

**Clinical Pharmacology BLA Review**

Office of Clinical Evaluation  
Office of Therapeutic Products

BLA	125720/0 Re-submission
Product	ROCTAVIAN (Valoctogene roxaparvovec-rvox, AAV5-hFVIII-SQ, BMN 270)
Sponsor	BioMarin Pharmaceutical Inc.
Indication	For the treatment of adults with severe hemophilia A (congenital factor VIII deficiency with factor VIII activity < 1 IU/dL) without pre-existing antibodies to adeno-associated virus serotype 5 detected by an FDA-approved test.
Date Received	September 29, 2022
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## 1 EXECUTIVE SUMMARY

On December 23, 2019, BioMarin Pharmaceutical Inc. submitted BLA 125720, seeking approval of ROCTAVIAN (valoctogene roxaparvovec-rvox, AAV5-hFVIII-SQ, BMN270)<sup>1</sup> 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. BMN270 is an adeno-associated virus (AAV) vector-based gene therapy. BMN270 is a sterile solution to be administered via intravenous (IV) infusion. The proposed BMN270 dosing regimen is a single target dose of  $6 \times 10^{13}$  vector genomes (vg) per kg of body weight.

The clinical data from an interim analysis of a Phase 3 study (Study 270-301) submitted in the original BLA submission were not sufficient to provide substantial evidence of the effectiveness of BMN 270 to support its approval. A surrogate endpoint of median FVIII activity level of (b) (4) IU/dL between weeks 23 and 26 post-administration of BMN 270, as assessed by the chromogenic substrate assay (CSA), was used as the primary endpoint to support the accelerated approval. However, in clinical study this endpoint was not demonstrated to be predictive of the clinically meaningful endpoint of annualized bleeding rate (ABR). Based on review of original submission of BLA 125720, a Complete Response Letter (CRL) was issued on August 18, 2020

On September 29, 2022, BioMarin Pharmaceutical Inc. submitted a Class 2 resubmission addressing the deficiencies listed to the Complete Response Letter for the original BLA submission.

The clinical pharmacology section of this biologics license application (BLA) included one in vitro drug-drug interaction study and two clinical studies: a Phase 1/2, dose-escalation study (Study 270-201, Study 201) evaluating the safety, tolerability, and efficacy of BMN 270 in patients with severe hemophilia A, and one a Phase 3 study (Study 270-301, Study 301) evaluating the efficacy and safety of BMN 270 in hemophilia A patients with residual FVIII  $\leq 1$  IU/dL receiving prophylactic FVIII infusions. Factor VIII (FVIII) activity levels (IU/dL) over time post-BMN 270 infusion in ITT population are reported by both chromogenic and one-stage assays. After administration of BMN 270 at the dose of  $6 \times 10^{13}$  vg/kg in Study 301, FVIII activity increased and reached the peak levels with the median [min, max] time of 26.0 [2.0, 111.0] weeks in intention-to-treat (ITT) population. The mean (SD) and median [min, max] peak FVIII activity (chromogenic assay) were 84.4 (81.9) IU/dL and 61.3 [4.0, 463.0] IU/dL, respectively. The mean FVIII activity level at Month 36 was 18.2 IU/dL (95% CI: 12.9, 23.4) using the chromogenic assay. Considerable high inter-subject variability was observed in FVIII activity and hFVIII-SQ protein levels.

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<sup>1</sup> In this review ROCTAVIAN is referred as to BMN 270.

The proposed dosing regimen of BMN 270 administered by intravenous (IV) infusion has demonstrated clinical effectiveness with a tolerable safety profile; therefore, the proposed dose is acceptable. From clinical pharmacology standpoint, the data presented in the BLA are adequate and acceptable to support approval.

## **2 RECOMMENDATIONS**

The clinical pharmacology information in this BLA is acceptable, provided that satisfactory agreement is reached between the sponsor and the FDA regarding the language in Section 12 of the package insert. Please refer to Section 4 for detailed Labeling Recommendations.

## **3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS**

### BMN270 vector DNA biodistribution and shedding

- Following administration of BMN270, vector DNA was detected in blood and all matrices evaluated (saliva, semen, stool, and urine). The peak concentrations of BMN270 vector DNA were observed between 1-9 days post-dose. The highest peak concentrations were in blood, followed by saliva, semen, stool, and urine. Following peak concentration, vector DNA steadily declined in all matrices steadily. The peak levels and duration of detection of BMN270 vector DNA increased in a dose-dependent manner within the doses ranging from  $6 \times 10^{12}$  (6E12) vg/kg to  $6 \times 10^{13}$  (6E13) vg/kg. After administration of BMN 270 at the dose of 6E13 vg/kg, the peak concentration observed to date in blood across Studies 201 and 301 was  $2 \times 10^{11}$  vg/mL. The maximum concentration observed in any shedding matrix was  $1 \times 10^{10}$  vg/mL.
- In subjects treated in Studies 201 and 301 (7 in Study 201 and 134 in Study 301), encapsidated (potentially transmissible) vector DNA was detectable in plasma up to 10 weeks after BMN 270 administration.
- All subjects treated in clinical studies achieved the first of 3 consecutive measurements below the lower limit of quantification (LLOQ) for vector DNA in semen by 36 weeks, and all except one subject achieved 3 consecutive measurements below limit of detection (BLOD) or negative by the time of the data cut. The maximum time to the first of 3 consecutive measurements BLOD for encapsidated (potentially transmissible) vector DNA in semen was 12 weeks.

- In Studies 201 and 301, all subjects achieved 3 consecutive measurements below the LLOQ for vector DNA in urine and saliva, and 126 (89%) subjects achieved 3 consecutive measurements below the LLOQ for vector DNA in stool by the time of the data cut. The maximum time to the first of 3 consecutive LLOQ measurements was 8 weeks for urine, 52 weeks for saliva, and 131 weeks for stool. All subjects in Study 201 achieved 3 consecutive measurements BLOD or negative in urine, saliva, and stool by five-year post-dosing. All subjects in Study 301 achieved 3 consecutive measurements BLOD or negative in urine, and saliva, and 85 (63%) subjects achieved 3 consecutive measurements BLOD or negative in stool by three-year data cut.

BMN270 transgene produced hFVIII-SQ (FVIII activity and hFVIII-SQ Protein) (Results from Phase 3 study, Study 301)

- FVIII activity levels were measured using both chromogenic and one-stage assays. FVIII activity were consistently 1.5 to 1.7-fold higher with the one-stage assay compared to the chromogenic assay. The one-stage assay utilizes an (b) (4) [REDACTED], while the chromogenic assay (b) (4) [REDACTED]. This observation demonstrates that BMN-270-produced hFVIII-SQ has higher activity than normal plasma during early stages of (b) (4) [REDACTED] reaction in the one-stage assay.
- After administration of BMN 270 at the dose of 6E13 vg/kg, FVIII activity increased and reached the peak levels with the median [min, max] time of 26.0 [2.0, 111.0] weeks. The mean (SD) and median [min, max] peak FVIII activity were 84.4 (81.9) IU/dL and 61.3 [4.0, 463.0] IU/dL, respectively.
- There were two subpopulations in the Phase 3 study: rollover population of 112 subjects whose baseline ABR and FVIII usage data were prospectively collected and directly enrolled population of 22 subjects who were enrolled directly without prospective ABR data collection. FVIII activity results were assessed separately for the two subpopulations due to differences in baseline ABR data collection, corticosteroids use, and duration of follow-up. FVIII activity levels after administration of ROCTAVIAN were summarized as following table:

Timepoint	Rollover Population N = 112		Directly Enrolled Population N = 22	
	CSA	OSA	CSA	OSA
<b>Month 3</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	34.9 (40.4)	54.6 (60.8)	31.4 (25.7)	48.3 (36.0)
Median (Q1, Q3)	20.7 (10.3, 40.5)	31.3 (15.3, 71.7)	20.9 (12.6, 45.7)	36.0 (22.4, 63.9)
Min, Max	0, 249.5	1.5, 335.8	0, 85.8	4.5, 126.0
<b>Month 6</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	55.4 (57.5)	84.9 (83.1)	40.0 (37.9)	63.0 (57.2)
Median (Q1, Q3)	38.8 (16.8, 76.5)	62.0 (28.0, 115.2)	33.2 (14.7, 46.3)	53.5 (23.7, 78.2)
Min, Max	0, 367.3	1.9, 483.9	0, 169.4	1.8, 261.9
<b>Month 10</b>	N = 111	N = 111	N = 20	N = 20
Mean (SD)	49.4 (49.5)	73.6 (70.5)	44.2 (49.6)	70.2 (70.9)
Median (Q1, Q3)	31.7 (17.1, 64.5)	51.3 (25.1, 96.2)	30.9 (14.1, 68.6)	55.4 (24.8, 101.4)
Min, Max	0, 265.3	1.2, 375.6	0, 223.6	2.4, 313.7
<b>Month 12</b>	N = 111	N = 111	N = 21	N = 21
Mean (SD)	43.6 (45.5)	64.7 (64.6)	38.2 (46.3)	59.7 (67.0)
Median (Q1, Q3)	24.0 (12.5, 63.7)	40.0 (20.4, 87.5)	23.9 (11.2, 52.8)	40.5 (17.4, 82.6)
Min, Max	0, 231.2	0, 311.1	1.6, 207.4	4.4, 294.1
<b>Month 18</b>	N = 99	N = 99	N = 18	N = 18
Mean (SD)	27.7 (32.3)	40.6 (45.9)	28.5 (28.9)	44.5 (43.9)
Median (Q1, Q3)	13.5 (6.9, 36.8)	22.5 (10.9, 55.30)	15.3 (10.8, 43.9)	24.4 (17.7, 60.4)
Min, Max	0, 167.9	0, 232.2	3.3, 117.0	4.2, 173.7
<b>Month 24</b>	N = 98	N = 99	N = 19	N = 18
Mean (SD)	25.0 (35.5)	38.9 (50.7)	22.0 (28.7)	36.0 (40.8)
Median (Q1, Q3)	12.7 (5.1, 26.5)	22.7 (7.9, 45.7)	8.9 (5.8, 25.9)	19.5 (7.9, 37.7)
Min, Max	0, 187.1	0, 271.3	0, 110.6	2.4, 146.7
<b>Month 36</b>	N = 96	N = 97	N = 15	N = 15
Mean (SD)	21.0 (34.0)	33.8 (47.6)	20.8 (24.4)	32.2 (33.1)
Median (Q1, Q3)	10.0 (4.3, 19.8)	17.7 (7.2, 35.1)	9.4 (6.6, 31.7)	20.6 (8.5, 46.7)
Min, Max	0, 217.7	0, 291.4	0, 74.5	1.9, 104.2

- The specific activity of BMN270-derived hFVIII-SQ was calculated as FVIII activity normalized to protein mass. The specific activity of BMN270-derived hFVIII-SQ measured by chromogenic assay was comparable to (b) (4)
- A trend of lower factor VIII activity levels was observed in Black subjects within the study population. The mean (SD) peak FVIII activity levels measured by chromogenic assay were 37.2 (27.5) IU/dL and 90.8 (84.6) IU/dL for black subjects and subjects of other races (Asian, white and others). Given the small sample size, the limited number of sites enrolling Black patients relative to the total population, the existence of potential confounding factors, and multiple post-hoc analyses, this trend was insufficient to allow meaningful conclusions about the differences in response rates based on race or other factors therein influencing factor VIII expression following ROCTAVIAN infusion.

### Drug-Drug Interactions

An in vitro primary human hepatocyte model was used to assess the effects of concomitant administration of isotretinoin, amphetamine, omeprazole, celecoxib, and selected highly active antiretroviral therapy (HAART) medications with BMN 270 on cytotoxicity and BMN 270 DNA and RNA expression. The results showed that:

- ***Isotretinoin*** suppressed BMN 270 transcription at clinically achievable concentrations. The suppression can be partially reversed after discontinuation of isotretinoin.
- ***Efavirenz*** dose-dependently decreased FVIII transcription without an impact on FVIII DNA or hepatotoxicity after treating human primary hepatocytes for 3 days in-vitro. FVIII RNA expression was not restored after discontinuation of efavirenz.

### Immunogenicity

- ***Anti-AAV5 TAb and AAV5 Transduction Inhibition (TI)***: following administration of ROCTAVIAN, all subjects developed anti-AAV5 TAb and reported positive AAV5 TI test results from the first assessment time point at 8-week post dosing and peaked around 36 - 40 weeks post dosing. All subjects had detectable anti-AAV5 TAb and AAV5 TI titers at the study cutoff dates.
- ***FVIII Total Binding Antibody and FVIII Neutralizing Antibody (inhibitors)***: ten subjects in Phase 3 study tested positive for FVIII TAb at one or more time points. Four subjects had a single transient positive Bethesda assay result (>0.6 BU). There were no apparent associations established between FVIII TAb positive results and FVIII activity and any liver enzyme elevations above the normal range.
- ***Cellular Immune Responses against AAV5 capsid***: Ninety-three percent subjects with available ELISpot testing results showed positive at one or more time points assessed through a maximum of 140 weeks of follow up. No association was observed between capsid specific cellular immune responses and FVIII activity levels (chromogenic assay).
- ***Cellular Immune Responses against hFVIII-SQ***: Sixty-five percent subjects in Phase 3 study had positive responses following stimulation with FVIII peptide pools. The majority of positive subjects were transiently positive. There was no trend toward higher ALT values nor lower FVIII activity measures at time points where a FVIII-specific cellular response was detected.

## 4 LABELING COMMENTS

### Reviewer's Comments to Applicant:

1. Please delete promotional language.
2. Please move factor VIII activity data from Section 14 Clinical Studies to Section 12.2 Pharmacodynamics.
3. Factor VIII activity over time results need to be listed separately for rollover and directly enrolled subpopulations.
4. Please include nonclinical data in Section 12.3 Pharmacokinetics Vector Biodistribution and Shedding part.

