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PRODUCT: BEQVEZ (fidanacogene elaparvovec)

APPLICANT: Pfizer, Inc.

PROPOSED INDICATION: Treatment of adults with moderate to severe hemophilia B (congenital FIX deficiency) currently on routine prophylaxis and without pre-existing antibodies to AAVRh74var capsid detected by an FDA-approved test.

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EXECUTIVE SUMMARY:

BEQVEZ (Fidanacogene Elaparvovec; PF-06838435) is a replication-deficient, recombinant adeno-associated viral vector derived from the Rh74 serotype (AAVRh74var) that expresses a transgene encoding a (b) (4) of the naturally occurring Padua variant of the human factor IX (hFIX39-Padua) protein. BEQVEZ is indicated for the treatment of adults with moderate to severe hemophilia B (congenital FIX deficiency) currently on routine prophylaxis and without pre-existing antibodies to AAVRh74var capsid detected by an FDA-approved test. Following intravenous (IV) infusion, BEQVEZ transduces liver cells,

with subsequent expression of hFIX39-Padua, resulting in secretion of functional hFIX39-Padua protein and increased circulating hFIX39-Padua levels. This results in restoration of hemostatic potential, thus reducing the frequency of bleeding episodes and the need for exogenous FIX replacement therapy. The recommended dose level of BEQVEZ is 5×10^{11} vector copies/kilogram of body weight (vg/kg BW). The nonclinical data provided to support this biologics licensing application are summarized below.

Transduction of a human hepatocyte cell line in vitro with BEQVEZ led to production of the Padua variant (R338) of human FIX (hFIX39-Padua) which correlated with increased FIX activity measured using a one-stage clotting assay (activated partial thromboplastin time; aPTT). In vivo pharmacology was evaluated using a surrogate BEQVEZ product, AAVRh74var-cFIX-R338L, which expresses a transgene encoding canine factor IX, in a juvenile hemophilia B dog model. Following IV administration of 5×10^{11} vg/kg to 5×10^{12} vg/kg AAVRh74var-cFIX-R338L, shortening in aPTT and whole blood clotting time (WBCT) was observed throughout the study duration, including at time points 370-547 days after administration. No bleeds were observed in recipient animals.

Several toxicology studies were conducted using a closely related predecessor product, AAVRh74var-(b) (4)-Padua, which is similar to BEQVEZ except the (b) (4). Administration of AAVRh74var-(b) (4)-Padua in healthy male mice at either 1.04×10^9 vg/animal or 1.93×10^9 vg/animal led to a dose-dependent increase in plasma expression of hFIX protein. This increased hFIX protein expression correlated with increased plasma FIX clotting activity. Out of 110 animals, 20 animals were found dead or euthanized in moribund condition during the study. Six of these unscheduled mortalities were considered related to AAVRh74var-(b) (4)-Padua, based upon anatomic pathology findings in the brain and/or skin. An additional eight mice from groups that received a predecessor product encoding the wild type FIX gene at approximately 3x higher dose level also experienced unscheduled mortalities with similar clinical findings. These findings were interpreted as likely test article-related exacerbations following tissue/vascular injury related to multiple blood collection procedures and administration of the product to healthy animals with endogenous FIX activity. The dose levels in this study were between 10 to 2-fold lower than the recommended human dose level for BEQVEZ, extrapolated based on body weight.

A toxicology study was also conducted evaluating IV administration of AAVRh74var (b) (4) Padua in nonhuman primates (NHP) at dose levels ranging from 1×10^{12} vg/kg to 5×10^{12} vg/kg. Vector administration resulted in detection of hFIX protein expression in plasma and increased mean plasma FIX clotting activity, as determined by aPTT, compared to concurrent controls. Plasma FIX clotting activity peaked 3 weeks post-dosing to 2-4x normal human activity and subsequently declined. Antibodies that targeted the hFIX protein and the AAV capsid were detected in most dosed animals. The levels of both anti-hFIX and anti-AAV antibodies increased with time, which correlated with a decline in plasma hFIX protein levels. No test article-related adverse findings were observed in this study, including any findings of thrombosis by microscopic histopathology. The dose levels in this study were between 2 to 10-fold higher than the recommended clinical dose level for BEQVEZ.

