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PRODUCT: ROCTAVIAN (valoctocogene roxaparvovec-rvox)

APPLICANT: BioMarin Pharmaceutical Inc.

PROPOSED INDICATION: For the treatment of adults with severe hemophilia A (congenital factor VIII deficiency) (b) (4) without antibodies to adeno-associated virus serotype 5 detected by an FDA-approved test and without a history of factor VIII inhibitors

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EXECUTIVE SUMMARY:

The in vivo pharmacology, biodistribution, germline transmission, and toxicology studies evaluating the intravenous (IV) administration of BMN 270 in hemophilia A mice, wild-type mice, and nonhuman primates were reviewed with the original BLA submission. Please refer to the pharmacology/toxicology review memo for BLA125720/0.

In this BLA resubmission, the nonclinical section includes (1) the analysis of BMN 270 vector presence in the brain of nonhuman primates and (2) the evaluation of BMN 270 vector integration in liver tissues from nonhuman primates.

Quantification of vector DNA in nonhuman primate brain tissues from previously conducted studies for BMN270 was performed to address the unusually high vector DNA levels observed in one of the mouse studies submitted in the original BLA. The data from the nonhuman primate study indicated that BMN270 vector DNA concentrations ranged between 0.46% and 0.64% of the vector DNA levels found in the liver at 13 weeks post-administration for dose levels of 2×10^{13} and 6×10^{13} vg/kg. These data confirmed that vector concentrations were significantly lower in the brain compared to the liver.

The BMN 270 vector integration study (BMN270-20-013) was conducted to address Comment #6 in the Complete Response Letter regarding the potential risk of mutagenicity and tumorigenicity of BMN 270. Specifically, (b) (4) methods were used to evaluate vector integration in liver samples of nonhuman primates administered BMN 270 intravenously at dose levels of 1.58×10^{13} vg/kg or 5.44×10^{13} vg/kg and collected at 13 and 26 weeks post-administration. BMN 270 vector DNA was mostly detected in the form of episomal DNA comprising 99.52% of vector sequences. Vector integration events were observed at low frequencies (0.48% of vector sequences) with an overall average of 1.55×10^{-3} integration sites per cell (IS/cell), with the ten most abundant IS with sequence counts ranging between 2 and 7 reads or a relative frequency of less than 1.62%. The total IS ranged between 701 and 1,044 sequence reads, while the unique IS counts were between 669 and 857 per liver sample. Overall, the integration events did not show preferential insertion to particular location(s), were broadly distributed across the host genome, and did not differ based on immunosuppression or time of assessment (13 and 26 weeks post-administration). There was no indication of clonal expansion or enrichment of IS.

The relative frequencies of each IS within (b) (4) of cancer genes (CG) (b) (4) were less than or equal to 0.59%. The cancer genes (b) (4) in monkey), (b) (4) in monkey), and (b) (4) in monkey) were observed to have the highest relative abundance of 0.44%, 0.54%, and 0.59%, respectively. The number of IS located within (b) (4) of a (b) (4) of all genes were 8.63-fold to 25.8-fold higher compared to frequencies of IS within (b) (4) of CG (b) (4). Further analysis of IS located within (b) (4) of CG (b) (4) indicated decreasing number of IS with decreasing distance to CG (b) (4).

In summary, the vector integration data in nonhuman primates and toxicology data obtained in mice and nonhuman primates (e.g., BMN270-16-045 and BMN270-16-046) indicate no evidence of BMN 270-related clonal expansion, preferential vector integration site(s), or tumor formation.