Below are the PI incorporating reviewer's recommendations.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Valoctocogene roxaparvovec-rvox is an adeno-associated virus serotype 5 (AAV5) based gene therapy vector, designed to introduce a functional copy of a gene encoding the B-domain deleted SQ form of recombinant human factor VIII (hFVIII-SQ). Transcription of this transgene occurs within the liver, using a liver-specific promoter, which results in the expression of hFVIII-SQ. The expressed hFVIII-SQ replaces the missing coagulation factor VIII needed for effective hemostasis.

### 12.2 Pharmacodynamics

Following ROCTAVIAN infusion, vector DNA is processed *in vivo* to form full-length, episomal transgenes that increase circulating hFVIII-SQ up to 5 years.

Liver samples from 5 patients in clinical studies collected 0.5-4.1 years post-dose were analyzed. Vector integration into human genomic DNA was observed in all samples. No preferential integration of the vector and no clonal outgrowth of cells as a result of vector integration was observed. ROCTAVIAN can also insert into DNA of other human body cells; vector insertion into parotid gland DNA samples without associated clinical manifestations was observed in one patient treated with ROCTAVIAN.

#### Factor VIII Activity

The pharmacodynamic effect of ROCTAVIAN was assessed by measuring circulating factor VIII activity levels.

Factor VIII activity levels (IU/dL) over time post-ROCTAVIAN infusion in ITT population are reported by both the CSA and OSA. The mean factor VIII activity levels at Month 36 was 18.2 IU/dL (95% CI: 12.9, 23.4) using the CSA, a statistically significant ( $p < 0.0001$ ) improvement from 1 IU/dL at baseline.

Table 7 shows factor VIII activity levels (IU/dL) over time post-ROCTAVIAN infusion in patients rolled over from a non-interventional study prospectively collecting patients' baseline annualized bleeding rate (ABR) and factor VIII usage data.

**Table 7: Factor VIII Activity Levels (IU/dL) Over Time**

Timepoint	Rollover Population N = 112		Directly Enrolled Population N = 22	
	CSA	OSA	CSA	OSA
<b>Month 3</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	34.9 (40.4)	54.6 (60.8)	31.4 (25.7)	48.3 (36.0)
Median (Q1, Q3)	20.7 (10.3, 40.5)	31.3 (15.3, 71.7)	20.9 (12.6, 45.7)	36.0 (22.4, 63.9)
Min, Max	0, 249.5	1.5, 335.8	0, 85.8	4.5, 126.0
<b>Month 6</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	55.4 (57.5)	84.9 (83.1)	40.0 (37.9)	63.0 (57.2)
Median (Q1, Q3)	38.8 (16.8, 76.5)	62.0 (28.0, 115.2)	33.2 (14.7, 46.3)	53.5 (23.7, 78.2)
Min, Max	0, 367.3	1.9, 483.9	0, 169.4	1.8, 261.9
<b>Month 10</b>	N = 111	N = 111	N = 20	N = 20
Mean (SD)	49.4 (49.5)	73.6 (70.5)	44.2 (49.6)	70.2 (70.9)
Median (Q1, Q3)	31.7 (17.1, 64.5)	51.3 (25.1, 96.2)	30.9 (14.1, 68.6)	55.4 (24.8, 101.4)
Min, Max	0, 265.3	1.2, 375.6	0, 223.6	2.4, 313.7
<b>Month 12</b>	N = 111	N = 111	N = 21	N = 21
Mean (SD)	43.6 (45.5)	64.7 (64.6)	38.2 (46.3)	59.7 (67.0)
Median (Q1, Q3)	24.0 (12.5, 63.7)	40.0 (20.4, 87.5)	23.9 (11.2, 52.8)	40.5 (17.4, 82.6)
Min, Max	0, 231.2	0, 311.1	1.6, 207.4	4.4, 294.1
<b>Month 18</b>	N = 99	N = 99	N = 18	N = 18
Mean (SD)	27.7 (32.3)	40.6 (45.9)	28.5 (28.9)	44.5 (43.9)
Median (Q1, Q3)	13.5 (6.9, 36.8)	22.5 (10.9, 55.30)	15.3 (10.8, 43.9)	24.4 (17.7, 60.4)
Min, Max	0, 167.9	0, 232.2	3.3, 117.0	4.2, 173.7
<b>Month 24</b>	N = 98	N = 99	N = 19	N = 18
Mean (SD)	25.0 (35.5)	38.9 (50.7)	22.0 (28.7)	36.0 (40.8)
Median (Q1, Q3)	12.7 (5.1, 26.5)	22.7 (7.9, 45.7)	8.9 (5.8, 25.9)	19.5 (7.9, 37.7)
Min, Max	0, 187.1	0, 271.3	0, 110.6	2.4, 146.7
<b>Month 36</b>	N = 96	N = 97	N = 15	N = 15
Mean (SD)	21.0 (34.0)	33.8 (47.6)	20.8 (24.4)	32.2 (33.1)
Median (Q1, Q3)	10.0 (4.3, 19.8)	17.7 (7.2, 35.1)	9.4 (6.6, 31.7)	20.6 (8.5, 46.7)
Min, Max	0, 217.7	0, 291.4	0, 74.5	1.9, 104.2

The proportion of patients achieving factor VIII activity level thresholds by year are presented in Table 8 by both the CSA and OSA. The majority (95%) of patients who reach factor VIII activity levels of  $\geq 5$  IU/dL using the CSA do so within 5 months post-infusion.

**Table 8: Patients Achieving Factor VIII Activity Thresholds by Year**

<b>Rollover Population (N = 112)</b>			
<b>Factor VIII Activity Threshold Achieved by Assay</b>	<b>Year 1 N = 111 n (%)</b>	<b>Year 2 N = 98 n (%)</b>	<b>Year 3 N = 96 n (%)</b>
<b>CSA</b>			
> 150 IU/dL	6 (5%)	2 (2%)	2 (2%)
40 - $\leq$ 150 IU/dL	37 (33%)	14 (14%)	9 (9%)
15 - < 40 IU/dL	37 (33%)	27 (28%)	23 (24%)
5 - < 15 IU/dL	18 (16%)	33 (34%)	35 (36%)
3 - < 5 IU/dL	3 (3%)	10 (10%)	8 (8%)
< 3 IU/dL	10 (9%)	12 (12%)	19 (20%)
	<b>Year 1 N = 111 n (%)</b>	<b>Year 2 N = 99 n (%)</b>	<b>Year 3 N = 97 n (%)</b>
<b>OSA</b>			
> 150 IU/dL	12 (11%)	5 (5%)	4 (4%)
40 - $\leq$ 150 IU/dL	44 (40%)	25 (25%)	17 (18%)
15 - < 40 IU/dL	37 (33%)	36 (36%)	36 (37%)
5 - < 15 IU/dL	10 (9%)	20 (20%)	26 (27%)
1 - < 5 IU/dL	6 (5%)	11 (11%)	12 (12%)
< 1 IU/dL	2 (2%)	2 (2%)	2 (2%)
	<b>Year 1 N = 21 n (%)</b>	<b>Year 2 N = 19 n (%)</b>	<b>Year 3 N = 15 n (%)</b>
<b>Directly Enrolled Population (N = 22)</b>			
<b>Factor VIII Activity Threshold Achieved by Assay</b>	<b>Year 1 N = 21 n (%)</b>	<b>Year 2 N = 19 n (%)</b>	<b>Year 3 N = 15 n (%)</b>
<b>CSA</b>			
> 150 IU/dL	1 (5%)	0 (0%)	0 (0%)
40 - $\leq$ 150 IU/dL	5 (24%)	3 (16%)	3 (20%)
15 - < 40 IU/dL	8 (38%)	4 (21%)	2 (13%)
5 - < 15 IU/dL	5 (24%)	8 (42%)	7 (47%)
3 - < 5 IU/dL	0 (0%)	1 (5%)	1 (7%)
< 3 IU/dL	2 (10%)	3 (16%)	2 (13%)
	<b>Year 1 N = 21 n (%)</b>	<b>Year 2 N = 18 n (%)</b>	<b>Year 3 N = 15 n (%)</b>
<b>OSA</b>			
> 150 IU/dL	1 (5%)	0 (0%)	0 (0%)
40 - $\leq$ 150 IU/dL	10 (48%)	4 (22%)	4 (27%)
15 - < 40 IU/dL	6 (29%)	6 (33%)	6 (40%)
5 - < 15 IU/dL	3 (14%)	6 (33%)	3 (20%)
1 - < 5 IU/dL	1 (5%)	2 (11%)	2 (13%)
< 1 IU/dL	0 (0%)	0 (0%)	0 (0%)

Specific Populations

A trend of lower factor VIII activity levels was observed in Black patients within the study population. The mean (SD) peak factor VIII activity levels measured by chromogenic assay were 37.2 (27.5) IU/dL and 90.8 (84.5) IU/dL for Black patients and patients of other races (Asian, White and others). Given the small sample size, the limited number of sites enrolling Black patients relative to the total population, the existence of potential confounding factors, and multiple posthoc analyses, this trend was insufficient to allow meaningful conclusions about the differences in response rates based on race or other factors therein influencing factor VIII expression following ROCTAVIAN infusion. Despite differences in factor VIII activity levels, ABR and annualized factor VIII usage was similar across races.

### **12.3 Pharmacokinetics**

#### Biodistribution (within the body) and Vector Shedding (excretion/secretion)

Valoctocogene roxaparvovec-rvox transgene DNA levels (total amount of vector DNA) in various tissues (evaluated in nonclinical studies), blood, and shedding matrices were determined using a quantitative polymerase chain reaction (qPCR) assay. This assay is sensitive to transgene DNA, including fragments of degraded DNA. It does not indicate whether DNA is present in the vector capsid, in cells or in the fluid phase of the matrix (e.g., blood plasma, seminal fluid), or whether intact vector is present. Plasma and semen matrices were further evaluated by measuring encapsidated (potentially infectious) vector DNA using an immunoprecipitation quantitative PCR assay in Studies 270-201 and 270-301.

#### Nonclinical Data

Biodistribution of ROCTAVIAN was assessed in adult male mice. Following intravenous administration of  $2.1 \times 10^{14}$  vg/kg, the highest vector DNA concentration was detected in the liver, followed by lower levels in the lung, heart, lymph nodes, kidney, spleen, bone marrow, testis, and brain through six months post-administration. The expression of the hFVIII mRNA transcripts were primarily detected in the liver, with no or minimal expression in extrahepatic tissues.

#### Clinical Data

ROCTAVIAN biodistribution and vector shedding were investigated on samples from blood, saliva, semen, stool, and urine. Administration of ROCTAVIAN at the dose of  $6 \times 10^{13}$  vg/kg resulted in detectable vector DNA in blood and all shedding matrices evaluated at the dose of  $6 \times 10^{13}$  vg/kg, with peak concentrations observed between 1 and 9 days post-administration. The peak vector DNA concentrations were observed in blood, followed by saliva, semen, stool, and urine. The peak concentration observed to date in blood across two clinical studies was  $2 \times 10^{11}$  vg/mL. The maximum concentration observed in any shedding matrix was  $1 \times 10^{10}$  vg/mL. After reaching the maximum in a matrix, the transgene DNA concentration declines steadily.

In patients treated in two clinical studies, encapsidated (potentially transmissible) vector DNA was detectable in plasma up to 10 weeks after ROCTAVIAN administration.

All patients treated in clinical studies achieved the first of 3 consecutive measurements below the lower limit of quantification (LLOQ) for vector DNA in semen by 36 weeks, and all except one patient achieved 3 consecutive measurements below limit of detection (BLOD) or negative by the time of the data cut. The maximum time to the first of 3 consecutive measurements BLOD for encapsidated (potentially transmissible) vector DNA in semen was 12 weeks.

In clinical studies, all patients achieved 3 consecutive measurements below the LLOQ for vector DNA in urine and saliva, and 126 (89%) patients achieved 3 consecutive measurements below the LLOQ for vector DNA in stool by the time of the data cut. The maximum time to the first of 3 consecutive LLOQ measurements was 8 weeks for urine, 52 weeks for saliva, and 131 weeks for stool. All patients in the first study achieved 3 consecutive measurements BLOD or negative in urine, saliva, and stool by five-year post-dosing. All patients in the second study achieved 3 consecutive measurements BLOD or negative in urine, and saliva, and 85 (63%) patients achieved 3 consecutive measurements BLOD or negative in stool by three-year data cut.

Magnitude and duration of shedding appear to be independent of the patient's attained factor VIII activity.

## **12.6 Immunogenicity**

The observed incidence of anti-drug antibodies is highly dependent on the sensitivity and specificity of the assay. Differences in assay methods preclude meaningful comparisons of the incidence of anti-drug antibodies in the studies described below with the incidence of anti-drug antibodies in other studies, including those of ROCTAVIAN or of other adeno-associated virus-based gene therapy products.

In clinical studies, all patients receiving treatment were required to screen negative for anti-AAV5 antibodies and negative (< 0.6 BU) for factor VIII inhibitors in a Nijmegen modified Bethesda assay following a lifetime minimum of 150 exposure days to factor VIII replacement therapy [*see Use in Specific Populations (8.7)*]. Following infusion of ROCTAVIAN, all patients remained negative for factor VIII inhibitors.

All patients seroconverted to anti-AAV5 antibody positive within 8 weeks of administration. Anti-AAV5 total antibody titers peaked by 36 weeks after administration with mean (SD) values of 12,528,983 (32,427,817), and remained stable until the last time point tested, Week 168 with mean (SD) values of 3,673,038 (3,344,713).

ROCTAVIAN-treated patients were tested for cellular immune responses against the AAV5 capsid and the factor VIII transgene product using an IFN- $\gamma$  ELISpot assay. AAV5 capsid-specific cellular immune responses were detected beginning at Week 2 following dose administration and often declined or reverted to negative over the first 52 weeks in the majority of patients with available data. Incidence peaked at Week 2 with 67 of 96 patients (70%) testing positive in the IFN- $\gamma$  ELISpot assay. This declined to 17 of 74 patients (23%) at Week 26, and 10 of 60 patients (17%) at Week 52.

Factor VIII-specific cellular responses were detected in 81 of 123 (65.9%) patients, often sporadically at a single time point and reverting to negative in most patients.

