3 (Mid)	25	25	4.0	12.0	0.8
4 (High)	25	25	13.2	39.6	2.6
Toxicokinetic (TK) Study Animals^e					
1 (Control)	3	3	0	0	0
2 (Low)	6	6	1.3	3.9	0.3
3 (Mid)	6	6	4.0	12.0	0.8
4 (High)	6	6	13.2	39.6	2.6

a Group 1 (Main or TK animals) received vehicle control article (Reverse Osmosis water) only.

b Animals were dosed three times daily, 6 hours (\pm 30 minutes) between doses based on the first animal dosed/sex/group from the previous dose.

c Dose levels and concentrations are expressed as BMN 125 free base (fb). A correction (conversion) factor of 1.9 has been applied to correct for salt content of the test article. The BMN 125fb dose levels listed in the table above are equivalent to BMN 125 phosphate salt dose levels of 7.5, 22.5 and 75 mg/kg/day (2.5, 7.5 and 25 mg/kg/dose), respectively.

d Animals were dosed at a dose volume of 5 mL/kg/dose.

e Time-mated females were used for the female TK animals. These animals were dosed from GD 6-17.

Observations and Results

Mortality [Twice daily]

No drug-related mortalities were reported. The sacrifice on one HD TK female on GD9 was preceded by clinical signs (i.e., front limb twitching audible/labored respiration, clear oral discharge, and hypoactivity) on GD8-GD9; this animal was presumed to have aspirated the drug (fluid was observed in the lungs at necropsy).

Clinical Signs [1 hr postdose, 3x/day]

CNS-related signs, primarily perioral twitching (16), front limb twitching (4), and excessive grooming (9), were observed in HDM on one to nine occasions. Clear oral discharge and excessive licking were also noted in 2 HDM. No drug-related signs were reported in females during the approximately 7-week dosing period.

Body Weight [Twice weekly; Mated females on GD0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 21]

There were no clearly drug-related effects on body weight or body weight gain, except in HDF during gestation. Mean body weight gain was slightly reduced (~35%) in HDF during GD0-GD18; however, mean body weight gain was similar to controls by GD21 and mean body weights were not affected. See the sponsor's Figures 1 and 3, below (summary gestation data are provided in the sponsor's Table 12, **Fertility Parameters**).

Figure 1
Male Body Weights

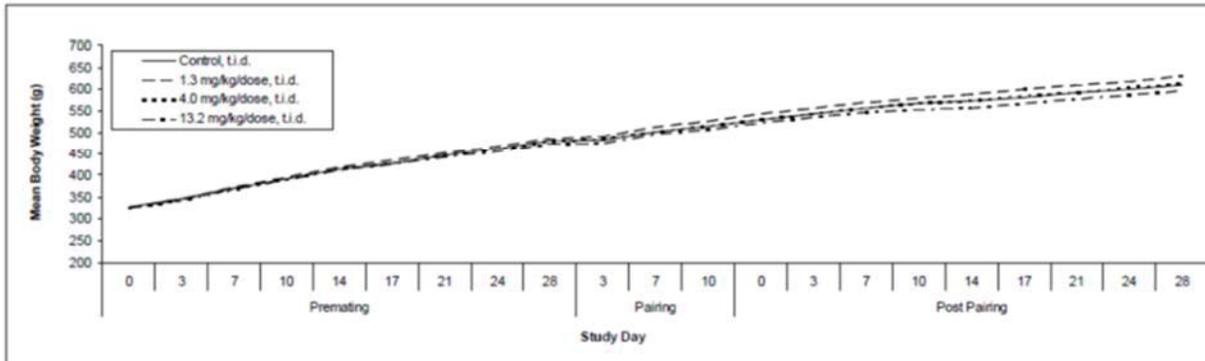
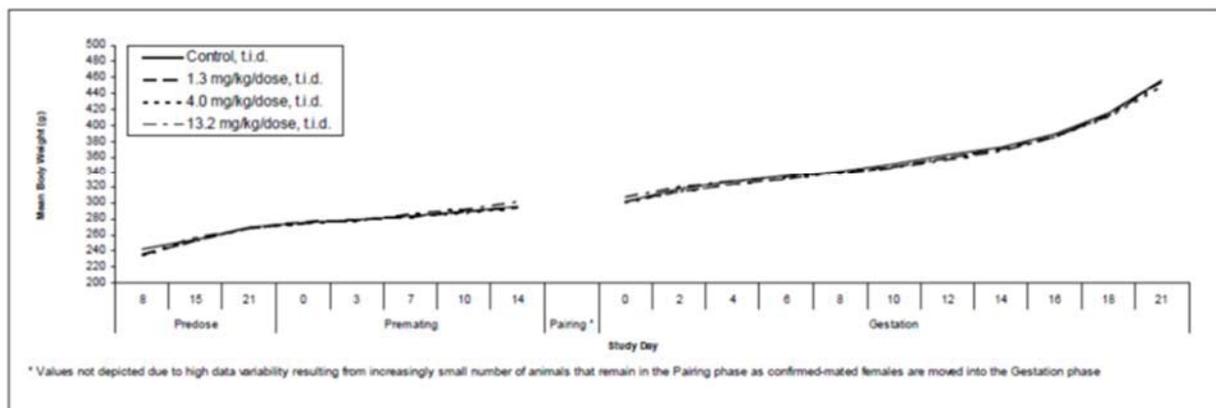


Figure 3
Female Body Weights



Food Consumption [Weekly; quantitative]

Dose-related reductions in food consumption were observed in MDF and HDF during the gestational period. Slight reductions (maximum of 16% during GD6-GD8) were observed periodically in MDF and from GD0 to GD14 in HDF; however, food consumption increased after GD14 and was similar to controls by GD21.

Toxicokinetics

Blood samples were collected from TK males at one hour postdose (first dose of 3) on the last day of dosing (D71), and from TK females on GD17 (12th day of dosing) at 0.5 and 2.0 hours after each TID dose. Plasma concentrations, but not TK parameters, were reported because of the limited number of blood collection timepoints.

Plasma concentrations of amifampridine and the 3-N-acetyl metabolite increased with increasing dose. Amifampridine was extensively converted to the 3-N-acetyl metabolite, resulting in higher plasma levels than parent. In pregnant females, mean plasma concentrations of amifampridine and the 3-N-acetyl metabolite were substantially higher

at 0.5 hours postdose than at 2 hours postdose and increased with each subsequent dose at 0.5 hours postdose (all dose levels) and 2 hours postdose (at the HD).

Dosing Solution Analysis

Formulation analysis showed that the mean concentrations were within $\pm 10\%$ of nominal concentrations.

Necropsy

No drug-related changes were observed.

Fertility Parameters

No effects on the mean number of estrous cycles or mean cycle lengths were observed. In males, there was no clear drug-related effect on sperm percent motility and an apparent increase in concentration at HD (see the sponsor's Tables 15 and 16, below). The observed lower sperm motility and concentrations in conM and MDM were primarily attributable to an outlier in each group (Con MB07099 [0% and ~90 M/g] and MDM B07162 [0% and ~21 M/g], respectively); these animals showed gross observations and were not successful in mating.

Table 15
Summary of Sperm Motility Data

SEX: MALES					
Group		Control	2	3	4
PERCENT MOTILITY	Mean	77.8	82.8	74.3	84.9
	S.D.	28.46	14.63	25.56	16.58
	N	12	12	15	14

Table 16
Summary of Sperm Concentration Data

SEX: MALES					
Group		Control	2	3	4
TOTAL CONCENTRATION (M/g)	Mean	809.8	951.4*	792.5	1069.3*
	S.D.	329.23	130.64	316.87	246.16
	N	12	12	15	14

*=Significantly greater than the corresponding vehicle control, $p \leq 0.05$
M/g = Million of sperm per gram of caudal epididymal tissue

In males, there was a slight reduction in the mating and fertility indices at MD and HD (not clearly dose-related; see selection from the sponsor's Table 10, below). Males that did not impregnate the paired dam totaled 2, 2, 5, and 4 in the control, LD, MD, and HD groups, respectively. Of the four HDM that did not impregnate the paired dam, only one

showed low sperm motility. In the main study females, 23, 23, 20 and 23 animals became pregnant in the control, LD, MD, and HD groups. No clear drug-related effect was observed on female fertility. The cesarean section data showed no clear drug-related adverse effects.

Table 10
Summary of Reproductive Performance (Males and Females)

Treatment Group	Male Mating Performance			
	Control	1.3 mg/kg	4 mg/kg	13.2 mg/kg
Total males	25	25	25	25
Unscheduled Deaths Prior to Cohabitation	0	0	0	0
Males Cohabitated	25	25	25	25
Unscheduled Deaths During Cohabitation	0	0	0	0
Males mating with at least 1 female	24	24	22	22
Males impregnating at least 1 female	23	23	20	21
Mating Index (%)	96	96	88	88
Fecundity Index (%)	96	96	91	95
Fertility Index (%)	92	92	80	84

Mating index % = (Number of males mating with at least 1 female / Number of males cohabitated with at least 1 female) x 100

Fecundity index % = (Number of males impregnating at least 1 female / Number of males mating with at least 1 female) x 100

Fertility Index % = (Number of males impregnating at least 1 female / Number of males cohabitated with at least 1 female) x 100

The fetal data showed few potentially drug-related fetal effects. Mean weight in males showed a slight dose-related increase in males (up to 6% in HDM) and in HDF (4%). No clearly drug-related malformations were observed. Skeletal variations of the ribs appeared slightly increased in amifampridine-treated fetuses; the sponsor considered the observation incidental because it lacked a clear dose relationship. See selected observations from the sponsor's summary table, below.