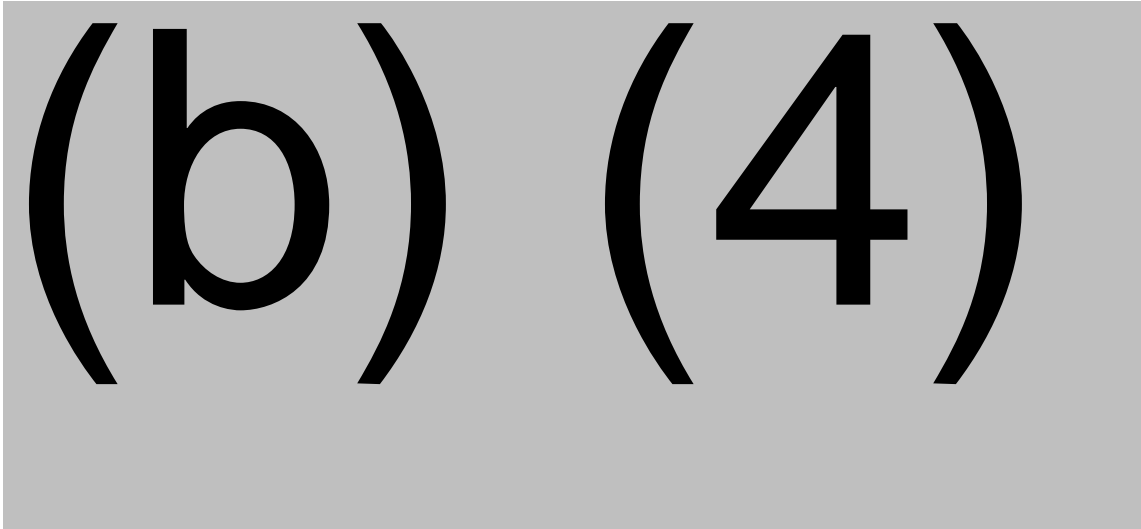
PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies in the pharmacology-toxicology studies, and there are no outstanding requests for additional nonclinical data. The nonclinical data provided in this submission support the approval of this biologics license application.

Formulation and Chemistry:

ROCTAVIAN (BMN 270, AAV5-hFVIII-SQ, AAV5-(b) (4) -FVIII Vector) is a replication-incompetent, (b) (4) recombinant adeno-associated virus serotype 5 (AAV5) encoding for the human B-domain deleted (BDD) Factor VIII (hFVIII-SQ) gene¹ that is driven by a human liver-specific promoter (HLP) (b) (4), followed by a (b) (4)

(Figure 1).



(b) (4)



¹ McIntosh J, Lenting PJ, Rosales C, et al. Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant. Blood. 2013;121(17):3335-3344. doi:10.1182/blood-2012-10-462200

(b) (4)

(b) (4)

The BMN 270 drug product is a sterile solution for intravenous infusion containing 2×10^{13} vg per milliliter (mL) supplied in a Cyclic Olefin Polymer, (b) (4) vial with a chlorobutyl rubber stopper, and aluminum crimp seal. Each vial contains an extractable volume of not less than 8 mL of clear and colorless to pale yellow sterile solution of BMN 270 equivalent to 2×10^{13} vg per mL. The formulation is composed of (b) (4) NaCl, (b) (4) mannitol, and (b) (4) Poloxamer 188 in (b) (4) phosphate buffer at (b) (4) BMN 270 is stored and supplied frozen at $\leq -60^{\circ}\text{C}$.

BMN 270 is administered as a single intravenous infusion at a recommended dose level of 6×10^{13} vg/kg.

Abbreviations

AAV	Adeno-associated virus
AAV5	Adeno-associated virus serotype 5
ALT	Alanine aminotransferase
BD	Biodistribution
BDD	B-domain deleted
(b) (4)	
DNA	Deoxyribonucleic acid
GLP	Good Laboratory Practice
hFVIII-SQ	Human Factor VIII-SQ
HLP	Human liver promoter
IV	Intravenous
(b) (4)	
NHP	Nonhuman primate
NOAEL	No-Observed Adverse Effect Level
qPCR/QPCR	Quantitative Polymerase Chain Reaction
rAAV5	Recombinant Adeno-associated virus serotype 5
ROA	Route of administration
SQ	SQ (b) (4)
vg	Vector genome

Related File(s)

Note: A Premarket Approval application (P190033) for a companion diagnostic to detect pre-existing AAV5 antibodies has been submitted to CDRH by ARUP Laboratories, Salt Lake City, UT.