## 5 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

### Summary of Clinical Pharmacology Section in Current Re-submission

In this re-submission, the clinical pharmacology section contains following:

- Updated report for Study 270-201 (Study 201) with new data cutoff date (March 29, 2021). All subjects have completed at least 208 weeks of follow-up post-BMN 270 infusion. Study 201 is a Phase 1/2, dose-escalation study that evaluated the safety, tolerability, and efficacy of BMN270, an adenovirus-associated virus-mediated gene transfer of human factor VIII in patients with severe hemophilia A. Subjects received BMN270 at four dose levels in Study 201: 6E12 vg/kg (Cohort 1, n=1), 2E13 vg/kg (Cohort 2, n=1), 6E13 vg/kg (Cohort 3, n=7), and 4E13 vg/kg (Cohort 4, n=6).
- Updated report for Study 301 with additional study subjects (n=134 for current submission, data cutoff date: November 15, 2021). Study 301 is a Phase 3 open-label, single-arm study (Study No. 270-301, Study 301) evaluating the efficacy and safety of BMN270, an adenovirus-associated virus-mediated gene transfer of human factor VIII in hemophilia A patients with residual FVIII  $\leq 1$  IU/dL receiving prophylactic FVIII infusions.
- In vitro drug-drug interactions study (Study # RS21-005): In vitro drug-drug interaction study: effects of concomitant administration of Isotretinoin, Amphetamine, Omeprazole, Celecoxib and selected highly active antiretroviral therapy (HAART) medications with AAV5-FVIII-SQ on cytotoxicity and AAV5-FVIII-SQ DNA and RNA expression in primary human hepatocytes.

In both Study 201 and 301, BMN270 was administered as a single intravenous infusion. To avoid breakthrough bleeding, subjects were continued on exogenous prophylactic FVIII replacement therapy for up to 4 weeks following infusion of BMN270.

#### **Reviewer's Comments:**

Due to differences in product manufacturing process and use of corticosteroids and/or other immunosuppressants for alanine aminotransferase (ALT) elevation and preservation of transgene expression between Studies 201 and 301, results of Study 201 were mainly used for vector biodistribution and shedding assessment. Pharmacodynamic (FVIII activity) evaluation focused on Study 301. In Study 201, some subjects at 6E13 vg/kg dose level had increased FVIII up to 260 weeks post-dosing.

## 5.1 General Pharmacology and Pharmacokinetic Characteristics

### 5.1.1 BMN270 Vector DNA Biodistribution and Shedding by qPCR

BMN270 vector DNA kinetic profiles of biodistribution and shedding were assessed in blood, semen, saliva, urine, and stool following BMN270 administration using a validated qPCR method. Blood, saliva, urine, stool, and semen were collected for evaluation of biodistribution and vector shedding at baseline and on Day 1 (between 2 and 24 hours after BMN 270 infusion), Day 4, Day 8, Day 15, Day 22, Day 29, Week 6, Week 8, Week 12, Week 16, Week 20, Week 24, Week 26, Week 32, Week 36, Week 40, Week 44, Week 48, Week 52 until at least 3 consecutive negative results were obtained. Testing of semen continued through Week 12 even if 3 consecutive negative results have been recorded in that compartment prior to that timepoint. Subjects who had not had 3 consecutive negative samples by Week 52 continued testing every 4 weeks during Year 2) and every 6 weeks (during Years 3-5) for semen and every 12 weeks for blood, saliva, stool, and urine until 3 consecutive negative samples were documented.

In general, peak BMN270 vector DNA concentrations were observed shortly after infusion across all matrices. Following peak vector DNA concentrations, BMN270 vector genomes declined in all matrices over the duration of follow-up. The kinetic profiles of BMN270 vector DNA biodistribution and shedding appear independent of FVIII activity response.

#### **Study 201**

After BMN 270 administration, vector DNA was detected in all matrices for all subjects. Following BMN 270 6E13 vg/kg, BMN 270 vector DNA was detected quickly in all matrices (range: 0.146 to 1.98 weeks post dosing). Median peak vector DNA levels were highest in blood (7.05E+09 vg/mL) followed by saliva (6.19E+07 vg/mL), semen (2.95E+06 vg/mL), stool (5.90E+05 vg/mg), and urine (2.70E+05 vg/mL). The median time for the peak vector DNA ranged from 1 to 9 days post dosing. Following peak vector DNA concentrations, BMN 270 vector genomes are steadily cleared from all matrices. Viral vector clearance was defined as the first time point of 3 negative consecutive samples. After administration of BMN 270 at 6E13 vg/kg, all subjects achieved vector clearance in saliva, semen, stool and urine with a median (min, max) time to clearance of 44.3 (40.0, 52.0) weeks, 20.0 (15.0, 83.1) weeks, 116 (87.1, 248), and 7.98 (5.98, 28.0) weeks, respectively. No subjects have achieved vector DNA clearance from blood following 6E13 vg/kg BMN 270 administration through at least 260 weeks of follow-up (the vector DNA levels were below the lower limit of quantification).

Following BMN 270 4E13 vg/kg, BMN 270 vector DNA biodistribution and viral shedding was similar to BMN 270 6E13 vg/kg, with lower peak vector DNA levels in all matrices. All subjects cleared vector DNA, defined as 3 consecutive negative qPCR samples, in saliva, semen, and urine with a median (min, max) time to clearance of 44.7 (20.0, 64.0) weeks, 28.1 (14.0, 60.1) weeks,

and 9.05 (7.84, 28.0) weeks, respectively. Four out of 6 subjects cleared stool with a median (min, max) time to clearance of 162 (151, 185). No subjects have cleared vector DNA from blood after 3 years following 4E13 vg/kg BMN 270 administration, although the vector DNA levels were below the lower limit of quantification (Table 1).

### **Study 301**

After BMN 270 administration, vector DNA was detected in all matrices for all subjects. Following BMN 270 6E13 vg/kg, peak vector DNA concentrations were observed shortly (median Tmax range: 1 – 8 days) in all matrices. Median peak vector DNA levels were highest in blood (4.73E+10 vg/mL) followed by saliva (6.63E+07 vg/mL), semen (1.75E+06 vg/mL), stool (2.65E+05 vg/mg), and urine (8.91E+04 vg/mL). Following peak vector DNA concentrations, BMN 270 vector genomes are steadily cleared from all matrices (Table 2).

At the data cutoff time of this application, 105 (78.4%), 121(91.0%), 4 (2.99%), and 132 (98.5%) subjects had 3 consecutive negative qPCR samples in saliva, semen, stool, and urine, respectively. For the subjects that achieved 3 consecutive negative samples, the median (min, max) time to the third negative sample was 52.7 (16.0, 132) weeks, 36.0 (12.0, 111) weeks, 114 (88.4, 155) weeks, and 20.0 (6.00, 57.1) weeks for saliva, semen, stool and urine, respectively. No subjects achieved defined as 3 consecutive negative qPCR samples in blood (Figure 1).

The Applicant assessed the clinical relevance of BMN 270 vector DNA level at the lower limit of quantification (LOQ). Based on the qPCR assay LOQ level at 50 vg/5mL DNA test sample, the back calculated quantities of vector genomes that remain at LOQ were approximately 6E3 vg/mL of blood, 7E3 vg/mL of saliva, 2.4E4 vg/mL of semen, 16 vg/mg of stool, and 1.54E4 vg/mL of urine. For a 70 kg subject at 6E12 vg/kg dose where minimal FVIII activity was measured (BLQ, < 3.0 IU/dL), the total amount of vector DNA was 4.2E14 vg, substantially higher than the total amount of BMN 270 vector DNA in blood at LLQ level (3.4E7 vg). Therefore, there was no clinical meaningful quantities of BMN vector DNA in blood and other matrices when the sample measurements were BLQ.

**Table 1. Vector DNA Biodistribution and Shedding Following 4E13 vg/kg BMN 270 Administration (Study 201)**

a. 4E13 vg/kg

		No. (%) of Detect. Subjects	Time to First Detect. Sample <sup>a</sup> (wk)	Peak Conc. <sup>b</sup> Median (vg/mL)	Time to Peak Conc. (wk)	Time to Last Detect. Sample <sup>c</sup> (wk)	Duration of Detect. Shedding <sup>d</sup> (wk)	Time to first BLQ/ negative sample confirmed by 2 consec. samples <sup>e</sup> (wk)	Number (%) of subjects with 3 consec. BLQ samples	Time to 3 consec. negative samples <sup>f</sup> (wk)	Time to first negative sample confirmed by 2 consec. samples <sup>g</sup> (wk)	Number (%) of subjects with 3 consec. negative samples
<b>Blood</b>	N	6 (100%)	6	6	6	0	0	6	6 (100%)	0	0	0 (0%)
	Min		0.00437	3.70E+09	0.00437	-	-	114		-	-	
	Median		0.145	5.39E+09	0.145	-	-	128		-	-	
	Max		0.148	1.18E+10	0.148	-	-	156		-	-	
<b>Saliva</b>	N	6 (100%)	6	6	6	6	6	6	6 (100%)	6	6	6 (100%)
	Min		0.00437	1.75E+07	0.00437	12.0	11.8	4.12		20.0	13.8	
	Median		0.142	3.52E+07	0.144	32.1	32.1	6.05		44.7	36.1	
	Max		0.145	1.14E+08	1.12	52.0	51.8	20.1		64.0	56.0	
<b>Semen</b>	N	6 (100%)	6	6	6	6	6	6	6 (100%)	6	6	6 (100%)
	Min		0.00437	6.02E+04	0.391	7.97	7.41	1.11		14.0	9.98	
	Median		0.135	1.45E+06	0.48	13.9	13.8	5.02		28.1	19.9	
	Max		0.551	7.98E+06	1.1	48.1	48.0	7.96		60.1	53.4	
<b>Stool</b>	N	6 (100%)	6	6	6	5	5	6	6 (100%)	4	4	4 (66.7%)
	Min		0.00437	7.84E+04	0.00437	134	134	27.8		151	140	
	Median		0.132	1.93E+05	0.906	146	146	38.7		162	147	
	Max		0.143	3.52E+06	1.11	155	154	61.4		185	162	
<b>Urine</b>	N	6 (100%)	6	6	6	6	6	6	6 (100%)	6	6	6 (100%)
	Min		0.00437	7.70E+03	0.124	0.836	0.706	0.396		7.84	4.11	
	Median		0.135	1.16E+05	0.142	5.05	4.91	3.98		9.05	5.11	
	Max		0.143	7.94E+05	0.979	16.1	16.0	4.13		28.0	20.1	

**b. 6E13 vg/kg**

		No. (%) of Detect. Subjects	Time to First Detect. Sample <sup>a</sup> (wk)	Peak Conc. <sup>b</sup> Median (vg/mL)	Time to Peak Conc. (wk)	Time to Last Detect. Sample <sup>c</sup> (wk)	Duration of Detect. Shedding <sup>d</sup> (wk)	Time to first BLQ/ negative sample confirmed by 2 consec. samples <sup>e</sup> (wk)	Number (%) of subjects with 3 consec. BLQ samples	Time to 3 consec. negative samples <sup>f</sup> (wk)	Time to first negative sample confirmed by 2 consec. samples <sup>g</sup> (wk)	Number (%) of subjects with 3 consec. negative samples
<b>Blood</b>	N	7 (100%)	7	7	7	0	0	6	6 (100%)	0	0	0 (0%)
	Min		0.146	4.84E+09	0.146	-	-	113		-	-	
	Median		0.149	7.05E+09	0.149	-	-	169		-	-	
	Max		0.151	1.51E+10	0.151	-	-	243		-	-	
<b>Saliva</b>	N	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	1.04E+07	0.147	20.0	19.8	3.97		40.0	27.0	
	Median		0.15	6.19E+07	0.151	28.0	27.8	6.98		44.3	36.0	
	Max		1.98	1.51E+08	1.98	42.0	40.0	12.0		52.0	44.0	
<b>Semen</b>	N	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	4.87E+05	0.423	13.0	12.8	4.85		15.0	12.0	
	Median		0.15	2.95E+06	1.29	20.0	19.8	5.97		20.0	14.8	
	Max		1.98	5.02E+07	2.98	68.9	68.7	22.0		83.1	76.5	
<b>Stool</b>	N	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.146	1.28E+05	0.151	73.1	73.0	20.0		87.1	79.1	
	Median		0.149	5.90E+05	0.973	155	155	28.4		116	108	
	Max		0.151	1.79E+06	1.98	246	246	78.0		248	237	
<b>Urine</b>	N	7 (100%)	7	7	7	7	7	7	7 (100%)	7	7	7 (100%)
	Min		0.147	4.49E+04	0.423	2.98	2.83	1.97		5.98	3.97	
	Median		0.15	2.70E+05	1.29	9.97	8.00	2.98		7.98	5.96	
	Max		1.98	1.82E+06	2.96	15.0	14.8	3.96		28.0	16.0	

<sup>a</sup> Defined to time to first positive shedding sample; <sup>b</sup> Units for stool reported as vg/mg; <sup>c</sup> Defined as time to last positive shedding sample followed by a negative; <sup>d</sup> Defined as time between first positive shedding sample and last positive shedding sample confirmed by a negative; <sup>e</sup> Confirmed by 2 consecutive negative or BLQ samples; <sup>f</sup> Reported as time of third sample; <sup>g</sup> Reported as time of first negative sample confirmed by 2 additional consecutive negative samples.