Variation in Fetal Skeletal Observations - Live and dead fetuses

	Dosage Group: Control 2 3 4				
	Number of Dams/Fetus:	25/156	25/166	25/136	25/166
	Number Examined Litter/Fetus:	23/156	23/166	20/136	23/166
Supernumerary Rib	Number Examined Litter/Fetus:	23/156	23/166	20/136	23/166
V-supernumerary rib - present.		1/1	3/9	6/11	0/0
	%Litter:	4	13	30*	0
	%Fetal:	0.62	5.12	7.56	0.00
V-rib-rudimentary.		0/0	2/3	1/1	4/4
	%Litter:	0	9	5	17
	%Fetal:	0.00	1.71	0.83	2.22

9.2 Embryonic Fetal Development

To support dose selection, the sponsor conducted a tolerability study in non-pregnant rabbits. Female rabbits received escalating oral doses for 3 days (given TID) with washout periods between doses; a final group was dosed for 13 days at the selected dose. Convulsions (i.e., continuous entire body tremors and clonic convulsions lasting less than one minute) were observed in an animal administered 15.8 mg/kg/dose of amifampridine at approximately one hour after the 2nd daily dose on D1; this animal died

shortly after this observation. Mortality was not observed at 0.53, 1.58 or 5.3 mg/kg/dose of amifampridine. Other CNS signs (including twitching of the head/nose and excessive licking) were observed in three animals at 15.8 mg/kg/dose (at approximately 1 hr after the 2nd daily dose on D1) and one animal at 5.3 mg/kg/dose (at approximately 1 hr after the 2nd daily dose on D13). Body weight loss (approximately 5% over D1 to D7) occurred at 5.3 mg/kg/dose TID for 13 days, and correlated with reduced food consumption (up to 36%; the largest reductions occurred from D1 to D7 and were partially reversible). Based on these data, the sponsor selected 5.3 mg/kg/dose as the MTD and the maximum dose for studies in rabbits.

Study BMN125-13-014 (8283617): Dose Range-Finding Oral Gavage Developmental and Reproductive Toxicity and Toxicokinetic Study in Rabbits: BMN 125 Effects on Embryo-Fetal Development (non-GLP)

Conducted by (b) (4) Initiated 5/29/13

Animals: time-mated Hra:(NZW)SPF rabbits

Drug: amifampridine phosphate, DAPP-018 & DAPP-022, (b) (4) % & (b) (4) % pure

See the sponsor’s summary design table, below.

GROUP DESIGNATION AND DOSE LEVELS

Group ^a	No. of Females	BMN 125fb Dose Level ^{b, c} (mg/kg/dose)	BMN 125fb Dose Level ^{b, c} (mg/kg/day)	BMN 125fb Dose Concentration ^{c, d} (mg/mL)	Dosing Schedule (Days of Gestation)
Main Study Animals					
1 (Control)	8	0	0	0	7-20
2 (Low)	8	1.58	4.74	0.32	7-20
3 (Mid)	8	5.3	15.9	1.1	7-20
4 (High)	8	10 ^e	30 ^e	2.0	7-20
Toxicokinetic (TK) Study Animals					
1 (Control)	3	0	0	0	7-20 ^f
2 (Low)	6	1.58	4.74	0.32	7-20 ^f
3 (Mid)	6	5.3	15.9	1.1	7-20 ^f
4 (High)	6	10 ^e	30 ^e	2.0	7-20 ^f

- a. Group 1 (Main or TK animals) received vehicle control article (Reverse Osmosis water) only.
- b. Animals were dosed three times daily, approximately 6 hours (± 30 minutes) between doses based on the first animal dosed/group from the previous dose.
- c. Dose levels and concentrations are expressed as BMN 125 free base (fb). A correction factor of 1.9 has been applied to correct for salt content of the test article. The BMN 125fb dose levels of 4.74, 15.9 and 30 mg/kg/day are approximately equivalent to 9, 30 and 57 mg/kg/day of BMN 125 phosphate salt, respectively.
- d. Animals were dosed at a dose volume of 5 mL/kg/dose.
- e. Due to adverse CNS-related clinical observations, dosing for Group 4 was suspended such that animals did not receive the third daily dose on GD 8 and the first daily dose on GD 9.
- f. For the purposes of fetal blood collection, on GD 20, TK animals received only the first daily dose.

Drug-related mortality occurred in one MD and one HD TK doe. Approximately 2 hr after the 2nd daily dose on GD7, one MD doe was euthanized after exhibiting continuous

entire body tremors followed by clonic and tonic (hindlimb) convulsions and irregular respiration. Approximately 3 hours after receiving the second daily dose on GD8, one HD TK doe was found with labored, irregular respiration and clear oral discharge, and in a sternal recumbent position; although no convulsion was observed, the clinical signs were attributed to convulsion and the animal was subsequently euthanized. The sponsor noted that “elevated plasma concentrations of BMN125” were observed in these animals, and posited that these animals may have been “slow-acetylator” phenotypes.

In addition to the severe CNS signs in the MD and HD mortalities, the MD and/or HD animals showed CNS clinical signs including perioral/nose twitching, head swaying, excessive grooming, or excessive licking. The sponsor stated that these observations generally occurred approximately 1 hour after the 2nd or 3rd daily dose and appeared to be both dose- and time-dependent, increasing in frequency with cumulative amifampridine exposure throughout the dosing period until GD20. Abnormal fecal production and abnormal respiration occurred in all groups but were increased at the MD and HD.

Body weight losses were observed at all doses at the initiation of dosing, but persisted in the MD and HD does. At the MD and/or HD, reduced maternal body weight compared to controls (up to 5% on GD21), body weight gain (30-70% over GD7 to GD21), and food consumption (up to 60%/day) were observed. Slight reductions (<10%) in RBC parameters were observed at the MD and/or HD. No macroscopic pathology changes were reported. Of the 8/group time-mated does, seven were found to be pregnant in the Control, MD, and HD groups. There were no clearly drug-related effects on embryofetal development. The sponsor provided summary TK data for amifampridine and the 3-N-acetyl metabolite on GD7 and GD19 (see the sponsor’s Tables 1 and 2, below).

Table 1
Toxicokinetic Parameters for BMN 125 in Plasma from Pregnant Rabbits

GD	Group	BMN 125fb Dose Level		Dose Number	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC ₀₋₆ (ng-hr/mL)	DN AUC ₀₋₆ [(ng-hr/mL)/ (mg/kg/dose)]	AUC _{0.25-6} (ng-hr/mL)	AUC _{0.25-8} (ng-hr/mL)	
		(mg/kg/dose)	(mg/kg/day)									
7	2	1.58	4.74	1	52.8	33.4	0.500	81.3	51.5	79.1	NA	
				2	36.7	23.2	1.00	NA	NA	NA	NA	
				3	31.4	19.9	0.500	NA	NA	66.7	69.5	
	3	5.3	15.9	1	197	37.1	0.500	414	78.1	394	NA	
				2	194	36.5	0.500	NA	NA	NA	NA	
				3	228	43.1	0.250	NA	NA	378	394	
	4	10	30	1	233	23.3	0.500	619	61.9	609	NA	
				2	220	22.0	2.00	NA	NA	NA	NA	
				3	326	32.6	1.00	NA	NA	816	872	
	19	2	1.58	4.74	1	70.0	44.3	0.500	88.4	56.0	85.7	NA
					2	45.2	28.6	0.500	NA	NA	NA	NA
					3	83.2	52.7	0.500	NA	NA	89.2	91.3
3		5.3	15.9	1	520	98.0	0.250	567	107	501	NA	
				2	339	63.9	0.500	NA	NA	NA	NA	
				3	321	60.5	0.250	NA	NA	463	479	
4		10	30	1	387	38.7	0.250	782	78.2	733	NA	
				2	400	40.0	0.500	NA	NA	NA	NA	
				3	477	47.7	0.500	NA	NA	825	852	

Table 2
Toxicokinetic Parameters for 3-N-acetyl BMN 125 in Plasma from Pregnant Rabbits

GD	Group	BMN 125fb Dose Level		Dose Number	C _{max} (ng/mL)	DN C _{max} [(ng/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC ₀₋₆ (ng-hr/mL)	DN AUC ₀₋₆ [(ng-hr/mL)/ (mg/kg/dose)]	AUC _{0.25-6} (ng-hr/mL)	AUC _{0.25-8} (ng-hr/mL)	
		(mg/kg/dose)	(mg/kg/day)									
7	2	1.58	4.74	1	843	533	1.00	3040	1920	3020	NA	
				2	876	554	1.00	NA	NA	NA	NA	
				3	985	623	1.00	NA	NA	2900	3240	
	3	5.3	15.9	1	2780	525	1.00	10000	1890	9970	NA	
				2	3230	608	1.00	NA	NA	NA	NA	
				3	3360	633	1.00	NA	NA	10800	12000	
	4	10	30	1	3250	325	1.00	14700	1470	14600	NA	
				2	4640	464	1.00	NA	NA	NA	NA	
				3	3920	392	1.00	NA	NA	16700	19100	
	19	2	1.58	4.74	1	690	437	1.00	2270	1440	2230	NA
					2	847	536	1.00	NA	NA	NA	NA
					3	992	628	1.00	NA	NA	2380	2560
3		5.3	15.9	1	2680	506	0.500	9730	1840	9490	NA	
				2	3740	705	0.500	NA	NA	NA	NA	
				3	3300	622	1.00	NA	NA	10200	11300	
4		10	30	1	4370	437	1.00	15000	1500	14600	NA	
				2	4880	488	0.500	NA	NA	NA	NA	
				3	5290	529	1.00	NA	NA	15500	16800	

Study title: Definitive Oral Gavage Developmental and Reproductive Toxicity Study in Rabbits: BMN 125 Effects on Embryo-Fetal Development

Study no.: BMN125-13-015
Study report location: EDR, SDN1
Conducting laboratory and location: (b) (4)
Date of study initiation: 6/28/13
GLP compliance: Yes (FDA), except:

- Drug characterization (GMP)
- Gestation D0 body weights and clinical signs at non-GLP facility

QA statement: Yes
Drug, lot #, and % purity: Amifampridine phosphate, Batch DAPP-022, (b) (4) % pure

Methods (see the sponsor's summary design table, below)

Route of administration: PO (gavage)
Species/Strain: Hra:(NZW)SPF rabbits

Deviations from the study protocol: Several deviations occurred on the study, most of which were unlikely to affect the interpretation or integrity of the study. However, two deviations occurred that could potentially affect the integrity of the study.