IND #17659; BioMarin Pharmaceutical Inc.; Adeno-Associated Virus Serotype 5 (AAV5) Expressing SQ Form of Human Factor VIII (hFVIII-SQ) (BMN 270); for Treatment of Hemophilia A

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INTRODUCTION

BMN 270 is a gene therapy product for the treatment of adults with severe hemophilia A (congenital factor VIII deficiency) (b) (4) without antibodies to adeno-associated virus serotype 5 (AAV5).

The original BLA was submitted on December 23, 2019, and nonclinical data provided in that submission were reviewed in the Pharmacology/Toxicology memo for STN 125620/0.0. A Complete Response Letter (CRL) was issued on August 18, 2020, for inadequate information to support clinical efficacy, including the durability of effect, corticosteroid use, and use of the surrogate endpoint. The CRL also included a recommendation for the applicant to provide additional data to address the risks of vector integration and insertional mutagenesis. In this BLA resubmission, the Applicant has submitted Study BMN270-20-013 that evaluated vector integration in archived liver tissues obtained from nonhuman primates from Study BMN270-16-046 (b) (4) from the original BLA (STN 125720/0.0). Study BMN270-20-013 is reviewed in further detail below. Additional data regarding vector biodistribution in the brain from nonhuman primate studies (BMN270-14-062 and BMN270-16-046 from the original BLA) were also provided in this resubmission and reviewed in detail below.

NONCLINICAL STUDIES

Overview of Biodistribution Study

Study #1

The following vector biodistribution study consisted of the evaluation of archived brain tissues collected from nonhuman primates dosed under toxicology studies (BMN270-14-062 and BMN270-16-046) from the original BLA submission. These were single-dose toxicology studies in nonhuman primates and evaluated the vector DNA concentrations in the liver, but did not assess other tissues/organs, including the brain. Frozen brain tissue samples were collected from two studies. Vector biodistribution was assessed in the nonhuman primate brain tissues to supplement the vector biodistribution data from the mouse studies (BMN270-14-075 and BMN270-16-045), and to verify that the high vector levels in the brain of a group of mice in Study BMN270-14-030 was an unexpected finding.

Report Number	BMN270-20-023 (b) (4)
Date Report Signed	December 29, 2020
Title	Sample Analysis of Primate Brain for the Presence of FVIII-SQ DNA
GLP Status	No
Testing Facility	(b) (4)

Objective(s)		To detect and quantify FVIII-SQ DNA in nonhuman primate brain tissue samples by quantitative PCR (qPCR)			
Study Animals	Species	(b) (4) monkeys			
	Age	2.7–4.5 years old			
	Body Weight	2.3–3.3 kilograms (kg)			
	#/sex/group	2-8 males/group/time point			
	Total #	10			
Test Article(s)		BMN 270 (Lot No. 16-097W) BMN 270 v2.0 (Lot No. PDPR-507-001) BMN 270 v3 (Lot No. 16-525)			
Control Article(s)		N/A			
Route of Administration		Intravenous (IV) administration			
Study Groups and Dose Levels		Sample	BMN 270 (vg/kg)	Timepoint	Study report
		1	2×10^{13}	Day 43	BMN270-14-062
		2	2×10^{13}	Day 43	BMN270-14-062
		3	2×10^{13}	Week 13	BMN270-16-046
		4	2×10^{13}	Week 13	BMN270-16-046
		5	2×10^{13}	Week 13	BMN270-16-046
		6	2×10^{13}	Week 13	BMN270-16-046
		7	6×10^{13}	Week 13	BMN270-16-046
		8	6×10^{13}	Week 13	BMN270-16-046
		9	6×10^{13}	Week 13	BMN270-16-046
		10	6×10^{13}	Week 13	BMN270-16-046
Dosing Regimen		Single IV bolus administration			
Randomization		Yes			
Description of Masking		Not described			
Scheduled Sacrifice Time Points		Day 43 Week 13			

Key Results:

The vector DNA levels in brain and liver tissues from nonhuman primates administered with a single IV administration of BMN 270 at various dose levels are summarized in Table 1.1 and Table 1.2, respectively

Table 1.1. FVIII-SQ DNA in brain tissues of nonhuman primates

Sample numbers	BMN 270 dose level (vg/kg)	Time point	FVIII-SQ DNA (copies/ μ g DNA)
1-2	2×10^{13}	Day 43	7,133
3-6	2×10^{13}	Week 13	2,259
7-10	6×10^{13}	Week 13	7,097