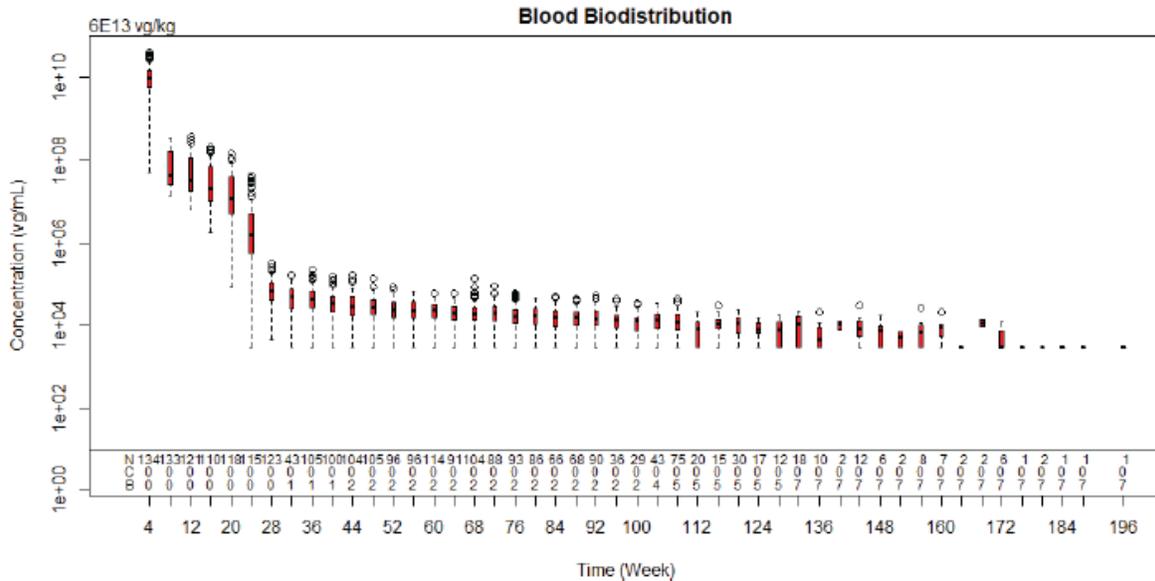
Source: Applicant. Study 201 Clinical Pharmacology Report.

**Table 2. Vector DNA Biodistribution and Shedding Following 6E13 vg/kg BMN 270 Administration (ITT Population) (Study 301)**

		No. (%) of Detectable Subjects	Time to First Detectable Sample <sup>a</sup> (wk)	Peak Conc. <sup>b</sup> Median (vg/mL)	Time to Peak Conc. (wk)	Time to Last Detectable Sample <sup>c</sup> (wk)	Duration of Detectable Shedding <sup>d</sup> (wk)	Time to first BLQ/negative sample confirmed by 2 consecutive samples <sup>e</sup> (wk)	Number (%) of subjects with 3 consecutive BLQ/negative samples	Time to 3 consecutive negative samples <sup>f</sup> (wk)	Time to first negative sample confirmed by 2 consecutive samples <sup>g</sup> (wk)	Number (%) of subjects with 3 consecutive negative samples
<b>Blood</b>	N	134 (100%)	134	134	134	2	2	7	7 (5.22%)	0	0	0 (0%)
	Median		0.143	4.73E+10	0.143	78	77.9	101		-	-	
	Min		0.143	1.55E+08	0.143	75.9	75.7	31.9		-	-	
	Max		1.00	2.01E+11	1.00	80.1	80.0	130		-	-	
<b>Saliva</b>	N	134 (100%)	134	134	134	126	126	134	134(100%)	105	105	105 (78.4%)
	Median		0.143	6.63E+07	0.143	42.1	41.9	6.57		52.7	43.4	
	Min		0.143	1.26E+06	0.143	6.00	4.86	3.14		16.0	7.86	
	Max		1.14	4.27E+09	2.29	116	115	68.7		132	122	
<b>Semen</b>	N	133 <sup>h</sup> (100%)	133	133	133	128	128	132	132 <sup>i</sup> (99.2%)	121	121	121 (91.0%)
	Median		0.143	1.75E+06	1.00	20.0	19.4	6.14		36.0	20.6	
	Min		0.143	1.20E+04	0.143	4.00	3.86	0.571		12.0	6.14	
	Max		2.14	1.02E+10	12.1	81.7	81.6	36.1		111	76.1	
<b>Stool</b>	N	134 (100%)	134	134	134	25	25	113	113 (84.3%)	4	4	4 (2.99%)
	Median		0.143	2.65E+05	1.14	87.9	87.7	44.0		114	101	
	Min		0.143	2.09E+02	0.143	52.1	52.0	12.0		88.4	68.4	
	Max		6.29	5.65E+06	6.29	140	140	88.1		155	144	
<b>Urine</b>	N	134 (100%)	134	134	134	134	134	134	134 (100%)	132	132	132 <sup>i</sup> (98.5%)
	Median		0.143	8.91E+04	0.214	8.00	7.86	2.29		20.0	12.1	
	Min		0.143	7.70E+03	0.143	1.14	1.00	0.286		6.00	3.00	
	Max		0.857	3.69E+07	2.29	44.1	44.0	8.14		57.1	48.0	

<sup>a</sup> Defined as time to first positive shedding sample; <sup>b</sup>Units for stool reported as vg/mg; <sup>c</sup>Defined as time to last positive shedding sample followed by a negative; <sup>d</sup>Defined as time between first positive shedding sample and last positive shedding sample confirmed by a negative; <sup>e</sup> Confirmed by 2 consecutive negative or BLQ samples; <sup>f</sup>Reported as time of third sample; <sup>g</sup>Reported as time of first negative sample confirmed by 2 additional consecutive negative samples; <sup>h</sup> One subject did not have available semen shedding assessments; <sup>i</sup> One subject had not achieved three consecutive BLQ samples through Week 16, the latest sample available at the time of the datacut date for this report *Source: Applicant. Study 301 Interim Clinical Pharmacology Report.*

**Figure 1. Vector DNA concentration vs. time profile in blood following 6E13 vg/kg BMN270 Administration (Study 301)**



Source: Applicant. Study 301 Interim Clinical Pharmacology Report.

### 5.1.2 Encapsidated BMN 270 Vector DNA Biodistribution and Viral Shedding by immunoprecipitation coupled qPCR in Plasma and Semen

The Applicant conducted further exploratory analysis to better understand the biodistribution and shedding of potentially transmissible vector DNA. Plasma and semen samples were evaluated for encapsidated vector DNA concentration by immunoprecipitating residual capsid with an AAV5-specific antibody and testing for vector genome by qPCR (b) (4)

In Study 201, following administration of 6E12 vg/kg to 6E13 vg/kg BMN 270, encapsidated vector DNA was detectable in plasma and semen from all 15 subjects. Median peak capsid levels increased with dose. The median (min, max) time to the first of 3 consecutive negatives samples were 3.16 (2.50, 9.01) and 3.33 (1.90, 9.02) weeks in plasma and semen, respectively.

In Study 301, after administration of BMN 270 6E13vg/kg, encapsidated vector DNA was detectable in plasma from 130 of 134 subjects (97.0%) evaluated, and in semen from 131 of the 133 subjects (98.5%) evaluated (Table 2). Peak encapsidated vector DNA concentrations in plasma and semen were observed within the first week post-dosing. The median peak capsid concentration was BLQ and 9.26E5 vg/mL in plasma and semen, respectively. The median (min, max) time to the first of 3 consecutive negatives samples were 3.29 (1.29, 10.10) and 3.00 (0.43, 12.10) weeks in plasma and semen, respectively (Table 3).

**Table 3. Encapsulated Vector DNA Biodistribution in Plasma and Semen by (b) (4) Following 6E13 vg/kg BMN 270 Administration (Study 301)**

a. Plasma

Dose (vg/kg)		No. (%) of Detectable Subjects	Peak Conc. (vg/mL)	Time to Peak Conc. (weeks)	Time to Last Detectable Sample (weeks)	No. (%) of Subjects that achieved 3 consecutive negatives	Time to first negative sample confirmed by 2 consecutive samples (weeks)
6E13 (n=134)	Min	130 (97.0%)	BLQ	0.857	0.857	134 (100%)	1.29
	Median		BLQ	1.14	2.21		3.29
	Max		1.13E+07	4.14	10.0		10.1

b. Semen

Dose (vg/kg)		No. (%) of Detectable Subjects	Peak Conc. (vg/mL)	Time to Peak Conc. (weeks)	Time to Last Detectable Sample (weeks)	No. (%) of Subjects that achieved 3 consecutive negatives	Time to first negative sample confirmed by 2 consecutive samples (weeks)
6E13 (n=133) <sup>a</sup>	Min	131 (98.5%)	BLQ	0.143	0.143	131 <sup>b</sup> (98.5%)	0.429
	Median		9.26E+05	0.571	1.86		3.00
	Max		3.79E+08	4.00	8.14		12.1

<sup>a</sup> one subject did not provide semen shedding assessments; <sup>b</sup> 2 subjects had insufficient sample quantity for vector DNA assessment by (b) (4); BLQ, below the limit of quantification.

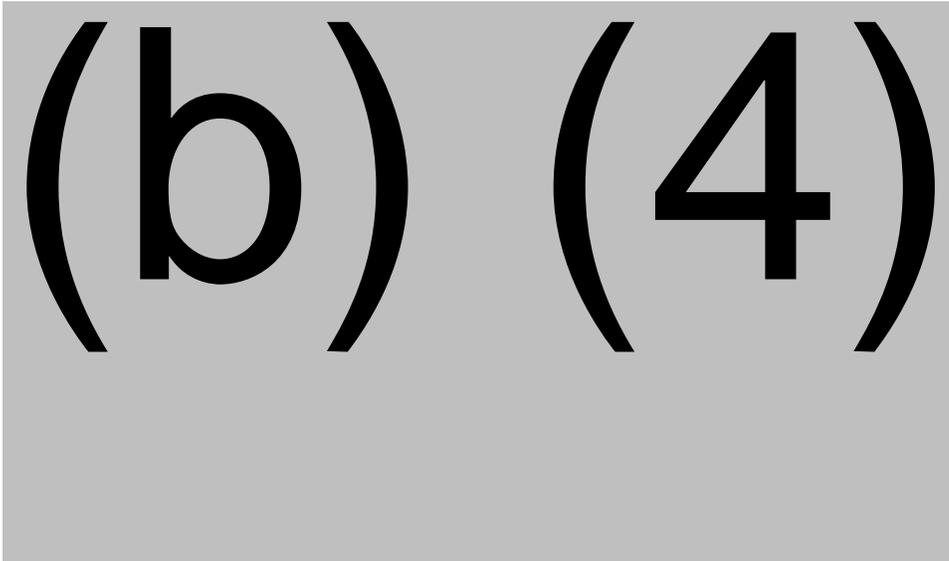
Source: Applicant. Summary of Clinical Pharmacology Studies.

### 5.1.3 BMN 270 Vector DNA Distribution in Blood

Exploratory analysis was performed to assess BMN270 vector DNA distribution in blood. Assessment of BMN 270 vector DNA distribution in blood in Study 201 has been reviewed in the review of original submission, and current review focuses on Study 301.

A (b) (4) assay was conducted to determination of the contiguity of BMN 270 vector genomes on a subset of blood and peripheral blood mononuclear cells (PBMC) samples. This method evaluates the (b) (4) of BMN 270 (b) (4) Figure 2a shows the individual profiles of the (b) (4) in PBMCs and whole blood. The transition of BMN 270 vector DNA from its initial (b) (4) over time was indicated. At earlier timepoints, PBMCs contained a similar (b) (4) as the whole blood (b) (4) period. These data suggested that, after RBCs expire, the residual vector DNA in whole blood resided as a complete transgene within the PBMC fraction.

**Figure 2. BMN 270 vector DNA in blood matrices following BMN270 administration (Study 301, 6E13 vg/kg)**



(b) (4)

Further assessment of the structural characteristics of (b) (4) transgene was performed using an (b) (4) on a subset of blood and PBMCs samples. This method assessed whether the (b) (4). The results showed that (b) (4) increased over time following BMN 270 administration. By 52 weeks post-dosing, the majority of vector DNA in whole blood (b) (4) (Figure 2b). The data indicated that the long-term form of DNA in blood was an (b) (4)

## 5.2 Pharmacodynamic Assessment

After administration, BMN270 is expected to be transduced into hepatocytes and transgene expression of FVIII is expected to occur through episomal expression. Pharmacodynamic analysis was conducted by monitoring both FVIII activity and B-domain deleted FVIII-SQ protein levels. Plasma samples for FVIII activity (chromogenic and one-stage assays) and hFVIII-SQ protein concentrations were scheduled to be collected at baseline, and on Day 8, weekly during weeks 2-36, every other week during weeks 38-52, every 4 weeks during Year 2 and every 6 weeks during Years 3-5.

FVIII activity was measured using validated chromogenic substrate assay (chromogenic (b) (4) assay, (b) (4) and one-stage clotting assay (b) (4)

. B-domain deleted FVIII-SQ protein was quantitatively measured in citrated human plasma using a validated (b) (4) assay. Recombinant B-domain deleted FVIII-SQ (ReFacto® AF drug product) was used as the calibrator and also in quality control (QC) samples.

### 5.2.1 FVIII Activity

#### 5.2.1.1 FVIII Activity – Chromogenic Assay

A total of 134 subjects enrolled in Study 301. As of the data cutoff date, 132 of 134 subjects remain on the study. One subject was lost to follow-up and one subject died by (b) (6) at Week 95. Two HIV-positive subjects on stable antiretroviral therapy (ART) regimens enrolled and were dosed in the study prior to Amendment 3, which excluded HIV-positive subjects from Study 301. Following BMN 270 administration, the two subjects continued their ART as prescribed and followed routine monitoring of CD4 count and viral load. Subject population excluding the two HIV-positive subjects was defined as mITT population.

Among the 134 subjects enrolled in Study 301, 112 subjects were rolled over from the non-interventional Study 902, in which subjects' baseline ABR and FVIII usage data were prospectively collected. These 112 subjects were defined as rollover population. Twenty-two subjects who enrolled in Study 301 did not participate Study 902. The 22 subjects were defined as directly enrolled population.

In the Phase 3 study (Study 301), FVIII activity was monitored using the chromogenic assay after administration of 6E13 vg/kg BMN270. These results were comparable between the ITT group and mITT group (Table 4). High inter-subject variability was also observed in FVIII activity profiles.