The biodistribution (BD) of AAVRh74var (b) (4)-Padua was assessed in NHPs. At days 30 and 92 after administration of 5×10^{12} vg/kg AAVRh74var (b) (4)-Padua, vector DNA was detected in all tissues assessed, including the brain and testes. The highest vector DNA concentrations were detected in the liver, spleen, and inguinal lymph nodes. In NHPs administered dose levels from 1×10^{12} vg/kg to 5×10^{12} vg/kg, dose-dependent vector DNA levels were present in liver through the final time point evaluated, at 542 days after administration.

Shedding of AAVRh74var (b) (4)-Padua in semen was evaluated in healthy (b) (4) rabbits. Vector DNA was detectable in semen until 4 months after dosing. No analysis of potential impact on fertility or transmission to offspring was performed.

Integration site analysis was performed on host genomic DNA isolated from liver tissue collected in juvenile hemophiliac dogs at 1-2 months post-administration of the canine surrogate vector, and in NHPs at 92 days or between 742 – 757 days following administration of BEQVEZ. For both species, most of the identified vector DNA sequences were episomal and were not integrated into the host DNA. In dogs, liver biopsies performed to quantify integration of AAVRh74var -cFIX-R338L DNA found (b) (4) unique integration sites (IS). A low level of integrated vector DNA was distributed throughout the host genome with no clear preference to specific integration sites, including in genes associated with malignant transformation in humans. In a 2-year vector integration study in NHPs administered 5×10^{12} vg/kg, there was no indication that integration of vector DNA into host cell DNA resulted in altered liver function, hepatocellular hyperplasia or carcinoma.

Studies to evaluate the safety pharmacology, developmental and reproductive toxicity, and carcinogenicity/tumorigenicity of BEQVEZ were not conducted. These studies are not warranted based on the product characteristics, results from the biodistribution and toxicology studies, and patient population.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of BEQVEZ. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

BEQVEZ is composed of a novel recombinant AAV vector, AAVRh74var, derived from the naturally occurring Rh74 AAV serotype. The vector (b) (4)

BEQVEZ is supplied in vials containing a nominal volume of 1 mL of concentrated solution at 1×10^{13} vg/mL. The volume of BEQVEZ to be prepared for the final product is calculated based on the patient's dose weight, which is equal to the patient's body weight whose body mass index (BMI) is less than 30 kg/m^2 , and equal to $30 \text{ kg/m}^2 \times [\text{Height (m)}]^2$ in patients whose BMI is above 30 kg/m^2 . BEQVEZ concentration solution is then diluted in 0.9% sodium chloride with 0.25% human serum albumin (HSA) to a final infusion volume of 200 mL. The final drug product is administered in a single IV infusion over approximately 60 minutes.

Abbreviations

AAV	Adeno-associated virus
(b) (4)	(b) (4)
AAVRh74var	Adeno-associated virus derived from AAVRh74 serotype; used in BEQVEZ
AAVRh74var-cFIX-R338L	AAVRh74 encoding a canine surrogate version of hFIX-R338L
AAVRh74var-hFIX-R338L	Identical to BEQVEZ
(b) (4)	(b) (4)
ALT	Alanine transaminase
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BD	Biodistribution
BMI	Body mass index
BW	Body weight
CIS	Common integration site
(b) (4)	(b) (4)
(b) (4)	(b) (4)
DNA	Deoxyribonucleic acid
DP	Drug product
DRG	Dorsal root ganglia
(b) (4)	(b) (4)
ELISpot	Enzyme-linked immunosorbent spot
E&L	Extractables and leachables
FIX	Factor IX
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
FIX-Padua	Factor IX variant (b) (4)
(b) (4)	(b) (4)
g	Grams