Source: Modified from Table 4 (page 10, Report BMN270-20-023, Module 4.2.2.3, STN 125720/0.69)

Table 1.2. FVIII-SQ DNA in liver tissues of nonhuman primates

Sample numbers	BMN 270 dose level (vg/kg)	Time point	FVIII-SQ DNA (copies/cell) ⁵	FVIII-SQ DNA (copies/μg DNA)
1-2	2×10^{13}	Day 43	9.50	1.73×10^6
3-6	2×10^{13}	Week 13	Not reported	4.89×10^5
7-10	6×10^{13}	Week 13	Not reported	1.12×10^6

Source: Modified from Table 12.7 (pages 932-933, Report BMN270-14-062, Module 4.2.3.1, STN 125720/0.0) and Table 10.3 (page 1438, Report BMN270-16-046, Module 4.2.3.1, STN 125720/0.0)

Reviewer comments:

- *In the original BLA submission, BMN270 vector biodistribution was only evaluated in the liver and not evaluated for other extrahepatic tissues in NHPs (Studies BMN270-14-062 and BMN270-16-046). For additional information on biodistribution assessment of BMN 270 vector DNA and FVIII-SQ transgene expression conducted in mice, please refer to the Pharm/Tox review of Studies BMN270-14-030 and BMN270-16-045 included in the original BLA review memo.*
- *In Study BMN270-14-030 in mice, the organs that consistently showed $\geq 20\%$ vector DNA concentration relative to the liver were spleen, lung, mesenteric lymph node, bone marrow, and kidney through Day 91 post-administration of BMN 270 at both dose levels (3.35×10^{12} vg/kg and 3.33×10^{13} vg/kg). However, a high average vector DNA level of 38.7% was observed in the brain relative to liver in mice in the high dose group (3.33×10^{13} vg/kg) at Day 91, which was significantly higher than the $<0.27\%$ observed at Day 28 (both high and low-dose groups) and BLOQ⁶ at Day 91 for the low-dose group (3.35×10^{12} vg/kg). Other mouse studies (BMN270-14-075 and BMN270-045) reviewed in the original BLA also indicate low vector DNA level in the brain relative to the liver. The assessment of the vector DNA levels in the brain tissues of NHPs (BMN270-20-023) provided in this submission was performed to address the observed increase in vector DNA in the mouse brain between Day 28 and Day 91 and the 38.7% relative abundance of vector DNA in the brain versus liver at Day 91.*
- *The BMN270 vector DNA concentrations in nonhuman primate brain samples at 43 days post-administration was 0.41% (Samples 1-2, Table 1.1) of the levels in the liver of the same animals (Table 1.2). At 13 weeks post-administration, the BMN270 vector DNA concentrations in nonhuman primate brain samples ranged between 0.46% (Samples 3-6, Table 1.1) and 0.64% (Samples 7-10, Table 1.1) of the vector DNA levels found in the liver (Table 1.2). In comparison, the vector DNA levels in the brain relative to liver of mice administered BMN 270 at dose levels 6.53×10^{13} vg/kg and 2.13×10^{14} vg/kg*

⁵ Genomic DNA (μg) per reaction calculated using RPLP0 Cp values was converted to diploid genome using the conversion factor of 5.5 pg DNA per monkey diploid genome (i.e., per cell) (Gao et. al.). The amount of FVIII-SQ vector genome detected was normalized to monkey diploid genome (per cell) for each sample tested.

⁶ Below the Level of Quantitation

ranged between 0.71% and 0.75% at 13 weeks post-administration (BMN270-16-045). These data indicate that vector DNA levels in the brain were significantly lower compared to levels detected in the liver of mice and nonhuman primates through 13 weeks post-administration. Per the applicant, the observed increase in vector DNA concentration in brain between Day 28 and Day 91, and the high concentration of DNA in brain relative to liver on Day 91 in Study BMN270-14-030 in mice, was concluded to be an atypical finding. Based on the data presented in Studies BMN270-14-030, BMN270-16-045, BMN270-14-062, BMN270-16-046, and BMN270-20-023, the applicant's conclusion is acceptable.