During the study, a skeletal processor malfunctioned and damaged a portion of the fetuses in 2 control litters (4 of 20 fetuses), 4 LD litters (10 of 32 fetuses), 3 MD litters (12 of 27 fetuses), and 1 HD litter (5 of 10 fetuses). The sponsor indicated that all fetuses within the remaining litters (numbering 19, 19, 18, and 18 litters in the con, LD, MD, and HD groups, respectively) were examined for skeletal abnormalities. Complete assessment of 18 litters is acceptable.

The sponsor also noted that many specimens for skeletal evaluation had been dissociated from their identification tags (affecting 40, 28, 40, and 26 fetuses in 9, 7, 9, and 7 litters in the control, LD, MD, and HD groups, respectively); however, the sponsor indicated that the litters to which the fetuses belonged could be identified. Since the litter (not fetus) is the unit of assessment, the impact is lessened.

Group ^a	No. of Females	BMN 125fb Dose Level ^{b,c} (mg/kg/dose)	BMN 125fb Dose Level ^{b,c} (mg/kg/day)	BMN 125fb Dose Concentration ^{c,d} (mg/mL)	Dosing Schedule (Days of Gestation)
Main Study Animals					
1 (Control)	25	0	0	0	7-20
2 (Low)	25	1.58	4.74	0.32	7-20
3 (Mid)	25	5.3	15.9	1.1	7-20
4 (High)	25	10	30	2.0	7-20
Toxicokinetic (TK) Study Animals					
1 (Control)	5	0	0	0	7-20
2 (Low)	5	1.58	4.74	0.32	7-20
3 (Mid)	5	5.3	15.9	1.1	7-20
4 (High)	5	10	30	2.0	7-20

- Group 1 (Main or TK animals) received vehicle control article (Reverse Osmosis water) only.
- Animals were dosed three times daily, approximately 6 hours (\pm 30 minutes) between doses based on the first animal dosed/group from the previous dose of that day.
- Dose levels and concentrations are expressed as BMN 125 free base (fb). A correction (conversion) factor of 1.9 has been applied to correct for salt content of the test article. The BMN 125fb dose levels of 4.74, 15.9 and 30 mg/kg/day are approximately equivalent to 9, 30 and 57 mg/kg/day of BMN 125 phosphate salt, respectively.
- Animals were dosed at a dose volume of 5 mL/kg/dose.

Observations and Results

Mortality [Twice daily]

Eight mortalities occurred in the study, involving five HD and three MD does. Of these the sponsor considered the five HD deaths drug-related. Two (F01250 and F01236) of the HDF early mortalities showed early adverse CNS signs (i.e., on GD7 within 3 hr after the 1st daily dose, and on GD8 approximately 1 hr after the 2nd daily dose, respectively) including entire body tremors and “circling violently around the cage before dropping into a lateral recumbent position.” At gross examination, one HDF had a discolored spleen and the other HDF had discolored kidneys. These animals were showed elevated plasma concentrations of amifampridine (which the sponsor indicated may be related to a “slow acetylator” polymorphism phenotype; see plasma concentration data from the sponsor, below). For reference, HD mean plasma exposures for BMN125 were 239 ng/mL at 0.5 hr and 118 ng/mL at 2 hr after the first dose; HD mean plasma exposures were 283 ng/mL at 0.5 hr and 120 ng/mL at 2 hr after the second dose.

Summary of Concentrations of BMN 125 and 3-N-acetyl BMN 125
Animals with test article-related mortalities

Dose Group	Animal No.	BMN 125fb Dose Level		Dose	GD	Hour Nominal	BMN 125	3-N-acetyl BMN 125
		Dose Level (mg/kg/dose)	Dose Level (mg/kg/day)				Concentration (ng/mL)	
4	F01250	10	30	1	7	3	1330	212
4	F01236	10	30	2	8	1	871	5000
4	F01243	10	30	1	17	4	104	3100

Note: Animal Nos. F01250 and F01236 were euthanized due to CNS-related clinical signs while Animal No. F01243 was euthanized after 6 consecutive days of low food consumption and total body weight loss.

The remaining three HDF deaths were considered drug-related by the sponsor based on reduced food consumption and body weight. HDF F01253 was found dead on GD9 (just prior to the 3rd daily dose) following CNS signs including (perioral) twitching, liquid/few feces, and pale ears, as well as reduced food consumption and body weight loss (approximately 10%) beginning on GD7. At necropsy, abnormal brown fluid was observed in the thoracic cavity. The remaining HDF mortalities did not show CNS signs prior to death, but showed body weight losses. HDF F01249 was found dead on GD12 immediately following unsuccessful attempts to administer the second daily dose; labored respiration was observed. This animal showed body weight loss (up to 7%), reduced food consumption, and a dark red discolored trachea at necropsy. HDF F01243 was euthanized moribund on GD17, showing low food consumption and body weight loss of 14%.

Three MD early mortalities occurred in the study, which were not considered drug-related by the sponsor. MDF F01208 was found dead on GD18 after unsuccessful attempts at dosing of the second daily dose. For this animal, no food consumption or body weight effects were reported, no CNS signs were reported, and no abnormalities were observed at necropsy (cause of death was undetermined); although the sponsor indicated that the death was not drug-related, a relationship to drug cannot be excluded. MDF F01210 aborted her litter and was euthanized; this animal showed reduced food consumption and body weight until sacrifice. Clinical signs of hypoactivity and audible/irregular respiration may have correlated with multi-colored discolored lung lobes at necropsy in this animal. MDF F01218 was euthanized moribund on GD27 after labored respiration, hypoactivity, purple skin, and cold ears were observed. Reductions in food consumption and body weight were observed on GD25 (i.e., during the recovery period), and correlated with gas-filled stomach and GI tract at necropsy. The sponsor stated that the moribundity in this animal was attributed to gas build-up in the GI tract causing low food consumption (i.e., not drug-related).

Clinical Signs [postdose 3 times daily; detailed daily beginning GD4]

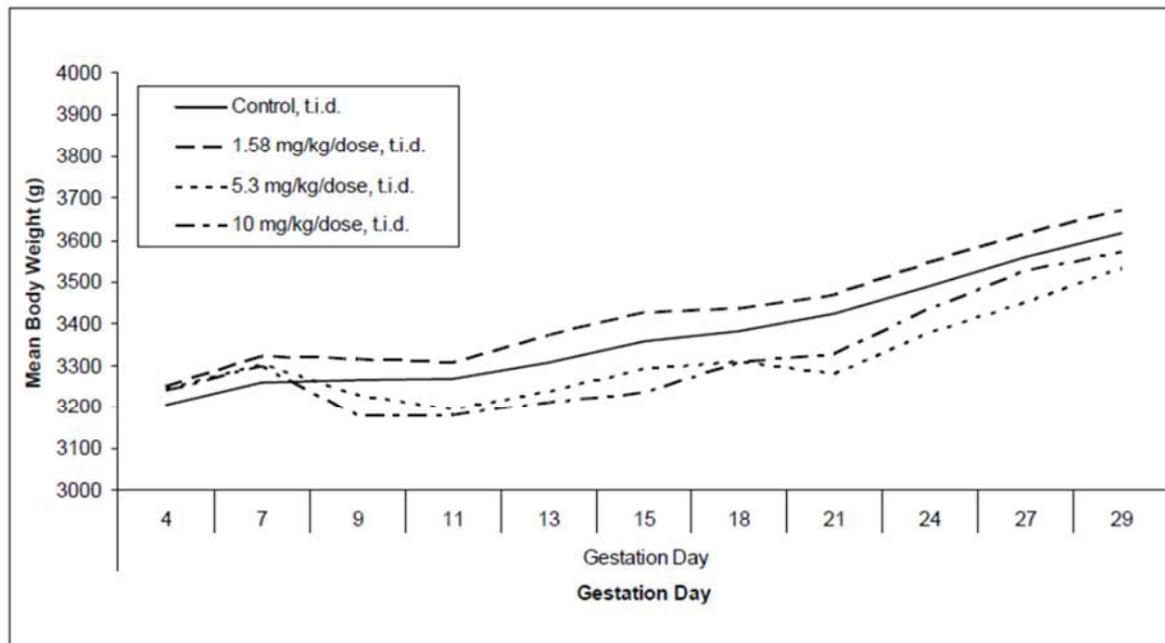
Clinical signs at the MD and HD were observed primarily during the dosing period, and included CNS signs, abnormal respiration, and abnormal fecal production. In addition to

the severe CNS signs that were observed in two HDF early mortalities (e.g., entire body tremors, circling violently around the cage), other CNS signs were observed, including: perioral twitching (\geq MD), excessive licking (\geq MD), excessive grooming (1 HD), and myoclonic jerking (1 HD). Abnormal respiration (i.e., audible, irregular, and/or rapid) was observed with increased incidence in treated animals. Abnormal fecal production (i.e., small, few, none, non-formed, and/or liquid) occurred in all groups, but with a dose-related incidence. The higher incidence in the MD and HD animals correlated with reductions in food consumption.