After administration of BMN 270, FVIII activity increased and reached the peak levels with the median [min, max] time of 26.0 [2.0, 111.0] weeks for both ITT population and mITT population. The mean (SD) and median [min, max] peak FVIII activity were 84.4 (81.9) IU/dL and 61.3 [4.0, 463.0] IU/dL, respectively for ITT population. The mean (SD) and median [min, max] peak FVIII activity were 85.2 (82.4) IU/dL and 61.3 [4.0, 463.0] IU/dL, respectively for mITT population.

For the rollover subject group, FVIII activity increased and reached the peak levels with the median [min, max] time of 26.4 [2.0, 111.0] weeks. The mean (SD) and median [min, max] peak FVIII activity were 88.5 (86.3) IU/dL and 61.8 [5.4, 463.0] IU/dL, respectively (Table 4, Figure 3).

In the directly enrolled population, FVIII activity increased and reached the peak levels with the median [min, max] time of 18.6 [3.9, 52.0] weeks. The mean (SD) and median [min, max] peak FVIII activity were 66.0 (52.0) IU/dL and 59.1 [4.0, 247.0] IU/dL, respectively (Table 4, Figure 3).

Based on the Applicant submitted three-year updates, at Week 104, the mean (SD) and median [min, max] peak FVIII activity were 25.0 (35.5) IU/dL (n=98) and 12.7 [0.0, 187.1] IU/dL, respectively for rollover population. At Month 36, the mean (SD) and median [min, max] peak FVIII activity were 21.4 (34.0) IU/dL (n=96) and 10.0 [0.0, 217.7] IU/dL, respectively for rollover population (Table 5).

For the directly enrolled population, based on the three-year updates, at Week 104, the mean (SD) and median [min, max] peak FVIII activity were 22.0 (28.7) IU/dL (n=19) and 8.9 [0.0, 110.6] IU/dL, respectively. At Month 36, the mean (SD) and median [min, max] peak FVIII activity were 20.8 (24.4) IU/dL (n=15) and 9.4 [0.0, 74.5] IU/dL, respectively for directly enrolled population (Table 5).

**Table 4. FVIII activity (chromogenic) PD Parameters after administration of 6E13 vg/kg BMN270 (Study 301)**

a. ITT Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ dL)	AUEC0- 104W (IU*week/ dL)	AUEC0- 156W (IU*week/ dL)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
<b>N</b>	134	134	134	134	134	130	129	19	134	134	134	130	129	19
<b>Mean</b>	29.4	84.8	392	907	2140	3080	3750	4330	24.5	34.9	41.2	39.4	36.0	27.8
<b>SD</b>	17.6	81.9	407	877	2100	3000	3810	4260	25.4	33.7	40.3	38.4	36.6	27.3
<b>Min</b>	2.00	4.00	0.00	1.93	19.2	51.7	53.8	78.0	0.00	0.0742	0.369	0.663	0.517	0.500
<b>Median</b>	26.0	61.3	261	602	1530	2090	2470	3030	16.3	23.2	29.4	26.9	23.7	19.4
<b>Max</b>	111	463	2500	4650	11300	17400	22500	18400	156	179	217	223	216	118
<b>CV%</b>	59.7	96.6	104	96.8	97.7	97.5	102	98.3	104	96.8	97.7	97.5	102	98.3

Source: Applicant. Clinical Pharmacology report for Study 301.

b. mITT Population

	tmax (week)	Emax (IU/dL)	AUEC 0-16W (IU*week/ dL)	AUEC0- 26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ dL)	AUEC0- 104W (IU*week/ dL)	AUEC0- 156W (IU*week/ dL)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
<b>N</b>	132	132	132	132	132	128	127	17	132	132	132	128	127	17
<b>Mean</b>	29.7	85.2	393	911	2160	3100	3790	4650	24.5	35.0	41.5	39.8	36.4	29.8
<b>SD</b>	17.6	82.4	409	883	2110	3010	3820	4380	25.6	33.9	40.5	38.6	36.8	28.1
<b>Min</b>	2.00	4.00	0.00	1.93	19.2	51.7	53.8	78.0	0.00	0.0742	0.369	0.663	0.517	0.500
<b>Median</b>	26.0	61.3	261	602	1530	2140	2590	3050	16.3	23.2	29.4	27.5	24.9	19.6
<b>Max</b>	111	463	2500	4650	11300	17400	22500	18400	156	179	217	223	216	118
<b>CV%</b>	59.2	96.7	104	96.9	97.5	97.1	101	94.2	104	96.9	97.5	97.1	101	94.2

Source: Applicant. Clinical Pharmacology report for Study 301.

c. Rollover Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
N	112	112	112	112	112	109	108	0	112	112	112	109	108	0
Mean	31.1	88.5	401.7	939.3	2216.9	3165.8	3848.2		25.1	36.1	42.6	40.6	37.0	-
SD	17.9	86.3	428.8	918.8	2156.0	3082.9	3914.2		26.8	35.4	41.5	39.5	37.6	-
Min	2.0	5.4	0.0	1.9	19.2	51.7	53.8		0.0	0.1	0.4	0.7	0.5	-
Median	26.4	61.8	267.5	596.0	1530.0	2190.0	2605.0		16.7	22.9	29.4	28.1	25.1	-
Max	111.0	463.0	2500.0	4650.0	11300.0	17400.0	22500.0		156.0	179.0	217.0	223.0	216.0	-
CV%	57.6	97.5	106.8	97.8	97.3	97.4	101.7		106.6	97.8	97.2	97.3	101.7	-

Source: Reviewer's analysis.

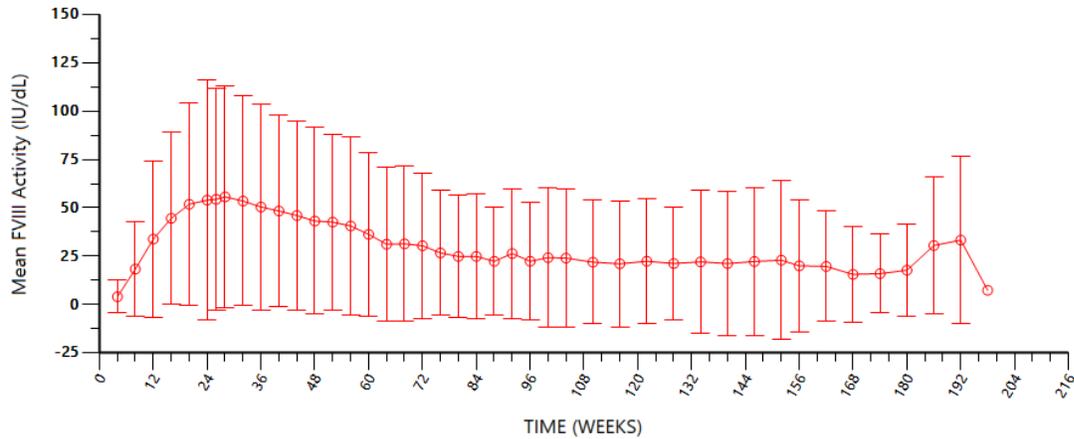
d. Directly Enrolled Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
N	22	22	22	22	22	21	21	19	22	22	22	21	21	19
Mean	20.7	66.0	344.3	739.7	1776.3	2610.7	3244.6	4331.3	21.5	28.4	34.2	33.5	31.2	27.8
SD	12.8	52.0	270.0	616.0	1756.2	2530.4	3263.1	4256.1	16.9	23.7	33.8	32.4	31.4	27.3
Min	3.9	4.0	18.0	21.4	24.5	78.0	78.0	78.0	1.1	0.8	0.5	1.0	0.8	0.5
Median	18.6	59.1	249.5	627.5	1480.0	2010.0	2340.0	3030.0	15.6	24.1	28.4	25.8	22.5	19.4
Max	52.0	247.0	1060.0	2610.0	8160.0	11800.0	15200.0	18400.0	66.2	100.0	157.0	151.0	146.0	118.0
CV%	61.7	78.8	78.4	83.3	98.9	96.9	100.6	98.3	78.4	83.2	98.9	96.8	100.5	98.3

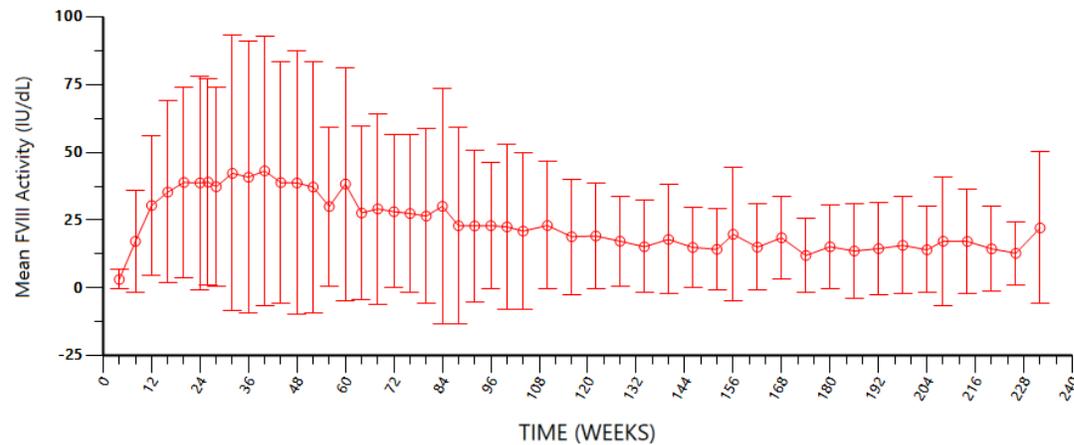
Source: Reviewer's analysis.

**Figure 3. FVIII activity (chromogenic) vs. time profiles following administration of 6E13 vg/kg BMN270 (Study 301, 3-year updates)**

a. Rollover



b. Direct Enrollment



Source: Reviewer's analysis based on Applicant's 3-year submission.

**Table 5. FVIII activity (chromogenic & one-stage) after administration of 6E13 vg/kg BMN270 (Study 301, 3-year updates)**

Timepoint	Rollover Population N = 112		Directly Enrolled Population N = 22	
	CSA	OSA	CSA	OSA
<b>Month 3</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	34.9 (40.4)	54.6 (60.8)	31.4 (25.7)	48.3 (36.0)
Median (Q1, Q3)	20.7 (10.3, 40.5)	31.3 (15.3, 71.7)	20.9 (12.6, 45.7)	36.0 (22.4, 63.9)
Min, Max	0, 249.5	1.5, 335.8	0, 85.8	4.5, 126.0
<b>Month 6</b>	N = 111	N = 111	N = 22	N = 22
Mean (SD)	55.4 (57.5)	84.9 (83.1)	40.0 (37.9)	63.0 (57.2)
Median (Q1, Q3)	38.8 (16.8, 76.5)	62.0 (28.0, 115.2)	33.2 (14.7, 46.3)	53.5 (23.7, 78.2)
Min, Max	0, 367.3	1.9, 483.9	0, 169.4	1.8, 261.9
<b>Month 10</b>	N = 111	N = 111	N = 20	N = 20
Mean (SD)	49.4 (49.5)	73.6 (70.5)	44.2 (49.6)	70.2 (70.9)
Median (Q1, Q3)	31.7 (17.1, 64.5)	51.3 (25.1, 96.2)	30.9 (14.1, 68.6)	55.4 (24.8, 101.4)
Min, Max	0, 265.3	1.2, 375.6	0, 223.6	2.4, 313.7
<b>Month 12</b>	N = 111	N = 111	N = 21	N = 21
Mean (SD)	43.6 (45.5)	64.7 (64.6)	38.2 (46.3)	59.7 (67.0)
Median (Q1, Q3)	24.0 (12.5, 63.7)	40.0 (20.4, 87.5)	23.9 (11.2, 52.8)	40.5 (17.4, 82.6)
Min, Max	0, 231.2	0, 311.1	1.6, 207.4	4.4, 294.1
<b>Month 18</b>	N = 99	N = 99	N = 18	N = 18
Mean (SD)	27.7 (32.3)	40.6 (45.9)	28.5 (28.9)	44.5 (43.9)
Median (Q1, Q3)	13.5 (6.9, 36.8)	22.5 (10.9, 55.30)	15.3 (10.8, 43.9)	24.4 (17.7, 60.4)
Min, Max	0, 167.9	0, 232.2	3.3, 117.0	4.2, 173.7
<b>Month 24</b>	N = 98	N = 99	N = 19	N = 18
Mean (SD)	25.0 (35.5)	38.9 (50.7)	22.0 (28.7)	36.0 (40.8)
Median (Q1, Q3)	12.7 (5.1, 26.5)	22.7 (7.9, 45.7)	8.9 (5.8, 25.9)	19.5 (7.9, 37.7)
Min, Max	0, 187.1	0, 271.3	0, 110.6	2.4, 146.7
<b>Month 36</b>	N = 96	N = 97	N = 15	N = 15
Mean (SD)	21.0 (34.0)	33.8 (47.6)	20.8 (24.4)	32.2 (33.1)
Median (Q1, Q3)	10.0 (4.3, 19.8)	17.7 (7.2, 35.1)	9.4 (6.6, 31.7)	20.6 (8.5, 46.7)
Min, Max	0, 217.7	0, 291.4	0, 74.5	1.9, 104.2

CSA: chromogenic assay, OSA: one-stage assay

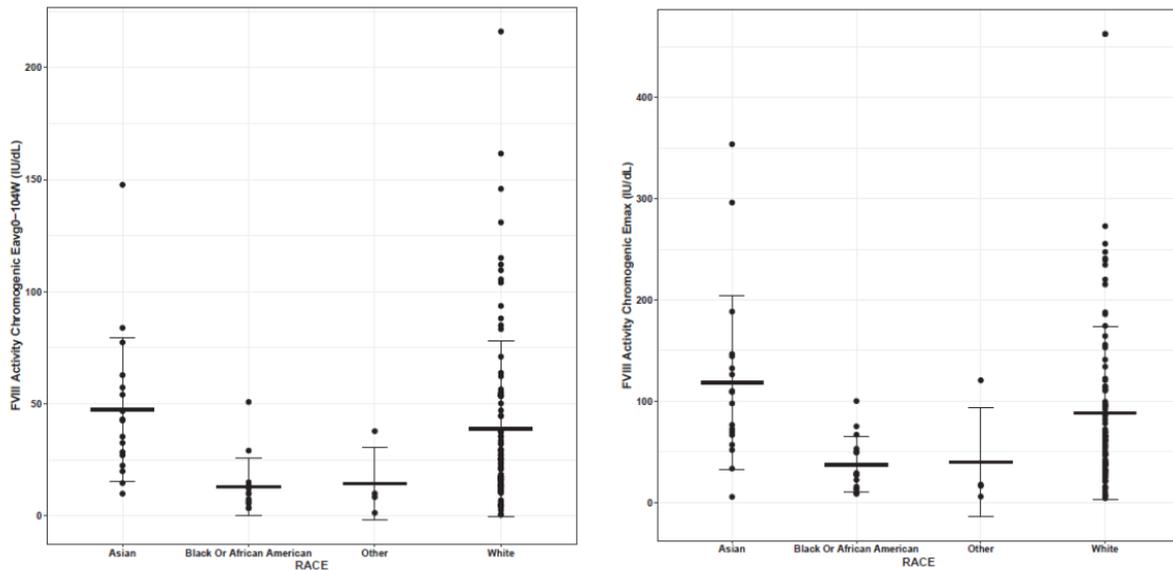
Source: Reviewer's analysis based on Applicant's 3-year submission.