Overview of Genotoxicity Study

Study #2

The following vector integration study was conducted in response to Comment #6 of the CRL regarding the potential risk of mutagenicity and tumorigenicity of BMN 270.

Report Number		BMN270-20-013
Date Report Signed		March 16, 2021
Title		BMN 270 DNA Integration Site Analysis of Liver Tissue from (b) (4) Monkeys
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To identify BMN 270 vector integration sites in nonhuman primate liver samples following intravenous (IV) administration of a single dose of BMN 270 or vehicle control. The tissue samples were obtained from Study BMN270-16-046 (b) (4)
Study Animals	Species	(b) (4) monkeys
	Age	2.7–3.6 years old
	Body Weight	2.3–3.3 kilograms (kg)
	#/sex/group	3-4 males/group/time point
	Total #	57
Test Article(s)		BMN 270 (Lot No. 16-097W) BMN 270 v2.0 (Lot No. PDPR-507-001) BMN 270 v3 (Lot No. 16-525)
Control Article(s)		Vehicle – (b) (4) sodium phosphate, (b) (4) sodium chloride, (b) (4) (w/v) mannitol, (b) (4) (w/v) Poloxamer 188, (b) (4)
Co-administered agents		Rituximab – (Lot Nos. 3115912, 640013, 3101440, 640012, 31825) Methylprednisolone ⁷ – (Lot Nos. 611552, 6110837, 6111330)
Route of Administration		Intravenous (IV) via cephalic vein

(b) (4)

Study Groups and Dose Levels	Group 1 – Vehicle (0 vg/kg); n=3 Group 2 – Vehicle (0 vg/kg); n=3 Group 3 – BMN 270 (1.58×10^{13} vg/kg); n=4 Group 4 – BMN 270 (5.44×10^{13} vg/kg); n=4 Group 5 – BMN 270 (5.44×10^{13} vg/kg); n=4 Groups 2 and 5 were co-administered with IV infusion of rituximab (10 mg/kg) and intramuscular methylprednisolone (10 mg/kg) weekly starting 1 week before BMN 270 administration for 4 doses and then decreased to 5 mg/kg weekly thereafter until Day 176.
Dosing Regimen	Single IV (slow bolus) administration
Randomization	Yes
Description of Masking	Not described
Scheduled Sacrifice Time Points	Week 13 (Day 92) – Groups 1, 3, 4 Week 26 (Day 183) – Groups 2, 5

Note: The liver tissues from Study BMN270-16-046 (b) (4) in the original BLA (STN 125720/0.0) are used for the DNA vector integration analysis in Study BMN270-20-013. The toxicology study evaluated the safety of IV administration of BMN 270 in (b) (4) monkeys. Please refer to the Pharm/Tox review memo of the original BLA for a more detailed review of the toxicology assessments.

Key Evaluations and Assessments:

- DNA was extracted from the liver (left medial lobe) samples from (b) (4) monkeys administered with a single administration of BMN 270 (Groups 3-5) or vehicle control (Groups 1-2) via a slow bolus IV administration under Study BMN270-16-046 (b) (4)
- Vector integration site analysis of the genomic DNA isolated from liver samples was conducted by (b) (4)
- The sequencing data generated by (b) (4) for the identification of integration sites. The sequencing data/integration sites were further analyzed for common integration sites, genomic distribution, proximity

to known cancer genes, breakpoint positions with respect to vector/genome and vector/vector junctions and were functionally annotated for integration at coding regions.

Key Results:

- Sequencing the libraries generated using samples from Groups 3-5 yielded a total of 62,263,872 raw read pairs and Groups 1-2 yielded a total of 33,288,707 raw read pairs. The read lengths ranged between 154 and 224 nucleotides.
- In Groups 3-5, the proportion of reads mapping to the (b) (4) ranged between 1.94% and 8.55% (% Chr Mapped Reads). In Groups 1-2, the % Chr Mapped Reads was greater than 99.33%.
- In Groups 3-5, the proportion of reads mapping to BMN 270 vector ranged between 86.01% and 95.48% (%BMN 270 Mapped Reads); while %BMN 270 Mapped Reads in Groups 1-2 ranged between 0.001% and 0.004%.
- Table 2.1 summarized the average number of integration sites. The total IS ranged between 701 and 1,044 sequence reads, while the unique IS counts were between 669 and 857 per liver sample.