Body Weight [GD0, 4, 7, 9, 11, 13, 15, 18, 21, 24, 27, and 29]

At the beginning of the dosing period, slight body weight losses (up to 2%) were observed in the MD and HD groups, which correlated with reduced food consumption. Body weight gains were reduced throughout the dosing period at the MD and HD, compared to controls, and were increased after the dosing period. Mean body weights were reduced at the MD and HD (up to 4%) throughout the dosing period (see the sponsor's Figure 1, below).

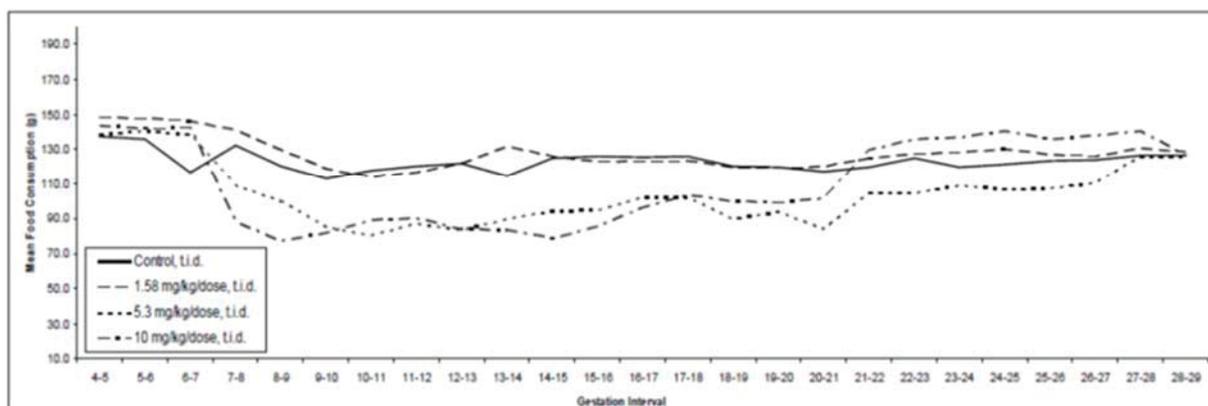
Figure 1
Maternal Body Weights



Food Consumption [beginning GD4, daily- quantitative]

Mean food consumption was reduced at the MD (17-32%) and HD (13-37%) compared to control throughout the dosing period. After the dosing period, food consumption was increased at the HD (although food consumption was not reduced at the MD, it was not increased compared to controls). See the sponsor's Figure 2, below.

Figure 2
Maternal Food Consumption



Dosing Solution Analysis [1st and last day of dosing]

Formulation concentration analyses showed that the concentrations of the dosing formulations ranged between 96.3% to 104.1% of the nominal concentrations.

Necropsy [GD29]

No clearly-drug-related changes were reported.

Cesarean Section Data

No drug-related effects were observed for corpora lutea, implantation sites, resorptions, pre- and post-implantation losses, or live fetuses. Gravid uterine weights were similar, although the MD and HD dams gained 19-27% less than controls.

Offspring (Malformations, Variations, etc.)

Incomplete ossification (partial or total) of the sternebra occurred with slightly increased incidence at \geq the MD (see selected from the sponsor's tables, below). No drug-related external, visceral, or skeletal alterations were reported by the sponsor. The sponsor reported that the incidence of the incomplete ossification of the sternebrae did not exceed the available historical control data (which were notably older; see the sponsor's Table 24, following).

Litters Completely (100%) Evaluated ^a

Variation in Fetal Skeletal Observations - Live and dead fetuses					
	Dosage Group:	Control	2	3	4
	Number of Dams/Fetus:	19/165	19/161	18/162	18/162
	Number Examined Litter/Fetus:	19/165	19/161	18/162	18/162
Sternebrae	Number Examined Litter/Fetus:	19/165	19/161	18/162	18/162
V-sternebra - incomplete ossification.		4/6	6/7	6/7	6/11
	%Litter:	21	32	33	33
	%Fetal:	3.21	4.61	5.05	6.07
V-sternebra - unossified.		8/16	7/22	12/30	11/27
	%Litter:	42	37	67	61
	%Fetal:	11.03	14.69	16.61	15.63

a. These litters were not affected by the unexpected malfunction of the skeletal processors that occurred on this study. Hence 100% of the fetuses from these litters were available for skeletal examination.

Litters Partially Evaluated ^a

Variation in Fetal Skeletal Observations - Live and dead fetuses					
	Dosage Group:	1	2	3	4
	Number of Dams/Fetus:	2/20	4/40	3/27	1/10
	Number Examined Litter/Fetus:	2/16	4/22	3/15	1/5
Sternebrae	Number Examined Litter/Fetus:	2/16	4/22	3/15	1/5
V-sternebra - unossified.		1/2	1/1	1/6	0/0
	%Litter:	50	25	33	0
	%Fetal:	9.09	4.17	25.00	0.00

a. These litters were affected by the unexpected malfunction of the skeletal processors that occurred on this study. Hence only a portion of the fetuses from these litters were available for skeletal examination.

Table 24
Historical Control Data

(b) (4)
HISTORICAL CONTROL DATA (2005-2009)
RABBIT - FETAL VARIATIONS (V) OR MALFORMATIONS (M)

SKELETAL ANOMALIES
NO. OF STUDIES: 44
NO. OF LITTERS: 819
NO. OF FETUSES: 6840

SKELETAL ANOMALIES – CONTINUED

STERNEBRA(E)	FETAL INCIDENCES				LITTER INCIDENCES			
	MEAN	SD	MIN	MAX	MEAN	SD	MIN	MAX
V 5 TH /6 TH STERNEBRA(E) INCOMPLETE OSSIFICATION	16.17	5.59	4.6	27	64.1	12.15	33.0	83

Toxicokinetics [GD7 and GD20]

Five females/time point/group were bled via the medial auricular artery. In treated animals, samples were taken at 0.5 and 2 hours postdose (controls at 0.5 hr postdose only). Plasma samples (but not TK measures) were assessed for amifampridine and the 3-N-acetyl metabolite. At the LD and MD, mean amifampridine plasma concentrations at 0.5 hr after the first dose on GD20 were approximately double those

after the first dose on GD7; plasma concentrations at 2 hr after the first dose as well as 0.5 and 2 hr after the 2nd and 3rd doses were similar. However, at the HD, mean amifampridine concentrations on GD20 were approximately double those at all time points on GD20. Mean 3-N-acetyl metabolite plasma concentrations at 0.5 hr after the first dose on GD20 were approximately double those after the first dose on GD7; mean plasma concentrations at other time points were similar between GD7 and GD20.

9.3 Prenatal and Postnatal Development

Study title: Oral Gavage Developmental and Perinatal/Postnatal Reproduction Toxicity Study of BMN 125 in Rats, Including Postnatal Behavioral/Functional Evaluation

Study no.: 8288665
(BMN125-13-025)

Study report location: EDR, SDN1

Conducting laboratory and location: (b) (4)

Date of study initiation: 8/7/18

GLP compliance: Yes (FDA)

QA statement: Yes

Drug, lot #, and % purity: amifampridine phosphate, batch DAPP-022, (b) (4) % pure

Methods (See the sponsor's summary design table, below)

Route of administration: PO, gavage

Species/Strain: female CrI:CD(SD) rats, time-mated
(b) (4)

11 weeks old; 221-293 g

STUDY DESIGN, GROUP DESIGNATION AND DOSE LEVELS

Group ^a	No. of Females	BMN 125fb Dose Level ^{b,c} (mg/kg/dose)	BMN 125fb Dose Level ^{b,c} (mg/kg/day)	BMN 125fb Dose Concentration ^{c,d} (mg/mL)	Dosing Schedule
Main Study Animals					
1 (Control)	25	0	0	0	GD 6 thru LD 20
2 (Low)	25	1.3	3.9	0.3	GD 6 thru LD 20
3 (Mid)	25	4.0	12.0	0.8	GD 6 thru LD 20
4 (High)	25	13.2	39.6	2.6	GD 6 thru LD 20
Toxicokinetic (TK) Study Animals					
1 (Control)	3	0	0	0	GD 6 thru LD 14
2 (Low)	6	1.3	3.9	0.3	GD 6 thru LD 14
3 (Mid)	6	4.0	12.0	0.8	GD 6 thru LD 14
4 (High)	6	13.2	39.6	2.6	GD 6 thru LD 14

- Group 1 (Main or TK animals) received vehicle control article (Reverse Osmosis water) only.
- Animals were dosed three times daily, approximately 6 hours (± 30 minutes) between doses based on the first animal dosed/group from the previous dose.
- Dose levels and concentrations are expressed as BMN 125 free base (fb). A correction factor of 1.9 was applied to correct for salt content of the test article. The BMN 125fb dose levels listed in the table above are equivalent to BMN 125 phosphate salt dose levels of 7.5, 22.5 and 75 mg/kg/day (2.5, 7.5 and 25 mg/kg/dose) respectively.
- Animals were dosed at a dose volume of 5 mL/kg/dose.