**Impacting Factor(s) for BMN 270 Transgene Product (FVIII Activity by Chromogenic Assay)**

The potential effect of intrinsic and extrinsic factors on FVIII activity (chromogenic assay) was evaluated. The factors included in the evaluation were baseline age, race, bodyweight, body mass index (BMI), baseline annualized bleed rate, baseline annualized FVIII usage, history of Hepatitis B, history of Hepatitis C, region, country, site, baseline ALT, FVIII genotype, von Willebrand factor levels, duration of study drug administration, concomitant medication use (including cumulative corticosteroid dose administration and alternative immune suppressants), and baseline creatine phosphokinase. Graphical and univariate regression analysis indicated that race may be a potential impacting factor for BMN 270 transgene product FVIII activity. The mean (SD) and median [min, max] peak FVIII activity in Black or African American subjects (n=15) were 37.19 (27.49) IU/dL and 27.60 [8.40, 100.00] IU/dL, respectively. The mean (SD) and median [min,

max] peak FVIII activity in Asian, White, and other races subjects (n=119) were 90.80 (84.51) IU/dL and 65.00 [4.00, 462.60] IU/dL, respectively. Black or African American subjects (n=15) also had lower FVIII activity (Emax and Eavg0-104w) compared to other races (Figure 4). Multivariate generalized additive modeling (GAM) analysis suggested potential association between race and FVIII activity: the treatment response of Black or African American subjects was lower compared to subjects of other races (Asian, White and others). However, due to the limited sample size and multiple potential confounding factors, this is not conclusive. The results should be interpreted with caution.

**Figure 4. Race and BMN 270 Pharmacodynamic Parameters**



Source: Applicant. Clinical Pharmacology report for Study 301.

### 5.2.1.2 FVIII Activity – One-Stage Assay

After administration of 6E13 vg/kg BMN270 in Study 301, FVIII activity was also measured using one-stage assay (Table 6). The results were similar between ITT and mITT populations.

After administration of BMN 270, FVIII activity increased and reached the peak levels with the median [min, max] time of 25.1 [3.9, 111.0] weeks and 25.2 [3.9, 111.0] weeks for ITT population and mITT population, respectively. The mean (SD) and median [min, max] peak FVIII activity were 124.0 (105.0) IU/dL and 92.7 [6.0, 500.0] IU/dL, respectively for ITT population. The mean (SD) and median [min, max] peak FVIII activity were 124.0 (105.0) IU/dL and 92.7 [6.0, 500.0] IU/dL, respectively for mITT population.

For the rollover subject group, FVIII activity increased and reached the peak levels with the median [min, max] time of 26.0 [2.1, 111.0] weeks. The mean (SD) and median [min, max] peak FVIII activity were 127.7 (107.9) IU/dL and 93.1 [6.4, 500.0] IU/dL, respectively (Table 6).

In the directly enrolled population, FVIII activity increased and reached the peak levels with the median [min, max] time of 16.5 [3.9, 47.5] weeks. The mean (SD) and median [min, max] peak FVIII activity were 106.2 (86.6) IU/dL and 91.8 [6.0, 399.0] IU/dL, respectively (Table 6).

Based on the Applicant submitted three-year updates, at Week 104, the mean (SD) and median [min, max] peak FVIII activity were 38.9.0 (50.7) IU/dL (n=99) and 22.7 [0.0, 271.3] IU/dL, respectively for rollover population. At Month 36, the mean (SD) and median [min, max] peak FVIII activity were 33.8 (47.6) IU/dL (n=96) and 17.7 [0.0, 291.4] IU/dL, respectively for rollover population (Table 5).

For the directly enrolled population, based on the three-year updates, at Week 104, the mean (SD) and median [min, max] peak FVIII activity were 36.0 (40.8) IU/dL (n=18) and 19.5 [2.4, 146.7] IU/dL, respectively. At Month 36, the mean (SD) and median [min, max] peak FVIII activity were 32.2 (33.8) IU/dL (n=15) and 20.6 [1.9, 104.2] IU/dL, respectively for directly enrolled population (Table 5).

#### Reviewer's Comment:

Similar to the observation in the original submission, FVIII activity levels measured using the one-stage assay were markedly higher than FVIII activity levels using the chromogenic assay. The applicant conducted an additional study to investigate this observation. Results indicate the difference is due to the difference in (b) (4) : one-stage assay utilizes (b) (4) , while chromogenic assay (b) (4) BMN270-produced hFVIII-SQ demonstrated higher activity than normal plasma during early stages of the (b) (4) reaction in the OS assay. Therefore, FVIII activity measured by chromogenic assay was used in clinical benefit prediction.

**Table 6. FVIII Activity (One-Stage) PD Parameters following administration of 6E13 vg/kg BMN270 (Study 301)**

a. ITT Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
<b>N</b>	134	134	134	134	134	130	129	19	134	134	134	130	129	19
<b>Mean</b>	28.8	124	615	1410	3280	4670	5700	6730	38.4	54.3	63.0	59.9	54.8	43.1
<b>SD</b>	18.0	105	610	1310	3050	4340	5490	6310	38.1	50.3	58.6	55.7	52.8	40.5
<b>Min</b>	3.88	6.00	29.2	61.0	104	178	226	263	1.83	2.35	2.00	2.28	2.17	1.68
<b>Median</b>	25.1	92.7	420	980	2370	3350	4030	4820	26.3	37.7	45.5	42.9	38.7	30.9
<b>Max</b>	111	500	3430	6500	15200	23800	31000	27200	215	250	293	305	298	174
<b>CV%</b>	62.4	84.3	99.2	92.5	93.0	92.9	96.4	93.7	99.2	92.5	93.0	92.9	96.4	93.7

Source: Applicant. Clinical Pharmacology report for Study 301.

b. mITT Population

	tmax (week)	Emax (IU/dL)	AUEC 0-16W (IU*week/ dL)	AUEC0- 26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
<b>N</b>	132	132	132	132	132	128	127	17	132	132	132	128	127	17
<b>Mean</b>	29.1	125	615	1420	3300	4710	5750	7210	38.4	54.6	63.5	60.4	55.3	46.2
<b>SD</b>	18.0	105	614	1320	3060	4360	5520	6490	38.4	50.6	58.9	56.0	53.0	41.6
<b>Min</b>	3.88	6.00	29.2	61.0	104	178	226	263	1.83	2.35	2.00	2.28	2.17	1.68
<b>Median</b>	25.2	92.7	420	980	2370	3370	4050	4920	26.3	37.7	45.5	43.3	38.9	31.5
<b>Max</b>	111	500	3430	6500	15200	23800	31000	27200	215	250	293	305	298	174
<b>CV%</b>	61.9	84.5	99.8	92.7	92.9	92.6	95.9	90.0	99.8	92.7	92.9	92.6	95.9	90.0

Source: Applicant. Clinical Pharmacology report for Study 301.

c. Rollover Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
N	112	112	112	112	112	109	108	0	112	112	112	109	108	0
Mean	30.4	127.7	627.8	1454.7	3363.5	4778.9	5819.4		39.2	56.0	64.7	61.3	55.9	-
SD	18.4	107.9	644.0	1366.3	3120.2	4439.0	5618.6		40.2	52.6	60.0	56.9	54.0	-
Min	7.1	6.4	29.2	61.0	104.0	178.0	226.0		1.8	2.4	2.0	2.3	2.2	-
Median	26.0	93.1	422.5	961.0	2370.0	3350.0	4020.0		26.4	37.0	45.6	43.0	38.7	-
Max	111.0	500.0	3430.0	6500.0	15200.0	23800.0	31000.0		215.0	250.0	293.0	305.0	298.0	-
CV%	60.4	84.5	102.6	93.9	92.8	92.9	96.5		102.6	93.9	92.8	92.9	96.5	-

Source: Reviewer's analysis.

d. Directly Enrolled Population

	tmax (week)	Emax (IU/dL)	AUEC0 -16W (IU*week/ dL)	AUEC0 -26W (IU*week/ dL)	AUEC0 -52W (IU*week/ dL)	AUEC0- 78W (IU*week/ d L)	AUEC0- 104W (IU*week/ d L)	AUEC0- 156W (IU*week/ d L)	Eavg0- 16W (IU/dL)	Eavg0- 26W (IU/dL)	Eavg0- 52W (IU/dL)	Eavg0- 78W (IU/dL)	Eavg0- 104W (IU/dL)	Eavg0- 156W (IU/dL)
N	22	22	22	22	22	21	21	19	22	22	22	21	21	19
Mean	20.5	106.2	550.4	1200.0	2842.1	4127.7	5081.0	6733.8	34.4	46.2	54.6	53.0	48.9	43.1
SD	13.3	86.6	399.9	951.8	2681.6	3852.8	4873.4	6315.7	25.0	36.7	51.7	49.5	46.8	40.4
Min	3.9	6.0	66.4	97.9	170.0	242.0	256.0	263.0	4.2	3.8	3.3	3.1	2.5	1.7
Median	16.5	91.8	403.5	1075.0	2440.0	3340.0	4030.0	4820.0	25.3	41.3	46.9	42.8	38.7	30.9
Max	47.5	399.0	1550.0	4200.0	12500.0	17900.0	22600.0	27200.0	97.2	162.0	241.0	230.0	217.0	174.0
CV%	64.7	81.5	72.7	79.3	94.4	93.3	95.9	93.8	72.7	79.4	94.5	93.5	95.8	93.7

Source: Reviewer's analysis.

### 5.2.1.3 hFVIII-SQ Protein

Following administration of 6E13 vg/kg BMN270 in Study 301, hFVIII-SQ concentration-time profiles were similar between ITT and mITT populations in Study 301 (Table 7). The peak levels of hFVIII-SQ were reached with a median [min, max] time of 10.0 [0.7, 47.5] weeks for both for ITT and mITT populations, respectively. The median Tmax of hFVIII-SQ was less than the Tmax of FVIII activity (26.4 and 25.1 weeks for chromogenic and one-stage assays of ITT population respectively). This may be due to 1) different sample size, 2) many subjects did not have hFVIII-SQ for longer time period, and 3) high inter-subject variability in Tmax.

The mean (SD) and median [min, max] peak hFVIII-SQ concentrations were 44.7 (50.8) IU/dL and 32.3 [0.0, 332.0] IU/dL, respectively for ITT population. The mean (SD) and median [min, max] peak hFVIII-SQ concentrations were 43.9 (51.1) IU/dL and 29.5 [0.0, 332.0] IU/dL, respectively for mITT population.

For the rollover subject group, hFVIII-SQ concentrations increased and reached the peak levels with the median [min, max] time of 6.0 [0.7, 30.1] weeks. The mean (SD) and median [min, max] peak hFVIII-SQ concentrations were 35.6 (41.2) ng/mL and 20.2 [0.0, 221.0] ng/mL, respectively (Table 7).

In the directly enrolled population, hFVIII-SQ concentrations increased and reached the peak levels with the median [min, max] time of 20.6 [1.0, 47.5] weeks. The mean (SD) and median [min, max] peak hFVIII-SQ concentrations were 69.8 (65.8) ng/mL and 58.6 [0.0, 332.0] ng/mL, respectively (Table 7).