Table 2.1. Integration site analysis

Group	Total raw read pairs (Average values)	Total insertion site (IS) reads (Average values)	Number of unique IS (Average values)
1	4,288,028	0.67	0.67
2	4,582,584	0.67	0.67
3	6,139,309	965.75	777.75
4	4,037,482	1,044.75	856.75
5	5,208,452	701.25	668.75

Source: Table 6 (pages 45-46, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- The number of multiple mappable IS (IS located within repetitive regions and aligning to more than one genomic location) were 30 (Group 3), 44 (Group 4), and 43 (Group 5) corresponding to 3.71% – 5.78% of the total number of IS. There were no multiple mappable IS in Groups 1 and 2.
- Integration site relative frequencies (Table 2.2) indicate that the ten most abundant IS per liver sample from Groups 3-5 cumulatively comprised 3.17% – 4.17% of total IS. For each individual IS found among the 10 most abundant IS, the sequence counts ranged from 2 to 7 reads and the IS relative frequency ranged between 0.14% and 1.62%. Data for the majority of reads (95.68%) indicate a polyclonal IS profile and absence of clonal expansion based on relative IS frequencies below 0.4%.

Table 2.2. Integration site relative frequencies

Group	Total Seq Counts (Average)	Seq Count of Top 10 IS (Average)	% Seq Count of Top 10 IS	Seq Count of Other IS (Average)	% Seq Count of Other IS
1	0.67	-	-	-	-
2	0.67	-	-	-	-
3	965.75	40.25	4.17 %	925.5	95.8 %
4	1,044.75	36.25	3.47 %	1,008.5	96.5 %
5	701.25	22.25	3.17 %	679	96.8 %

Source: Average of values from Figures 16-27 (pages 48-54, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- Functional annotation of genes mapped to IS was conducted for the top 10 IS in each sample. Functions of the genes mapped to the top IS include protein, ion, and/or nucleic acid binding, enzymatic/kinase/phosphatase activities, and receptor binding.
- Common Integration Site (CIS) analysis⁹ indicate that there were a total of 1,281 CIS identified from liver samples in Groups 3-5. These CIS were grouped based on orders that ranged between 2 and 26 (Table 2.3). A CIS of order n is defined as an n-tuple of IS such that the maximum distance between the elements is no greater than a fixed bound d_n (i.e., (b) (4) in this study).¹⁰ CIS order below 5 indicate low probability of integration in specific genomic regions.

Table 2.3. Common Integration Site (CIS) for Groups 3-5

Order	Number of CIS	% of total CIS
2	1,058	82.59 %
3	161	12.57 %
4	38	2.97 %
>5	24	1.87 %

Source: Summarized from Section 3.1.3.4 (pages 64-65, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- The top CIS identified in Groups 3-5 with order greater than 7 include gene loci for (b) (4) (Table 2.4).

⁹ Common integration site (CIS) analysis aims to identify recurring integration sites that fall within a (b) (4) genomic region.

¹⁰ Abel U, Deichmann A, Nowrouzi A, Gabriel R, Bartholomae CC, Glimm H, von Kalle C, Schmidt M. Analyzing the number of common integration sites of viral vectors--new methods and computer programs. PLoS One. 2011;6(10):e24247. doi: 10.1371/journal.pone.0024247. Epub 2011 Oct 14. PMID: 22022353; PMCID: PMC3194800.

Table 2.4. Top 10 common integration sites (CIS) identified in the NHP samples receiving the BMN 270 vector.