F₀ Dams Observations and Results

Survival: One HD dam (B10167) was euthanized moribund (“general debilitation”) on LD0; vaginal prolapse was reported in the clinical observations. Total litter loss led to the euthanization of 2 HD dams (B10164 and B10170), 2 MD dams (B10136 and B10149), and one control dam (B10064) on LD0; one LD dam was euthanized following prolonged delivery on LD0. Another MD dam (B10146) was euthanized on GD26 for “failure to produce a viable litter.” The sponsor considered none of the deaths drug-related.

Clinical signs: Gestation Phase

CNS signs were observed in 12 HD dams beginning on GD9, including: excessive chewing (3), grooming (3), and licking (7); twitching (3); squinting eyes (2); and tonic convulsions (1 HD, B10164; GD20). Other signs observed at the HD included oral discharge, nasal discharge, and rapid respiration. Similar CNS signs (including hypoactivity) were observed in two MD dams.

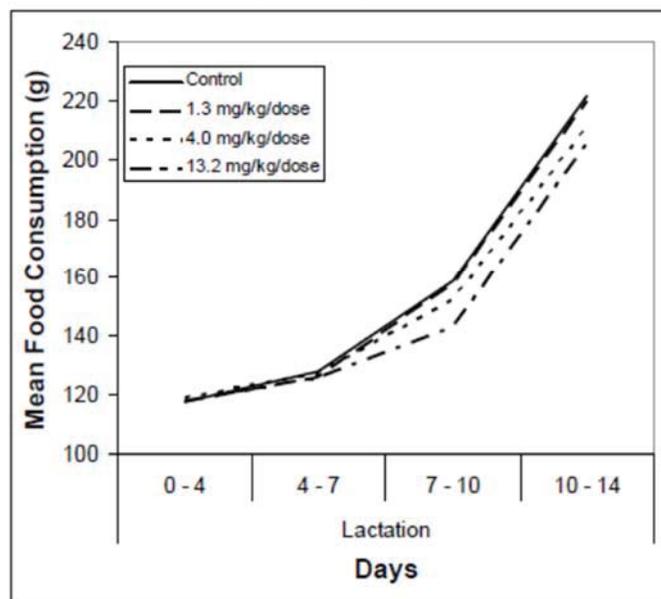
Lactation Phase

CNS signs were primarily observed in the HD group, including: excessive chewing, grooming, and licking; twitching; limb paddling; and hypoactivity.

Body weight: No drug-related effects in body weight were reported during the gestation or lactation phases.

Food consumption: Food consumption was slightly reduced (up to 10%) on LD7 through LD14 in the MD [nss] and HD [ss] dams. See the sponsor’s Figure 4, below.

Figure 4
Mean Food Consumption During Lactation– F₀ Generation



Uterine content: A dose-related increase in the percentage of dams with stillborn pups (8%, 17%, and 20% in LD, MD, and HD dams, compared to 4% in control dams) was observed. The percentage of dams with stillborn pups at the MD and HD exceeded the historical control mean of 12%. An increase in the mean number of stillborn pups at the MD and HD (75% and 269% of controls) was also observed. Live birth and LD4 viability indices were slightly reduced at the MD and HD. See selected from the sponsor's summary Table 10, below.

Table 10
Natural Delivery Data and Litter Data Summary

	DOSE LEVEL	CONTROL 0 mg/kg/dose	GROUP 2 1.3 mg/kg/dose	GROUP 3 4.0 mg/kg/dose	GROUP 4 13.2 mg/kg/dose
Females: Mated	N	25	25	25	25
Pregnant	N	25	25	25	25
Delivering	%	100	100	100	100
	N	25	25	24	25
	%	100	100	96	100
Duration of Gestation:	MEAN	23.0	23.1	23.0	23.0
	S.D.	0.2	0.4	0.2	0.0
	N	25	25	24	25
Females with Liveborn Pups	N	25	25	24	25
Gestation Index	%	100	100	96	100
with Stillborn Pups	N	1	2	4	5
	%	4.00	8.00	16.67	20.00
Females with no Liveborn Pups	N	0	0	0	0
	%	0.00	0.00	0.00	0.00
Pups Delivered	MEAN	281	287	275	287
	S.D.	11.24	11.48	11.46	11.48
	N	1.59	1.76	1.82	2.42
	N	25	25	24	25
Liveborn	MEAN	277	285	257	276
	S.D.	11.08	11.40	10.71	11.04
	S.D.	1.98	1.78	2.42	2.92
Stillborn	MEAN	4	2	18	11
	S.D.	0.16	0.08	0.75	0.44
	S.D.	0.80	0.28	2.67	1.16
Females: Delivering Live Pups		25	25	24	25
Died or Killed During Lactation		0	1	0	1
Removed due to Total Litter Loss		1	0	2	2
Surviving to Scheduled Sacrifice		24	24	22	22
Pup Survival Indices					
Livebirth Index	MEAN%	98	99	95	95
Day 4 Viability Index	MEAN%	95	97	90	89
Weaning Index	MEAN%	96	99	91	92
Pup Disposition					
Culled day 4	TOTAL	78	71	63	77
Dead Pup		2	19	9	10
Pup Removed/Dam Unscheduled Death		0	0	0	12
Other		0	0	0	0
Cannibalized		5	0	8	3
Missing		0	4	3	9
Pups Surviving at 21 days	TOTAL	192	191	174	177
Pups Dead Pup, Other, Missing and/or Cannibalized+					
Days 0-4		7	22	18	21
Days 5-21		0	1	2	1
Entire Litter Dead Pup, Other, Missing and/or Cannibalized+					
Days 0-4	N	1	1	2	2
Days 5-21	N	0	0	0	0

N = Number of Females or Litters

TOTAL = Number of Pups or Implants+ Excludes pups where the dam has died or was killed moribund

Necropsy observation: No drug-related findings were observed.

Toxicokinetics: Plasma samples were BLQ for amifampridine and the 3-N-acetyl metabolite in all control animals. TK parameters were not assessed for the study; exposures were given only as plasma concentrations.

Plasma concentrations were quantifiable at 0.5 hr postdose (1st and 3rd daily dose) on GD6 and LD14, and increased with increasing dose. Plasma concentrations of the 3-N-acetyl metabolite exceeded those of parent. Plasma concentrations on GD6 and LD14 increased approximately 1 to 3-fold following the 3rd dose of the day compared to the first dose. Accumulation was not observed.

Amifampridine and 3-N-acetyl-amifampridine concentrations were quantifiable in milk at 0.5 hr postdose (1st daily dose), and increased with increasing doses. Mean milk concentrations on LD14 of amifampridine were 9.46, 36.5, and 298 ng/mL and concentrations of the 3-N-acetyl metabolite were 264, 589, and 2438 ng/mL for the 1.3, 4.0, and 13.2 mg/kg/dose groups, respectively.

Dosing Solution Analysis Formulation analysis showed that concentrations were within 10% of the nominal concentrations.

F1 Generation Observations and Results

Survival: Reduced pup viability was observed at the MD and HD (see sponsor's Table 10, above in **Uterine Content**), mostly through PND4. The number of pups surviving at LD21 was reduced at the MD and HD (8-9%).

Clinical signs: One HDM showed tilt from PND12 until the end of the study; the brain was not examined. The sponsor reported no drug-related clinical signs.

Body weight: Pup body weights were slightly reduced in HDM (6%, [ss]) and HDF (7%, [nss]) on LD21.

Food consumption: Food consumption was variable. Clear drug-related differences were not observed.

Physical development: Vaginal opening was delayed by approximately 1 day [ss] at the MD and HD. The sponsor reported that the observed delay was "within biological parameters" and did not consider it drug-related. Balanopreputial cleavage was also slightly [nss] delayed. See the sponsor's Table 16, below. No other landmarks showed drug-related alterations.

Table 16
Pup Landmark Development Summary Mean Day to Reach Criteria

DOSE LEVEL		Control 0 mg/kg/dose	GROUP 2 1.3 mg/kg/dose	GROUP 3 4.0 mg/kg/dose	GROUP 4 13.2 mg/kg/dose
Balanopreputial Cleavage	MEAN BODY WEIGHT	229.0	225.9	231.6	229.7
	MEAN	43.6	43.3	44.6	44.4
	S.D.	2.2	1.8	2.1	1.7
	N	24	24	22	22
Vaginal Opening	MEAN BODY WEIGHT	118.8	113.0	118.2	117.5
	MEAN	32.3	32.0	33.3* τ	33.2* τ
	S.D.	1.5	1.4	1.7	1.5
	N	24	24	22	22

N = Number of pups.

* τ = Wilcoxon Rank Sum Test Significant at 0.05 level.

Neurological assessment: Locomotor activity was evaluated on PND22 and PND56. HDM tended to show slightly increased locomotor activity on PND56. The sponsor did not consider the slight increase drug-related.