**Table 7. hFVIII-SQ Concentration (b) (4) following administration of 6E13 vg/kg BMN270 (Study 301)**

a. ITT Population

	tmax (week)	Emax (ng/mL)	AUEC 0-16W (ng*week/ mL)	AUEC 0-26W (ng*week/ mL)	AUEC 0-52W (ng*week/ k/mL)	AUEC 0-78W (ng*week/ mL)	Eavg 0-16W (ng/mL)	Eavg 0-26W (ng/mL)	Eavg 0-52W (ng/mL)	Eavg 0-78W (ng/mL)
<b>N</b>	83	83	38	29	16	2	38	29	16	2
<b>Mean</b>	11.9	44.7	335	684	1220	1660	20.9	26.3	23.5	21.3
<b>SD</b>	11.6	50.8	316	679	1060	1830	19.7	26.1	20.4	23.5
<b>Min</b>	0.713	0.00	0.00	0.00	0.00	367	0.00	0.00	0.00	4.71
<b>Median</b>	10.0	32.3	254	524	988	1660	15.9	20.2	19.0	21.3
<b>Max</b>	47.5	332	1470	3520	3320	2960	91.8	135	63.9	37.9
<b>CV%</b>	98.0	114	94.3	99.4	86.4	110	94.3	99.4	86.4	110

Source: Applicant. Clinical Pharmacology report for Study 301.

b. mITT Population

	tmax (week)	Emax (ng/mL)	AUEC 0-16W (ng*week/ mL)	AUEC 0-26W (ng*week/ mL)	AUEC 0-52W (ng*week/ k/mL)	AUEC 0-78W (ng*week/ mL)	Eavg 0-16W (ng/mL)	Eavg 0-26W (ng/mL)	Eavg 0-52W (ng/mL)	Eavg 0-78W (ng/mL)
<b>N</b>	81	81	36	27	14	2	36	27	14	2
<b>Mean</b>	11.6	43.9	336	699	1280	1660	21.0	26.9	24.6	21.3
<b>SD</b>	11.2	51.1	325	701	1110	1830	20.3	27.0	21.3	23.5
<b>Min</b>	0.713	0.00	0.00	0.00	0.00	367	0.00	0.00	0.00	4.71
<b>Median</b>	10.0	29.5	247	524	988	1660	15.4	20.2	19.0	21.3
<b>Max</b>	47.5	332	1470	3520	3320	2960	91.8	135	63.9	37.9
<b>CV%</b>	96.4	116	96.4	100	86.8	110	96.4	100	86.8	110

Source: Applicant. Clinical Pharmacology report for Study 301.

c. Rollover Population

	tmax (week)	Emax (ng/mL)	AUEC 0-16W (ng*week/ mL)	AUEC 0-26W (ng*week/ mL)	AUEC 0-52W (ng*wee k/mL)	AUEC 0-78W (ng*week/ mL)	Eavg 0-16W (ng/mL)	Eavg 0-26W (ng/mL)	Eavg 0-52W (ng/mL)	Eavg 0-78W (ng/mL)
<b>N</b>	61	61	16	7	0	0	16	7	0	0
<b>Mean</b>	8.5	35.6	363.7	722.4	-	-	22.7	27.8	-	-
<b>SD</b>	8.1	41.2	309.8	467.6	-	-	19.3	18.0	-	-
<b>Min</b>	0.7	0.0	4.8	140.0	-	-	0.3	5.4	-	-
<b>Median</b>	6.0	20.2	269.5	805.0	-	-	16.9	31.0	-	-
<b>Max</b>	30.1	221.0	1130.0	1420.0	-	-	70.5	54.7	-	-
<b>CV%</b>	96.0	115.8	85.2	64.7	-	-	85.1	64.8	-	-

Source: Reviewer's Analysis.

d. Directly Enrolled Population

	tmax (week)	Emax (ng/mL)	AUEC 0-16W (ng*week/ mL)	AUEC 0-26W (ng*week/ mL)	AUEC 0-52W (ng*wee k/mL)	AUEC 0-78W (ng*week/ mL)	Eavg 0-16W (ng/mL)	Eavg 0-26W (ng/mL)	Eavg 0-52W (ng/mL)	Eavg 0-78W (ng/mL)
<b>N</b>	22	22	22	22	16	2	22	22	16	2
<b>Mean</b>	21.3	69.8	314.1	670.9	1223.4	1663.5	19.6	25.8	23.5	21.3
<b>SD</b>	14.6	65.8	326.2	743.6	1057.9	1833.5	20.4	28.5	20.4	23.5
<b>Min</b>	1.0	0.0	0.0	0.0	0.0	367.0	0.0	0.0	0.0	4.7
<b>Median</b>	20.6	58.6	235.5	524.0	986.5	1663.5	14.7	20.2	19.0	21.3
<b>Max</b>	47.5	332.0	1470.0	3520.0	3320.0	2960.0	91.8	135.0	63.9	37.9
<b>CV%</b>	68.4	94.2	103.8	110.8	86.5	110.2	103.8	110.6	86.5	110.2

Source: Reviewer's Analysis.

#### 5.2.1.4 hFVIII-SQ Specific Activity

To assess the relationship between FVIII activity and hFVIII-SQ protein levels, FVIII activity was normalized to protein mass as specific activity of BMN270-derived hFVIII-SQ. Xyntha<sup>®</sup>/Refacto<sup>®</sup>, a recombinant B-domain-deleted (BDD) FVIII-SQ protein approved for FVIII replacement therapy was used as reference for specific activity of assessment.

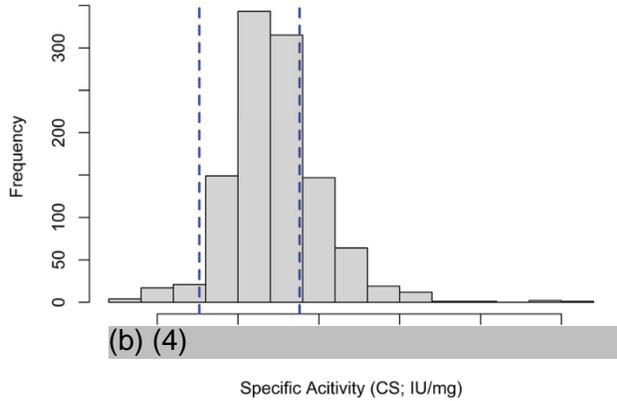
Specific activity of BMN270 transgene product as measured by the chromogenic assay was comparable to that that of Refacto<sup>®</sup> (Figure 5a). Specific activity of BMN270 transgene product as measured by the one-stage assay was remarkably higher than that of Xyntha<sup>®</sup> (Figure 5b). The applicant conducted additional studies for the one-stage assay and found an accelerated rate of early (b) (4) formation for BMN270 transgene-produced hFVIII-SQ compared to native FVIII and recombinant BDD FVIII-SQ. This may explain the higher one-stage FVIII activity as this assay utilizes (b) (4), while the chromogenic assay (b) (4). These observations were noted and discussed during a meeting in drug development. Both FDA reviewers and the applicant agreed that BMN270-produced hFVIII-SQ demonstrated functional differences in comparison with both the normal pooled plasma and the commercial FVIII-SQ concentrate (Xyntha<sup>®</sup>/Refacto<sup>®</sup>). The hemostatic effect of BMN-270-produced FVIII-SQ cannot be directly predicted from the clinical investigations of the plasma-derived FVIII or FVIII-SQ concentrates.

Figure 6 shows the specific activity of BMN 270 transgene product over time following BMN 270 administration. The result is consistent with the activity comparison with Refacto<sup>®</sup>/Xyntha<sup>®</sup>.

Comparison between specific activity to FVIII activity and hFVIII-SQ levels indicated that higher variabilities of BMN270 transgene product specific activity were observed at lower FVIII activity and hFVIII-SQ levels.

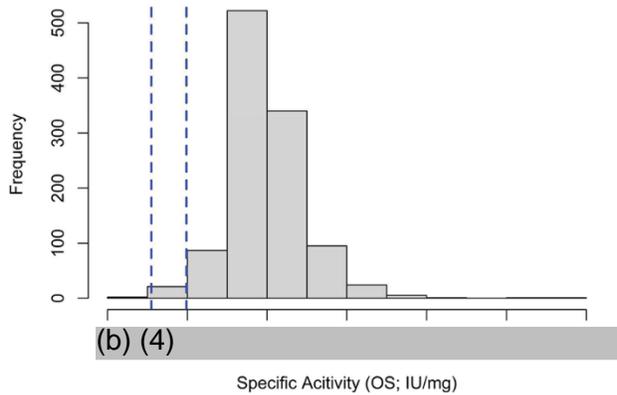
**Figure 5. Histogram of specific Activity of BMN270 transgene product (Study 301, ITT population)**

a. Histogram of specific activity compared to (b) (4) Range – Chromogenic Assay



Blue dashed line is the specific activity specification range (b) (4) IU/mg reported for ReFacto®

b. Histogram of specific activity compared to Xyntha® Range – One-Stage Assay

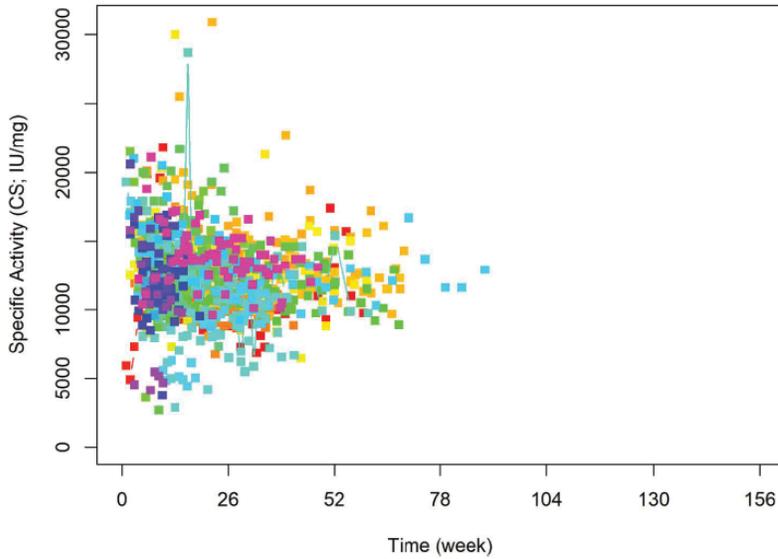


Blue dashed line is the specific activity specification range (b) (4) IU/mg reported for Xyntha®

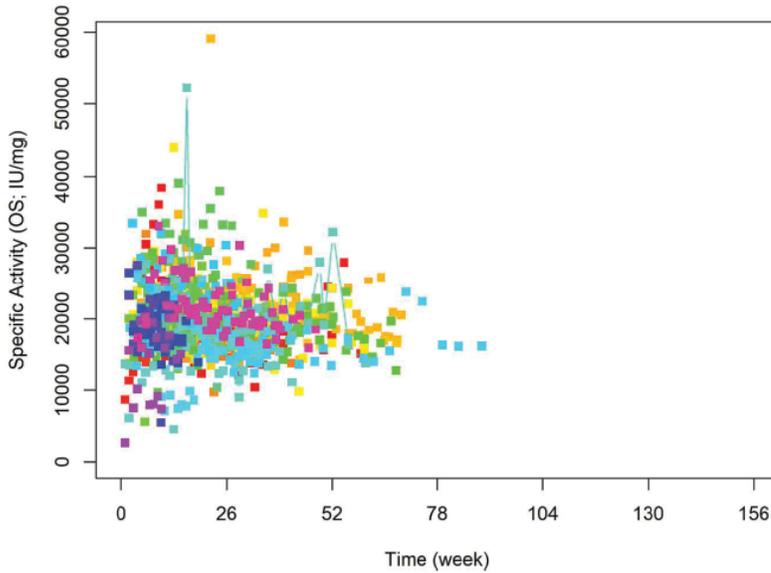
Source: Applicant. Clinical Pharmacology Report for Study 301.

**Figure 6. Specific activity of BMN270 transgene product over time after BMN 270 administration for individual subject (Study 301, ITT population)**

a. Chromogenic Assay



b. One-stage Assay



Source: Applicant. Clinical Pharmacology Report for Study 301.

### 5.3 Drug-Drug Interactions

The Applicant evaluated the potential impact of concomitant drugs on hepatotoxicity and/or BMN 270 expression. An in vitro primary human hepatocyte model was used to assess the effects of concomitant administration of isotretinoin, amphetamine, omeprazole, celecoxib, and selected

highly active antiretroviral therapy (HAART) medications with BMN 270 on cytotoxicity and BMN 270 DNA and RNA expression. The results are summarized in Table 8.

#### Effects of Isotretinoin on BMN 270 Expression and Cytotoxicity

At the two concentrations (5 and 500 ng/mL, corresponding to 0.01x to 1x of reported C<sub>max</sub>) evaluated, isotretinoin had no direct effect on BMN 270 DNA and cytotoxicity. Isotretinoin suppressed BMN 270 transcription 1.7- to 2.7-fold at the concentration of 5 and 500 ng/mL, respectively. After removal of isotretinoin treatment, the suppression was partially reversed.

#### Effects of Amphetamine, Omeprazole and Celecoxib on BMN 270 Expression and Cytotoxicity

There was no cytotoxicity for the combinations of BMN 270 and any of amphetamine, omeprazole and celecoxib.

Omeprazole and celecoxib, but not amphetamine, dose-dependently reduced FVIII-SQ transcript. Significant reduction of FVIII-SQ transcript was observed at very high doses for both omeprazole and celecoxib, which may not be possible to achieve in the clinic. Therefore, no clinically relevant impact of co-administration of amphetamine, omeprazole and celecoxib on BMN 270 expression.