CIS	Gene	Chromosome	CIS order	Number of contributing samples
1	(b) (4)	(b) (4)	26	10/12
2			14	6/12
3			13	6/12
4			12	7/12
5			10	7/12
6			9	6/12
7			8	5/12
8			8	4/12
9			7	3/12
10			7	4/12

Source: Modified from Table 9 (pages 64-65, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- The genomic distribution of IS (Groups 3-5) across all monkey chromosomes indicate widespread distribution without preferred locus/loci. Specifically, IS were located upstream (48.36%) or downstream (47.69%) of a gene, while 3.93% of all IS were located within genes (3.3% in introns and 0.20% in exons).
- The number of IS located within (b) (4) of cancer gene (CG) (b) (4) are summarized in Table 2.5. The cancer genes (b) (4) were found to have IS within (b) (4) in 4 (out of 12) samples.

Table 2.5. Number and percentage of integration sites located within (b) (4) of genes listed in the Cancer Gene Census database.

Group	Total number of IS (Average)	Total number of IS within (b) (4) of CG (b) (4) (Average)	% of IS within (b) (4) of CG (b) (4) (Average)
3	777.75	46.75	5.95 %
4	856.75	56.25	5.91 %
5	668.75	55	8.30 %

Source: Modified from Table 12 (pages 69, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- The relative frequencies of each IS within (b) (4) CG (b) (4) were less than or equal to 0.59%. The cancer genes (b) (4) in monkey), (b) (4) in monkey),

and (b) (4) in monkey) were observed to have the highest relative abundance of 0.44%, 0.54%, and 0.59% in Groups 3-5, respectively.

- The number of IS located within (b) (4) of a (b) (4) of all genes ranged between 81.26% and 87.84% in Groups 3-5. These frequencies were 8.63-fold to 25.8-fold higher compared to frequencies of IS within (b) (4) of CG (b) (4).
- Further analysis of IS located within (b) (4) of CG (b) (4) indicated decreasing number of IS with decreasing distance to CG (b) (4) (Table 2.6).

Table 2.6. Number and percentage of integration sites within (b) (4) of CG (b) (4)

Group	Total number of IS (Average)	Number of IS (% IS) within (b) (4) (Average)	Number of IS (% IS) within (b) (4) (Average)	Number of IS (% IS) within (b) (4) (Average)
3	777.75	27.25 (3.49%)	18.25 (2.35%)	10.25 (1.26%)
4	856.75	38.25 (3.96%)	25.5 (2.48%)	13.75 (1.35%)
5	668.75	32.25 (4.74%)	22.5 (3.36%)	14.5 (2.16%)

Source: Modified from Tables 4, 5, and 6 (pages 615-616, BMN270-20-013, Module 4.2.3.1, STN 125720/0.69)

- The positional distribution of IS near cancer genes indicated that 53.94% were located upstream and 39.62% were located downstream of a CG, while 4.96% were located within a CG (5.51% in introns and 0.69% in exons).
- (b) (4) analyses of liver samples from Groups 3-5, indicate that the proportion of vector sequences that integrated averaged at 0.48%, while the proportion of episomal vector sequences averaged at 99.52%.
- Vector breakpoint analysis indicated that vector positions involved in integration events were generally broadly distributed across the BMN 270 vector sequence. The overall distribution and frequencies of IS vector breakpoint were observed highest in the ITR regions (> 100 IS reads).
- BMN 270 (b) (4) in Groups 3-5 liver samples were characterized by highest break points in the (b) (4) (75.29%), then followed by breaks in the (b) (4) (9.71%), (b) (4) (7.77%), (b) (4) (7.23%).
- The average unique IS/cell ranged between 3.09×10^{-4} and 4.50×10^{-3} , with an overall average of 1.55×10^{-3} IS/cell.
- The average unique IS/vg ranged between 2.63×10^{-5} and 1.61×10^{-4} , with an overall average of 6.10×10^{-5} IS/vg.