Auditory startle assessment was conducted on PND28 and PND62 (both ± 4 days). The mean maximum amplitude was variable among the groups and habituation across the blocks was minimal in all groups (only males showed evidence of habituation on PND62). The lack of habituation in the assay (particularly on PND62) may reflect a general insensitivity in the assay, as conducted. All groups showed increased pre-pulse inhibition (PPI) as the decibel level increased; no clear drug-related differences were observed for PPI.

The learning and memory was assessed using an M-water maze. Testing was performed on PND38 (± 3 days), with memory and reversal (change in the platform location) evaluations 7 days later. The M-water maze is generally considered a simple maze; however, the reversal portion of the assay lends some complexity by allowing the possibility of perseverative errors. In general, the data were highly variable and a few outliers resulted in apparent differences in the summary data. In HDM, early performance in the reversal portion of the assay suggested slight effects on learning. While reported by the sponsor, this change was not considered drug-related by the sponsor because: it was not statistically significant, did not occur in females, and performance was similar to controls in the next session. It was noted that, particularly in the reversal evaluation, elapsed time tended to decrease over the first trials (presumably showing learning) but then appeared to increase in later trials in the drug-treated animals. The reason for this pattern in performance is unclear (see sponsor's Figures 18 and 20, below).

Figure 18
Water Maze – Males - Test 2 (Memory and New Learning)

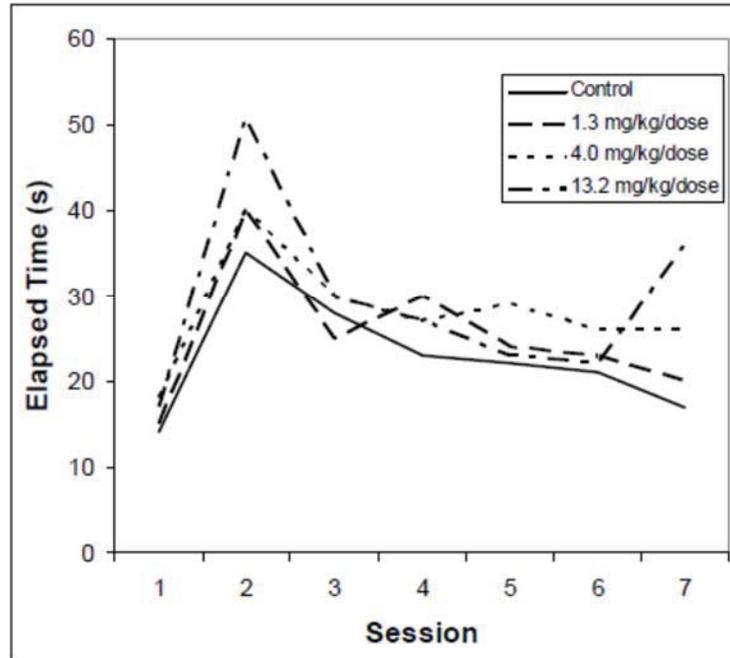
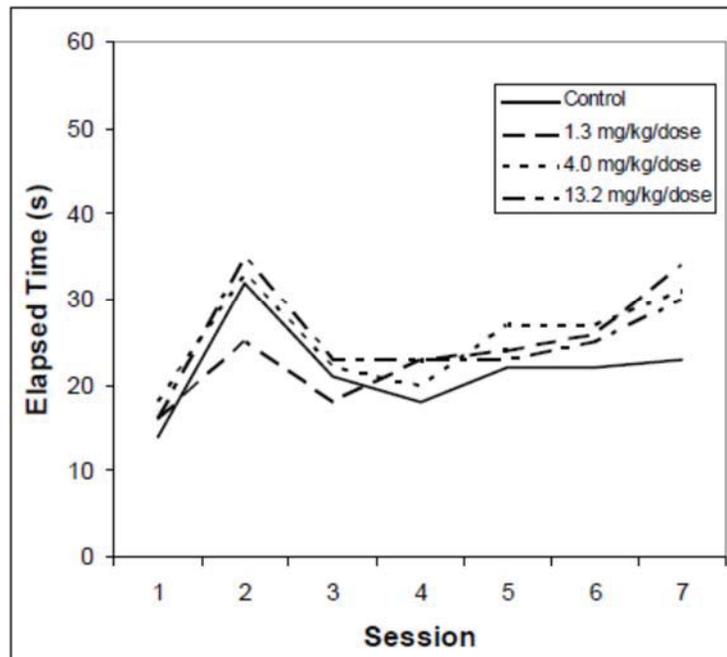


Figure 20
Water Maze – Females – Test 2 (Memory and New Learning)



Reproduction: No drug-related alterations in estrous cyclicity were observed. A slight reduction in the male mating index was observed at the HD (86% vs. 92% in controls), but the fertility index was unaffected. See below from the sponsor.

Table 44
Summary of Reproductive Performance

Treatment Group	Males			
	Control	1.3 mg/kg	4 mg/kg	13.2 mg/kg
Total males	24	24	22	22
Unscheduled Deaths Prior to Cohabitation	0	0	0	0
Males Cohabitated	24	24	22	22
Unscheduled Deaths During Cohabitation	0	0	0	0
Males mating with at least 1 female	22	22	21	19
Males impregnating at least 1 female	20	21	21	18
Mating Index (%)	92	92	95	86
Fecundity Index (%)	91	95	100	95
Fertility Index (%)	83	88	95	82

Mating index % = (Number of males mating with at least 1 female / Number of males cohabitated with at least 1 female) x 100
Fecundity index % = (Number of males impregnating at least 1 female / Number of males mating with at least 1 female) x 100
Fertility Index % = (Number of males impregnating at least 1 female / Number of males cohabitated with at least 1 female) x 100

Treatment Group	Females			
	Control	1.3 mg/kg/dose	4 mg/kg/dose	13.2 mg/kg/dose
Total Females	24	24	22	22
Unscheduled Deaths Prior to Cohabitation	0	0	0	0
Females Cohabited	24	24	22	22
Unscheduled Deaths During Cohabitation	0	0	0	0
Females Mated	23	23	22	19
Pregnant Females	21	22	22	18
Non Pregnant Females	3	2	0	4
Matings Per Day Periods Of Cohabitation				
Day 1	5	6	4	7
Day 2	6	6	4	5
Day 3	5	3	8	3
Day 4	6	6	5	4
Day 5	0	1	0	0
Day 10	0	0	1	0
Mating Index %	96	96	100	86
Fecundity Index %	91	96	100	95
Fertility Index %	88	92	100	82

Mating index % = Mated females/females cohabited (excluding females sacrificed during Cohabitation) x 100
Fecundity Index % = Pregnant females/mated females (excluding females with an undetermined pregnancy status) x 100
Fertility Index % = Pregnant females/females cohabited (excluding females sacrificed during Cohabitation or with an undeterm

Other: No clearly drug-related macroscopic changes were observed.

F₂ Generation Observations and Results

- Survival: No drug-related effects were observed.
- Body weight: No drug-related effects were observed.
- External evaluation: No clearly drug-related changes were observed.
- Male/Female ratio: While variable, there was no clear drug-related difference in the percentage of male offspring.

10 Special Toxicology Studies

Studies for Impurity Evaluation

10.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Mutagenicity Test on *Salmonella Typhimurium His-* (5 Strains) Using B.N. Ames's Technique with (b) (4)

Study no.: (b) (4)-080408
Study report location: EDR, SDN1
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/27/08
GLP compliance: Yes (OECD), except:
Drug treatment concentration analysis
QA statement: Yes
Drug, lot #, and % purity: (b) (4) batch (b) (4)-025
(source identified as (b) (4)
(b) (4) % pure

Methods

Strains: *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and TA102
Concentrations in definitive study: See the sponsor's results tables, below
Basis of concentration selection: Maximum recommended concentration in the absence of cytotoxicity/precipitation, except where cytotoxicity was detected by reduced revertant frequency (see excerpt from the sponsor, below)
Negative control: DMSO
Positive control: See the sponsor's results tables, below
Formulation/Vehicle: DMSO
Incubation & sampling time: 48 hr at 37°C

- For toxic test items, irrespective of the solubility, the top concentration used is the dose provoking a moderate thinning of the lawn and/or a reduction in the number of revertants close to 75 % in comparison with the controls as recommended by J.F. de Serre (1979).

Study Validity

Standard methodology was generally used, with a few exceptions. The test formulation concentrations were not verified. The metabolic activation system was prepared on-site, using liver S9 fraction (in DMSO) from Aroclor 1254-treated (500 mg/kg IP, single injection) male Sprague Dawley rats. The activity of the rat liver S9 was determined in an Ames test using 2-amino anthracene and benzo[a]pyrene, and in an *in vitro* micronucleus test on L5178Y lymphoma mouse cells. The first assay was conducted by

the plate incorporation method and the confirmatory assay was conducted by the pre-incubation method.

Results- Negative, as conducted

No increases in revertant frequencies were observed (see the sponsor's Tables 2 and 3, below).