GLP	Good Laboratory Practice
(b) (4)	(b) (4)
IU	International units
IVIg	Human intravenous immunoglobulin
hFIX	Human factor IX
(b) (4)-Padua	(b) (4)
(b) (4)	(b) (4)
hFIX-R338L	(b) (4)
(b) (4)	(b) (4)
IFN	Interferon
IgG	Immunoglobulin G
IS	Integration site
kg	Kilogram
LLoQ	Lower limit of quantitation
mL	Milliliter
ng	Nanogram
NHP	Nonhuman primate
PF-06838435	Identical to BEQVEZ
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PT	Prothrombin time
RNA	Ribonucleic acid
(b) (4)	(b) (4)
(b) (4)	(b) (4)
vg	Vector genome copies
WBCT	Whole blood clotting time
WT	Wild-type
µg	Microgram

Related File(s)

IND #16437 (primary), Adeno-Associated Viral Vector (b) (4)
 [redacted] Expressing Human Factor IX (hFIX) Gene;
 Treatment of Hemophilia B; Pfizer Inc.

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INTRODUCTION

Hemophilia B is an X-linked, recessive genetic disorder caused by deficiency of factor IX (hFIX) and characterized by increased bleeding, including intra-articular and intramuscular bleeding that may lead to hemophilic arthropathy^{1,2}. Patients with hemophilia B are also at risk of occult organ bleeding. The disease mainly affects males, but females with two affected alleles may exhibit hemophilia symptoms. The current standard of care for patients with hemophilia B consists of administration of exogenous hFIX replacement therapy as needed (i.e., 'on demand') or at regular intervals. However, per the applicant, there remains an unmet medical need for long-term management of patients with hemophilia B due to risks of breakthrough bleeding and the burden of routine prophylaxis IV infusions.

¹ Bolton-Maggs, P.H.B, and Pasi, J.K. Haemophilias A and B. The Lancet (2003); 361; pages 1801-1809

² Rodriguez-Merchan, E. C. Common orthopaedic problems in haemophilia. Haemophilia (1999); 5 (Suppl. 1); pages 53-60

BEQVEZ is a gene therapy product for the treatment of patients with hemophilia B and who are negative for the presence of AAVRh74var neutralizing antibodies. BEQVEZ administration is intended to provide sustained expression of hFIX following a single IV administration, thereby reducing spontaneous bleeding and the need for hFIX replacement therapy.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of BEQVEZ to treat the proposed clinical indication.

Primary Studies

Study Number	Study Title	Report Number
1	In Vivo Treatment of Juvenile Hemophilia B Dogs with a (b) (4) AAV Vector Expressing Canine FIX-R338L	34212
2	In vivo comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L potency in hemophilia b mice	11243

Supplementary Studies

Study Number	Study Title	Report Number
<i>In Vitro</i>		
3	In Vitro Comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L Potency in a (b) (4)	125358
4	In Vitro Comparison of the Principal Molecular Structures of AAVrh74var and (b) (4) Capsids	123704
5	T Cell Response to (b) (4)	0822003
6	Analytical Investigation of the Root-Causes for the Observed Variability in the Range of FIX Activity in Different FIX Assays	044149
7	Field Study to Assess Variability of Factor IX Assays in Determining Activity Levels in Plasma From Participants Treated with Fidanacogene Elaparvovec	041307
8	Measurement of (b) (4) Levels in Plasma Treated with Fidanacogene Elaparvovec	043814
9	Analysis of FIX Activity Following Addition of Exogenous FIX to Plasma from Patients Treated with Fidanacogene Elaparvovec	041438
<i>In Vivo</i>		
10	Studies in male (b) (4) mice to evaluate the potency of (b) (4) expressing human factor IX in the presence or absence of circulating anti-AAV neutralizing antibodies	014530 – Appendix L
11	Studies in male (b) (4) mice to evaluate the potency of (b) (4) for liver-specific expression of human FIX	015802 – Appendix M