Reviewer's comments:

- The applicant targeted (b) (4) (covering (b) (4) in (b) (4), respectively, to allow evaluation of both nonhuman primate and human samples, based on >99 % homology of these sequences in nonhuman primates and humans.
- The applicant used a positive control that consisted of (b) (4) BMN 270 (b) (4) NHP gDNA and a negative control composed of (b) (4) NHP gDNA only.
- The %Chr and %BMN 270 Mapped Reads indicate the binding of RNA baits and their respective targets in controls (Groups 1-2) and BMN 270-administered (Groups 3-5) nonhuman primates.
- For each individual IS found among the 10 most abundant IS, the sequence counts ranged from 2 to 7 reads and the IS relative frequency ranged between 0.143% and 1.622%.
- The relative frequencies of IS indicate a polyclonal IS profile and absence of clonal enrichment. The average unique IS/cell ranged between 3.09×10^{-4} and 4.50×10^{-3} , with an overall average of 1.55×10^{-3} IS/cell.
- The IS relative frequencies did not differ significantly in the presence or absence of immunosuppression or between the 13-week and 26-week sacrifice time points. The applicant's justification (described in Module 1.11.4, Complete Response Letter (CRL) 24Jun2022 submitted in BLA125720/0.69) for the study duration based on time of assembly/presence of linear and episomal AAV DNA with respect to time of transduction/vector administration was acceptable.
- Of the 120 top IS identified in Groups 3-5, there were 99 unique IS and there were 10 IS that overlapped in 2 or 3 samples including (b) (4). Given the very low frequencies, it is unclear if these IS have biological significance or appeared due to chance. The data indicate that there is no IS common to all samples. (b) (4) was the top CIS in the liver, which was not surprising, since this is the most active gene in the liver.
- IS analysis indicated decreasing integration events nearer the (b) (4) of CG.
- There were no IS identified in proximity of cancer genes (b) (4) which were previously reported in (b) (4). However, low frequency (0.034% - 0.068%)

(b) (4)

integration events, equivalent to 1-2 reads, were observed near the (b) (4) gene, which was also identified in the (b) (4) in the (b) (4) study.

- The applicant assumed that all vector-vector sequence junctions (b) (4) were episomal. It is unclear what proportions of vector (b) (4) may have integrated in the cellular genomes.
- The vector integration study conducted using liver samples from nonhuman primates indicate non-targeted (random) and low frequency integration of BMN 270 vector in the host genome. Although the vector integration study observed IS and CIS within (b) (4) of gene (b) (4) (including cancer genes), there was no evidence of clonal expansion as measured by (b) (4) methods. In summary, the vector integration data in nonhuman primates and toxicology data obtained in mice and nonhuman primates (e.g., BMN270-16-045 and BMN270-16-046) indicate no evidence of BMN 270-related clonal expansion, preferential vector integration site(s), or tumor formation.
- The applicant's discussion of the overall nonclinical data submitted in the original BLA and in this resubmission as regards the tumorigenic potential of systemically administered BMN 270 (Section 2.4.1.3.2, Module 2.4, Nonclinical Overview, BLA125720/069) indicated a low probability of liver tumors based on (a) findings in a one-year mouse study evaluating an AAV vector backbone with similar regulatory elements; (b) differences between BMN 270 and BMN 370 nonclinical data (in which hepatocellular adenomas/carcinomas were observed at 1 year post-administration in Study TB18-61) with regard to disease models, vector backbones and transcription cassettes, and dose levels administered; (c) vector integration data from livers of nonhuman primates collected at 13-weeks and 26-weeks post-administration; (d) limitations in designing long-term carcinogenicity studies in rodents and large animals. The applicant's justifications were deemed acceptable.
- IS analysis of the livers from nonhuman primates (BMN270-20-013) and the five human liver biopsies obtained from Studies 270-201, 270-301, and 270-303 subjects, did not identify integration events in (b) (4), which were identified in the hepatocellular adenomas/carcinomas observed in Study TB18-61 evaluating BMN 370 in immunodeficient mice with induced (b) (4).

APPLICANT'S PROPOSED LABEL

Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), 201.57(c)(14).

Section 12.3 ('Pharmacokinetics') should be revised to accurately reflect the available data.

Section 13 ('Nonclinical Toxicology') should be revised to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies in this resubmission and in the original BLA did not identify any safety concerns that could not be adequately addressed in labeling. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

AAV, AAV5, BMN 270, gene therapy, Hemophilia A, hFVIII-SQ, ROCTAVIAN, mice, monkeys, biodistribution, genotoxicity, vector integration, mutagenicity, carcinogenicity