TABLE 2
MUTAGENICITY ACTIVITY ASSAY
RECAPITULATION : ASSAY 1

Sponsor: (b) (4)
Test item: (b) (4) Solvent: DMSO

	TA 1535			TA 1537			TA 98			TA 100			TA102		
	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)
Positive control	(b)	344.7	33.5	(b)	722.0	90.3	(b)	427.3	31.2	(b)	384.7	4.4	(b)	797.3	4.4
TEST ITEM	0	10.2	-	0	6.5	-	0	13.8	-	0	71.5	-	0	156.0	-
	50	6.3	0.6	15	5.3	0.8	50	10.0	0.7	15	69.0	1.0	15	127.7	0.8
	150	9.0	0.9	50	3.0	0.5	150	11.7	0.8	50	78.3	1.1	50	132.7	0.9
WITHOUT S9-mix	500	7.7	0.8	150	7.3	1.1	500	11.7	0.8	150	66.3	0.9	150	122.0	0.8
	1500	8.0	0.8	500	5.0	0.8	1500	9.0	0.7	500	66.3	0.9	500	89.3	0.6
	5000	4.3	0.4	1500	4.0	0.6	3000	9.7	0.7	1500	60.3	0.8	1500	60.0	0.4
Positive control	(c)	330.7	20.3	(c)	353.3	66.7	(c)	2656.0	117.0	(c)	2216.0	29.0	(c)	748.7	3.2
TEST ITEM	0	13.5	-	0	6.0	-	0	21.8	-	0	79.0	-	0	239.5	-
	50	11.7	0.9	50	6.3	1.1	50	17.7	0.8	50	68.3	0.9	15	236.3	1.0
	150	8.0	0.6	150	9.0	1.5	150	20.0	0.9	150	64.3	0.8	50	220.7	0.9
WITH S9-mix	500	8.3	0.6	500	6.7	1.1	500	22.0	1.0	500	68.7	0.9	500	207.0	0.9
	1500	9.0	0.7	1500	6.7	1.1	1500	16.7	0.8	1500	55.3	0.7	1500	173.3	0.7
	5000	4.7	0.3	5000	3.3	0.6	5000	15.3	0.7	3000	44.3	0.6	3000	139.3	0.6

(a) Induction Ratio = number of revertants in the treated / number of revertants in the control
Reference positive compounds ($\mu\text{g}/\text{plate}$):
(b) TA1535 and TA100: Sodium azide¹; TA1537: 9-amino-acridine 50; TA98: 2-nitrofluorene²; TA102: Mitomycin C³ 0.125
(c) TA1535, TA1537, TA98, TA100: 2-anthramine¹; TA102: benzo(a)pyrene²
Solvents used for positive controls: ¹ DMSO; ² distilled water

TABLE 3
MUTAGENICITY ACTIVITY ASSAY
RECAPITULATION : ASSAY 2

Sponsor: (b) (4)
Test item: (b) (4) Solvent: DMSO

	TA 1535			TA 1537			TA 98			TA 100			TA102		
	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)	DOSES in $\mu\text{g}/\text{plate}$	revertants /plate	Induction Ratio (a)
Positive control	(b)	344.0	39.5	(b)	487.3	131.7	(b)	378.3	31.5	(b)	459.7	6.4	(b)	911.3	4.7
TEST ITEM	0	12.0	-	0	4.7	-	0	12.5	-	0	62.8	-	0	166.5	-
	50	9.0	0.8	15	3.0	0.6	50	9.0	0.7	15	69.7	1.1	5	148.0	0.9
	150	8.3	0.7	50	3.3	0.7	150	8.7	0.7	50	77.7	1.2	15	184.0	1.1
WITHOUT S9-mix	500	7.7	0.6	150	4.7	1.0	500	8.7	0.7	150	64.0	1.0	50	171.7	1.0
	1500	5.7	0.5	500	5.7	1.2	1500	6.3	0.5	500	75.7	1.2	150	152.0	0.9
	5000	4.3	0.4	1500	4.0	0.9	3000	9.3	0.7	1500	58.0	0.9	300	142.0	0.9
Positive control	(c)	333.0	49.7	(c)	177.0	24.2	(c)	1589.3	113.5	(c)	2011.3	33.5	(c)	877.3	3.7
TEST ITEM	0	7.8	-	0	8.0	-	0	15.3	-	0	64.5	-	0	232.7	-
	50	6.3	0.8	50	10.0	1.3	50	21.0	1.4	15	56.3	0.9	15	195.0	0.8
	150	7.0	0.9	150	8.7	1.1	150	15.3	1.0	50	63.0	1.0	50	229.7	1.0
WITH S9-mix	500	7.0	0.9	500	8.3	1.0	500	17.3	1.1	150	61.0	0.9	150	218.3	0.9
	1500	6.3	0.8	1500	7.3	0.9	1500	16.0	1.0	500	59.0	0.9	500	204.7	0.9
	3000	7.3	0.9	5000	2.0	0.3	5000	14.0	0.9	1500	42.0	0.7	1500	149.0	0.6

(a) Induction Ratio = number of revertants in the treated / number of revertants in the control
Reference positive compounds ($\mu\text{g}/\text{plate}$):
(b) TA1535 and TA100: Sodium azide¹; TA1537: 9-amino-acridine 50; TA98: 2-nitrofluorene²; TA102: Mitomycin C³ 0.125
(c) TA1535, TA1537, TA98, TA100: 2-anthramine¹; TA102: benzo(a)pyrene²
Solvents used for positive controls: ¹ DMSO; ² distilled water

11 Integrated Summary and Safety Evaluation

Amifampridine phosphate (3,4-diaminopyridine phosphate) has been developed for the (b) (4) treatment (i.e., improvement of neuromuscular function) of Lambert Eaton Myasthenic Syndrome (LEMS).

Pharmacology of amifampridine phosphate

The proposed mechanism of action and EPC for amifampridine phosphate is as a voltage-gated potassium channel blocker, similar to that for structurally-related 4-aminopyridine (dalfampridine, Ampyra®). Although amifampridine has been generally accepted as a potassium channel blocker for decades (cf., Kirsch & Narahashi, 1978, Judge & Bever, 2006; like structurally-related 4-aminopyridine), recent literature has suggested other potential activity (e.g., see Wu et al., 2009).

The in vitro assays submitted by the sponsor showed a weak concentration-dependent inhibition of several voltage-gated potassium channels (i.e., Kv1.1-1.5, and Kv1.7; IC_{50} s= 300-1900 μ M). The single major human metabolite of amifampridine phosphate, the 3-N-acetyl metabolite, did not inhibit any of the potassium channels tested (IC_{50} > 3000 μ M), and concurrent positive control 4-aminopyridine showed approximately 60-90% inhibition at 1 to 2 mM. Additionally, in vitro binding assays of 29, 40, and 44 receptors, channels, and/or enzymes showed no activity at 10 μ M amifampridine phosphate or its 3-N-acetyl metabolite. However, it is noted that the concentration tested in the secondary pharmacology assays (i.e., 10 μ M) did not meet or exceed the concentrations at which amifampridine showed potassium channel blockade.

General Toxicology

In acute and subchronic studies, mortality and CNS toxicity were observed. CNS and respiratory clinical signs included: tremors, convulsion, nonresponsiveness to stimuli, stiffness of limbs, outstretched limbs, hyperesthesia, and/or labored breathing.

In the acute and subchronic studies in rodents, mortality and/or convulsions occurred at doses \geq 26 mg/kg in mice and \geq 40 mg/kg/day (given TID) in rats. The observed NOAELs were 13 mg/kg in mice and 5.3 mg/kg in rat ("agitated movements" of the forelimbs and excessive grooming occurring at 13 mg/kg). In the 13-week study in rats, adverse CNS signs and mortality occurred at 40 mg/kg/day (the HD). Slightly increased liver, adrenal, and mandibular salivary gland weights were observed at the HD; salivary gland hypertrophy was observed at the HD, and showed recovery.

The 26-week study of amifampridine phosphate (0, 3.9, 11.8, and 39.5 mg free base/kg/day, given TID) by oral gavage in SD rats showed drug-related CNS and respiratory clinical signs. Slightly increased liver, adrenal, and ovary weights were reported, without clear histologic correlates. Enlarged salivary glands with increased weights, correlating histologically with acinar cell hypertrophy, were observed at HD; this effect showed reversibility after the recovery period. Amifampridine exposure increased with increasing dose, and females generally showed slightly higher exposures than males. Systemic exposure to the 3-N-acetyl metabolite increased with increasing dose, but in a less-than-dose-proportional manner.

Among the acute and subchronic studies in dogs, 2 mg/kg/day (given TID) was identified as a maximum tolerated dose, with tremors, emesis, and convulsions occurring at doses \geq 3 mg/kg/day (given TID), and deaths occurring at 4 mg/kg/day (given TID). In one 4-week study at doses that exceeded the MTD (i.e., 3 and 4 mg/kg/day), mortality, severe CNS signs, congestion (in GI tract, liver, kidney, adrenal, and/or heart), minimal to moderate myodegeneration/regeneration (in skeletal muscle, tongue, and/or larynx), and increased white filaments in the posterior lens capsule (HDF) were observed. A second 4-week study testing up to 3.3 mg/kg/day (given TID) showed dose-related CNS and respiratory clinical signs and reduced body weights, but drug-related myopathy was not clearly observed.

The 9-month study of amifampridine phosphate (0, 0.53, 1.0, and 2.0 mg free base/kg/day, given TID) by oral capsule in beagle dogs showed drug-related CNS and respiratory clinical signs. Dose-related clinical signs included: hypersalivation, panting, tremors, aggressive behavior, and/or convulsions (\geq MD). Reduced body weights were observed. Salivary gland weights were increased in the MD and HD females. Clearly drug-related histopathological alterations were not observed. Increased amifampridine exposures were observed with increasing doses, without clear sex differences; dogs did not show systemic exposure to the 3-N-acetyl metabolite.