**Table 8. Summary of In Vitro Drug-Drug Interaction (DDI) Results**

<b>Drug</b>	<b>Fold C<sub>max</sub>**</b>	<b>Concentration</b>	<b>Cytotoxicity (Fold-increase vs vehicle)</b>	<b>Effect on BMN 270 Transduction (fold -increase vs. vehicle)</b>	<b>Effect on BMN 270 Expression (fold -decrease vs vehicle)</b>	<b>Effect on BMN 270 Expression following Drug Withdrawal (fold -decrease vs vehicle)</b>
<b>Accutane (Isotretinoin)</b>	1x	500 ng/mL	1.15	1.13	2.7*	1.53*
	0.01x	5 ng/mL	1.15	1.10	1.65*	1.25
<b>Levo-Amphetamine</b>	20x	640 ng/mL	0.87	0.73	1.18	NT
	5x	160 ng/mL	1.04	NT	NT	
	1x	32 ng/mL	0.75	1.03	1.25	
	0.2x	6.4 ng/mL	0.63	NT	NT	
<b>Lamivudine</b>	20x	28 µg/mL	1.31	1.35	0.20	NT
	5x	7 µg/mL	1.38	NT	NT	
	1x	1.4 µg/mL	1.32	0.81	1.22	
	0.2x	0.28 µg/mL	1.12	NT	NT	
<b>Omeprazole</b>	20x	30 µg/mL	1.02	0.83	1.97 *	2.35 *
	5x	7.5 µg/mL	1.01	1.30	1.24	1.44 *
	1x	1.5 µg/mL	1.03	0.72	0.87	0.8
	0.2x	0.3 µg/mL	1.03	NT	NT	NT
<b>Celecoxib</b>	20x	14 µg/mL	1.37	1.18	2.95 *	1.11
	5x	3.5 µg/mL	1.10	0.79	1.47 *	0.77
	1x	0.7 µg/mL	0.97	1.26	1.17	0.88
	0.2x	0.14 µg/mL	1.07	NT	NT	NT

<b>Drug</b>	<b>Fold Cmax**</b>	<b>Concentration</b>	<b>Cytotoxicity (Fold-increase vs vehicle)</b>	<b>Effect on BMN 270 Transduction (fold -increase vs. vehicle)</b>	<b>Effect on BMN 270 Expression (fold -decrease vs vehicle)</b>	<b>Effect on BMN 270 Expression following Drug Withdrawal (fold -decrease vs vehicle)</b>
<b>Efavirenz</b>	20x	81.44 µg/mL	38.78 *	Toxic	Toxic	Toxic
	5x	20.36 µg/mL	30.45 *	Toxic	Toxic	Toxic
	1x	4.1 µg/mL	1.72	1.24	6.24*	4.09*
	0.5x	2.05 µg/mL	NT	1.32	3.29*	2.35*
	0.2x	0.82 µg/mL	1.68	NT	NT	NT
	0.1x	0.41 µg/mL	NT	1.60	1.10	1.21
<b>Tenofovir Disproxil Fumarate</b>	20x	59.20 µg/mL	1.07	1.01	1.17	NT
	5x	14.80 µg/mL	1.24	1.03	0.73	
	1x	2.96 µg/mL	1.24	1.76*	0.37*	
	0.2x	0.59 µg/mL	1.51	NT	NT	
<b>Tenofovir Alafenamide Fumarate</b>	20x	43 µg/mL	1.16	0.87	2.31	NT
	5x	10.75 µg/mL	0.90	0.74	1.29	
	1x	2.15 µg/mL	0.94	1.23	0.55*	
	0.2x	0.43 µg/mL	1.04	NT	NT	
<b>Raltegravir</b>	20x	1396 µg/mL	1.36	1.67*	2.35*	NT
	5x	349 µg/mL	1.17	1.54*	1.27	
	1x	69.8 µg/mL	1.19	1.29	1.47	
	0.2x	13.96 µg/mL	1.26	NT	NT	
<b>Bictegravir</b>	20x	1230.00 µg/mL	4.80*	3.41*	4.56*	NT
	5x	307.50 µg/mL	1.52	3.35*	2.77*	

<b>Drug</b>	<b>Fold C<sub>max</sub>**</b>	<b>Concentration</b>	<b>Cytotoxicity (Fold-increase vs vehicle)</b>	<b>Effect on BMN 270 Transduction (fold -increase vs. vehicle)</b>	<b>Effect on BMN 270 Expression (fold -decrease vs vehicle)</b>	<b>Effect on BMN 270 Expression following Drug Withdrawal (fold -decrease vs vehicle)</b>
	1x	61.50 µg/mL	1.12	1.57	0.88	
	0.2x	12.30 µg/mL	1.03	NT	NT	
<b>Dolutegravir</b>	20x	782.00 µg/mL	4.96*	1.85*	1.11	NT
	5x	195.50 µg/mL	1.05	3.74*	0.80	
	1x	39.10 µg/mL	0.99	3.03*	0.96	
	0.2x	7.82 µg/mL	1.05	NT	NT	
<b>Darunavir</b>	20x	1388.00 µg/mL	1.34	0.89	6.87*	NT
	5x	347.00 µg/mL	1.49	0.79	1.24	
	1x	69.40 µg/mL	1.28	1.13	1	
	0.2x	13.88 µg/mL	1.19	NT	NT	

\*statistically significant NT=not tested; \*\*various concentrations including mean C<sub>max</sub> (1x) at label suggested doses were evaluated

Source: Applicant.

### Effects of HAART Medications on BMN 270 Expression and Cytotoxicity

The effects of selected HAART medications (tenofovir, lamivudine, raltegravir, dolutegravir, bictegravir, darunavir, and efavirenz) on BMN 270 expression and synergistic cytotoxicity were evaluated.

Concomitant treatment with BMN 270 did not increase cytotoxicity of above selected HAART medications, except for bictegravir and dolutegravir. At high dose concentrations corresponding to 20 times C<sub>max</sub> (at label suggested doses), co-treatment of BMN 270 with bictegravir or dolutegravir showed cytotoxicity. Considering that the high concentrations are not possibly achieved in the clinic, there is observation may not be clinically relevant.

At 1x reported C<sub>max</sub> levels (clinically achievable concentrations), both Dolutegravir and tenofovir increased AAV5-hFVIII-SQ transcription. This observation is not expected to compromise the efficacy of BMN 270.

Treatment of efavirenz had a dose-dependent decrease in AAV5-hFVIII-SQ transcription at both 0.5x and 1x reported C<sub>max</sub>. Drug removal for 72 hours did not restore FVIII-SQ RNA levels. Considering the concentrations of efavirenz evaluated are clinically achievable, this observation may be clinically relevant for BMN 270 treatment.

## **5.4 Immunogenicity Assessment**

Plasma and PBMC samples were collected at screening and/or baseline and at regular intervals post dosing to evaluate humoral and cellular immune responses against BMN 270:

- Humoral immune responses: AAV5 total antibody (TA<sub>b</sub>), AAV5 transduction inhibition, FVIII TA<sub>b</sub>, and FVIII neutralizing antibodies (inhibitors)
- Cellular immune responses: cellular responses against AAV5 capsid and hFVIII-SQ

### **5.4.1 AAV5 Total Antibody (anti-AAV5 TA<sub>b</sub>)**

AAV5 TA<sub>b</sub> was measured using a validated (b) (4) assay.

#### AAV5 TA<sub>b</sub> Screening Assay

In Study 201, subjects were screened and excluded from enrollment based on testing results for two different assays used to assess pre-existing AAV5 immunity: anti-AAV5 TA<sub>b</sub> and AAV5 transduction inhibition. The results of Study 901 (Study 270-901, a global prospective laboratory study for AAV seroprevalence in hemophilia A patients previously treated with Factor VIII concentrations) showed that about 29.7% of hemophilia A patients have preexisting antibodies against the AAV5 capsid. A nonclinical study in (b) (4) monkeys showed that animals with detectable AAV5 TA<sub>b</sub> inhibited efficacy and pharmacodynamic effect following administration

of BMN 270. Therefore, the Applicant determined that identification of AAV5 TAb was an appropriate screening assessment for selection of subjects for BMN 270 clinical studies. For subsequent clinical studies (203, 301, 302, and 303), the enrollment exclusion criteria for pre-existing AAV5 TI were removed and the AAV5 TAb screening assay was selected for further development as the companion diagnostic assay.

In Study 201, all treated subjects were negative at Baseline and seroconverted from negative at baseline to positive at the first post-dosing timepoint (Week 8) assessed. There was no apparent dose relationship of BMN270 and post-dosing anti-AAV5 TAb titer. Anti-AAV5 TAb titer peaked at Week 40 with an overall mean (SD) of 8,791,437 (10,567,127). Following the peak, anti-AAV5 TAb titers remained stable with a no meaningful reduction in titer through Week 156 with a mean (SD) of 7,812,166 (11,023,806). There was no association of the anti-AAV5 TAb titer at Week 8, nor the maximal anti-AAV5 TAb titer with median FVIII activity measures at Week 23-26. There was no association of anti-AAV5 TAb titer at Week 8 nor maximal anti-AAV5 TAb titer with peak ALT measures. There was no association identified between anti-AAV5 TAb titers and the safety and efficacy after BMN 270 treatment.

In Study 301, three subjects screened negative but developed detectable AAV5 antibodies between screening and Day 1 just prior to dose administration. The peak FVIII activity levels measured by chromogenic assay were 12.85 IU/dL (Week 32), 29.2 IU/dL (Week 16), and 13.3 IU/dL (Week 68), respectively. The median FVIII activity levels were 4.8, 4.8, and 5.4 IU/dL at Week 104 post-dosing. Because of the small sample size, there is no definitive conclusion regarding impact of pre-existing anti-AAV5 antibodies on efficacy. The safety profile for these subjects was no different from that seen in other subjects; the most commonly reported AEs in these three subjects included non-serious Grade 1 ALT elevations with a time to onset between 6-10 weeks. Two of the 3 subjects were given prednisolone for ALT elevation, while 1 of the 3 subjects took Tacrolimus for ALT elevation due to a medical history of diabetes mellitus. No SAEs were reported for any of the subjects.

Following administration of BMN270, all subjects developed anti-AAV5 TAb from the first assessment time point (8-week post dosing). Anti-AAV5 concentrations increased during the study and reached the peak level around 36 - 40 weeks post dosing and were measurable as of the data cutoff dates. There was no apparent dose-response relationship between BMN270 and AAV5TAb. AAV5 TAb at week 8 and peak levels do not appear to be associated with FVIII activity or ALT levels. There was no clear, definitive association between AAV5TAb titers and the efficacy and safety of BMN270.

#### **Reviewer's Comments:**

Three subjects developed anti-AAV5 antibodies prior to administration of BMN 270. The peak FVIII activity levels after dosing of BMN 270 were less than 30 IU/dL. Because of the small

sample size, there is no definitive conclusion regarding impact of pre-existing anti-AAV5 antibodies on efficacy. The Applicant is conducting a study (Study 270-203) to evaluate the safety and efficacy of BMN 270 in subjects with pre-existing AAV5 TAb.

#### **5.4.2 AAV5 Transduction Inhibition**

AAV5 transduction inhibition (TI) was measured using a cell-based assay.

As of the data cutoff dates, 12 of 148 (8.1%) subjects in the safety population across studies (one in Study 203 and 11 in Study 301) screened and confirmed positive in the TI assay at Baseline. All subjects with reported TI data post-dosing (270-203, 270-301 and 270-302), regardless of Baseline results, seroconverted to AAV5 TI titer positive by Week 8 (n=126). Mean AAV5 TI titers peaked by Week 36 at 216,236 (n=108) and were sustained over time through Week 104 in all subjects with reported data.

The 11 subjects testing positive at Baseline in the AAV5 TI assay had median FVIII activity measures using the chromogenic assay from Week 104 ranging from 1.6 IU/dL to 78.25 IU/dL. The median FVIII activity at Week 104 in the 11 baseline AAV5 TI positive subjects overlapped that in 119 baseline AAV5 TI negative subjects. No clear association could be established between Baseline AAV5 TI positivity or baseline titers and median FVIII activity measures at Week 104 show overlapping median FVIII activity at Week 104.

#### **5.4.3 FVIII Total Binding Antibody**

FVIII TAb was measured using a validated (b) (4) assay. As of the data cutoff date, 11 of 170 (7.3%) subjects (one in Study 201 and 10 in Study 301) across studies tested positive at one or more time points for FVIII TAb. Many of these reported FVIII TAb titers are at or near the MRD of the assay with some occurring prior to dosing (Screening or Baseline). At each time point for which there were positive FVIII TAb results, corresponding results in the Nijmegen-modified Bethesda assay for FVIII inhibitors were negative. There was no apparent association of FVIII TAb positive results or titer levels with either ALT or FVIII activity measures.

#### **5.4.4 FVIII Neutralizing Antibody (Inhibitors)**

FVIII neutralizing antibody (inhibitors) were measured using Nijmegen modified Bethesda assay with a series sampling time point. As of the data cutoff date, all subjects except 4 subjects in Study 301 tested negative for FVIII inhibitors (below 0.6 BU) at all time points monitored following administration of BMN 270.

In Study 301, 4 subjects had a single (transient) positive Bethesda assay result (>0.6 BU). One subject had a positive result occur during Screening and prior to dose administration (Day -70). The other 3 subjects all had a single positive result that was both preceded and followed by a series of negative inhibitor results.

#### **5.4.5 Cellular Immune Responses**

Detection of cellular immune responses against AAV5 and hFVIII-SQ in human peripheral blood mononuclear cells (PBMCs) was performed using an IFN- $\gamma$  (b) (4) Assay (Study 201) or a validated (b) (4) IFN- $\gamma$  ELISpot assay (Studies 203, 301, 302, & 303). Because a different assay was used in Study 201, this review focuses on results of Study 301 using the IFN- $\gamma$  ELISpot assay.

##### **5.4.5.1 AAV5 Capsid Specific Cellular Immunity**

As of the data cutoff of this report, AAV5 capsid specific ELISpot data was available for 124 of 134 ITT subjects in Study 301, with 115 of 124 (92.7%) testing positive at one or more time points assessed through a maximum of 140 weeks of follow up. Positive responses following stimulation with AAV5 capsid peptides were detected in only 5 of 95 (5.3%) baseline samples tested, however the majority of subjects with available results (67 of 96 [69.8%]) tested positive at Week 2 post-dosing. The incidence of positive responses declined to 17 of 74 (23.0%) tested samples at Week 26, and 9 of 52 (17.3%) at Week 52. Overall, most positive responses were detected as early as two weeks following dose administration and diminished over the following time point assessments, eventually reverting to negative in the majority of subjects tested.

Correlation analysis was conducted evaluating cumulative ELISpot results over the first 52 weeks after BMN 270 administration and a cumulative measure of ALT levels over the same duration, the results were not statistically significant ( $r^2=0.0365$ ,  $p=0.147$ ).

Because of varied temporal relationship between cellular responses, use of corticosteroids in some subjects, no clear relationship was established between AAV5 capsid specific cellular immune responses and safety and efficacy of BMN 270.

No association was observed between capsid specific cellular immune responses and FVIII activity levels (chromogenic assay).

#### **5.4.5.2 hFVIII-SQ Specific Cellular Immunity**

As of the data cutoff date, ELISpot data were available for 125 subjects in Studies 203 (n=1), 301 (n=123) and 302 (n=1). Positive responses following stimulation with FVIII peptide pools were detected in 80 of 123 (65.0%) subjects in Study 301. The majority of positive subjects were positive only at single time points including some at Baseline and reverted to negative at the next time point.

There was no trend toward higher ALT values nor lower FVIII activity measures at time points where a FVIII-specific cellular response was detected. The mean (SD) of ALT values was 31 (33.7) U/L for all timepoints testing negative (n=1197) compared to 31 (31.5) U/L for timepoints testing positive (n=145). The mean (SD) of FVIII activity measures was 39 (44.5) IU/dL for timepoints testing negative (n=1076) compared to 38 (43.3) IU/dL for timepoints testing positive (n=125).