Genotoxicity & Carcinogenicity

Amifampridine was negative in an in vitro bacterial reverse mutation (Ames) assay, and in the in vivo rat micronucleus and rat hepatocyte unscheduled DNA synthesis (UDS) assays; amifampridine was positive in an in vitro mouse lymphoma assay in the absence of metabolic activation.

The sponsor conducted a 2-year carcinogenicity bioassay in rats, using dietary administration (at doses of approximately 0, 8, 25, and 55 mg/kg/day). Drug-related uterine endometrial carcinomas and combined adenomas/carcinomas were observed at the MD and HD. The sponsor attributed this finding to prolonged reproductive senescence. The endometrial tumors were usually observed late (i.e., 4 of 27 from Week 86 to Week 99, and the remaining 23 at or after Week 100), were generally described as poorly-differentiated and highly invasive, and were often the cause of unscheduled death. Notably, an effect on survival was less pronounced at the MD but was clearly associated with increased endometrial tumors. The uterine endometrial tumors exceeded the incidence in the concurrent controls and were statistically significant at MD and HD; see Table 2 below from Dr. Mbodj's review).

Table 2: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons. Treated Groups and Control Group in Rats

Sex	Organ Name	Tumor Name	0 mg	8 mg	25 mg	55 mg
			Cont (N=60) P - Trend	Low (N=60) P - C vs. L	Med (N=60) P - C vs. M	High (N=60) P - C vs. H
Female	Uterus	M-Carcinoma	0/60 (47) 0.0039*	3/59 (51) 0.1369	13/60 (53) <0.001*	9/60 (54) 0.0025*
		B-Adenoma/M-Carcinoma / M-Carcinoma, squamous cell	0/60 (47) 0.0031*	4/59 (51) 0.0692	13/60 (53) <0.001*	10/60 (54) 0.0012*
	Cervix/Harderian Gland/ Heart/ Mandibular Salivary Gland/ Uterus	M-Schwannoma/M-Malignant schwannoma/M-Endocardial schwannoma	0/60 (47) 0.0251 [#]	1/60 (52) 0.5253	2/60 (53) 0.2784	4/60 (55) 0.0803

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.

*: Statistically significant at 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

[#]: not statistically significant at 0.025 for rare tumors, in dose response relationship (trend) tests

The incidence of Schwannomas appeared dose-related, particularly in females, but of low incidence in the study. No Schwannomas were observed in the concurrent controls (i.e., the most relevant control data) and the pathologist stated that “No benign or malignant Schwannomas were observed among 170 males and 170 females from two historical studies conducted with Harlan SD rats at the test facility.” While both the concurrent controls and the relevant test facility historical controls suggest a zero incidence of spontaneous Schwannomas, these data are comprised of a relatively small number of observations. Available sources of historical control data in Sprague Dawley rats (although not directly relevant to this test facility) suggest a low, but non-zero, spontaneous incidence of malignant Schwannomas. Brix et al. (2005) showed historical control incidences for malignant Schwannoma in females that ranged from 0.3% (1/371) to 0.5% (2/371) in a few tissues (i.e., mesentery, skin/subcutaneous tissue, uterus, vagina, cervix) as well as 0.8% (3/369) in heart. Dinse et al., 2010 also showed low incidences of Schwannoma in heart (3/471; 0.64%), mesentery (1/473; 0.21%), skin (2/473; 0.42%), and uterus (4/473; 0.85%) in female Sprague Dawley rats. The NTP 2017 Historical Control data (from studies in 2007-2012) also showed malignant Schwannomas at relatively low incidences in a few tissues, with most incidences less than 1% (notably uterus was 0.83% [2/240] and vagina was 1.25% [3/240]); in uterus, the tumors were observed in one study (2/90, or approximately 2%), while the tumors in vagina were observed in three studies (i.e., 1/90, 1/50, and 1/50; approximately 1-2%). While the reason for the seemingly dose-related observation in this study is unclear, it did not reach statistical significance when analyzed (with methods that account for survival, the rarity of the tumor, etc.) by individual tissue or with the tissues combined and there are data suggesting a (non-zero) spontaneous incidence rate of Schwannomas in Sprague Dawley rats.

Non-neoplastic findings observed in the two year study included retinal atrophy in the eye in HDF, and dose-related alterations in female reproductive tissues (e.g., uterine dilatation, ovarian cysts).

Reproductive Toxicology

Reproductive and developmental toxicity was evaluated in a combination fertility and embryofetal development study in rats, an embryofetal development study in rabbits, and a pre- and postnatal development study in rats.

In a combination fertility and developmental toxicity study of amifampridine phosphate (0, 3.9, 12, and 39.6 mg free base/kg/day, given TID PO, dosed from 4 weeks prior to mating in males and two weeks prior to mating in females until termination, i.e., GD17 in F) in rats, a slight reduction in male mating and fertility indices occurred at ≥ 12 mg/kg/day. No clear drug-related effects on estrous cyclicity, sperm, or female fertility were observed. Reduced maternal body weight gains were observed in HDF during gestation, but were similar to controls by GD21.

In an embryofetal development study of amifampridine phosphate (0, 4.74, 15.9, and 30 mg free base/kg/day, given TID PO, from GD7 to GD20) in rabbits, mortality and reduced body weight gains occurred at ≥ 15.9 mg/kg/day. Clearly drug-related adverse embryofetal effects were not observed.

In a pre- and postnatal development study of amifampridine phosphate (0, 3.9, 12, and 39.6 mg free base/kg/day, given TID PO, from GD6 to LD14) in rats, mortality, convulsions (HD), and/or total litter loss was observed at \geq the MD. Dose-related CNS clinical signs occurred at \geq the MD, as well as slight reductions in food consumption (without clear effects on body weights). A dose-related increase in dams with stillborn pups occurred (i.e., 8%, 17%, and 20% at the LD, MD, and HD, compared to 4% in controls); this exceeded the historical control mean of 12% at \geq the MD. The mean number of stillborn pups was increased (75% and 269% of controls at the MD and HD), and the live birth and LD4 viability indices reflected this effect. Pup body weights were slightly reduced on LD21 but did not persist. A slight delay in sexual maturation was observed in the MD and HD females. Slight behavioral effects were suggested in the neurobehavioral assays, but clearly drug-related adverse effects were not observed in the assays as conducted. Reproductive performance was not clearly affected.

Impurities

Four potential genotoxic impurities of amifampridine were referred for nonclinical evaluation, including:

(b) (4) is structurally-related to 3,4-diaminopyridine. (b) (4) was shown to lack structural alerts for mutagenicity. (b) (4) is being controlled at NMT (b) (4)%, which is below the TTC of (b) (4) μ g/day at a MRHD of 80 mg/day.

The sponsor conducted an in vitro Ames assay for (b) (4). Although the drug formulation concentrations were not verified, concentration-dependent cytotoxicity (based on reductions in revertant frequencies compared to solvent control) was observed in several strains. (b) (4) was not mutagenic in the assay.

12 Appendix/Attachments

Appendix 1: References

- Brix et al. (2005) Toxicologic Pathology, 33: 477-483.
 De Vera Mudry et al. (2013) Toxicologic Pathology, 41: 813-825.
 Dinse et al. (2010) Toxicologic Pathology, 38: 765-775.
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 NTP (2013). (<https://ntp.niehs.nih.gov/results/dbsearch/historical/index.html>)
 NTP (2017). (https://ntp.niehs.nih.gov/ntp/historical_controls/ntp2000_2017/r_hcrpt_allrte20171100.pdf). Report dated 1/20/2018.
 Wu et al. (2009) The Journal of Biological Chemistry, 284(52): 36453-36461.

Appendix 2: Histopathology inventory

Study	26W	2Y	39W
Species	Rat	Rat	Dog
Adrenals	X*	X	X*
Aorta	X	X	X
Bone Marrow smear	X (femur) (Preserved, not eval)	X (femur)	X (sternum)
Bone (femur)	X	X	X
Brain	X*	X	X*
Cecum	X	X	X
Cervix	X	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			oviducts
Gall bladder	n/a	n/a	X (w/liver)
Gross lesions	(at discretion of pathologist)	X	(at discretion of pathologist)
Harderian gland	X	X	
Heart	X*	X	X*
Ileum	X	X	X
Injection site	n/a	n/a	n/a
Jejunum	X	X	X
Kidneys	X*	X	X*
Lachrymal gland			X

Larynx			X
Liver	X*	X	X*
Lungs	X*	X	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X (F only)	X (F only)	X (F only)
Nasal cavity			
Optic nerves	X	X	X
Ovaries	X*	X	X*
Pancreas	X	X	X
Parathyroid	X	X	X
Peripheral nerve	X (sciatic)	X (sciatic)	X (sciatic)
Pharynx			
Pituitary	X*	X	X*
Prostate	X*	X	X*
Rectum	X	X	X
Salivary gland	X*	X	X*
Sciatic nerve	X	X	X
Seminal vesicles	X*	X	X
Skeletal muscle	X (biceps femoris)	X (biceps femoris)	X (abdominal) X (NOS) X (quadriceps femoris)
Skin	X (abdomen)	X	X (abdominal)
Spinal cord	X	X	X
Spleen	X*	X	X*
Sternum	X	X	X
Stomach	X	X	X
Testes	X*	X	X*
Thymus	X*	X	X*
Thyroid	X*	X	X*
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X*	X	X*
Vagina	X	X	X
Zymbal gland			n/a

X, histopathology performed

*, organ weight obtained

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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11/28/2018

LOIS M FREED
11/28/2018