Note: Study Nos. 3 – 9 are briefly summarized in this review memo under ‘Overview of Supplementary In Vitro Studies.’ Study Nos. 3 – 5 are not reviewed in depth in this memo because their data are not directly applicable to the intended clinical use of BEQVEZ. Study Nos. 6 – 9 are not reviewed in depth because they are primarily reports validating methodologies used in clinical trials for BEQVEZ. Study Nos. 10 and 11 are briefly summarized in this review memo under ‘Overview of Supplementary In Vivo Studies.’ These studies contained preliminary data derived from early predecessor products and are not directly applicable to the intended clinical use of BEQVEZ.

Overview of Pharmacology Studies

Overview of In Vivo Studies

Study #1: In Vivo Treatment of Juvenile Hemophilia B Dogs with a (b) (4) AAV Vector Expressing Canine FIX-R338L

Report Number		034212
Date Report Signed		03-MAY-2022
Title		In Vivo Treatment of Juvenile Hemophilia B Dogs with a (b) (4) AAV Vector Expressing Canine FIX-R338L
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To 1) assess the durability of cFIX-R338L transgene expression over a time during which growth in the treated dogs mimics growth seen in humans during the 6 to <12-year age period and the 2 to 6-year age period at different doses, 2) evaluate potential vector integration, and 3) evaluate the potential of dilution of FIX expression by the increase in blood volume and hepatic growth in male juvenile hemophilia B dogs.
Study Animals	Strain/Breed	Hemophilia B dogs (FIX-deficient) <i>NOTE: All animals were tested and negative for preexisting AAV antibodies (<1:5).</i>
	Species	Dogs
	Age	3- and 6-months old
	Body Weight	1.4 – 5.1 kg
	#/sex/group	6 males/age group
Total #		12
Test Article(s)		AAVRh74var cFIX-R338L, which is a canine surrogate encoding canine factor IX (rcFIX-R338L), Lot: (b) (4) 00713278-0008-M01
Control Article(s)		N/A
Route of Administration		IV

Description of the Disease/Injury Model and Implant Procedure	<p>The hemophilia B (FIX deficient) dog models at (b) (4) contain a missense mutation causing severe hemophilia phenotype. Both male and female dogs have less than 1% normal coagulant bleeding and potentially fatal spontaneous bleeding into soft tissue and joints.</p> <p>All hemophilia B dogs had at least one exposure to rcFIX-R338L protein prior to gene therapy with an IV infusion of rcFIX-R338L and limited fall off sampling of plasma and serum, aPTT, WBCT, and TEG. Before the dogs received a single IV dose of the AAV-canine cFIX-R338L vector, they also received a dose of rcFIX-R338L protein (from (b) (4)) to provide hemostasis during the infusion.</p>
Study Groups and Dose Levels	<p>Per age group (3 or 6 months old):</p> <p>Group 1 – 5×10^{11} vg/kg</p> <p>Group 2 – 2.5×10^{12} vg/kg</p> <p>Group 3 – 5×10^{12} vg/kg</p> <p>Approximately 10-30 U rcFIX-R338L was administered on the day of infusion, on the day of and several days following liver biopsy, and as needed to treat bleeds.</p>
Dosing Regimen	Single administration
Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	All time points were in-life assessments. In-life assessments were conducted for a duration of 24 months.

Key Evaluations and Assessments:

- Clinical observation: Prior to injection, weekly after injection
- Body weight: Prior to injection, weekly to Week 12, monthly to Month 16; Months 18, 21, and 24
- Liver ultrasound: Prior to injection; monthly to Month 16; Months 18, 21, and 24
- Hemostatic measurements: Prior to injection, weekly to Week 12, monthly to Week 52
 - o One-stage aPTT ((b) (4))
 - o Whole blood clotting time (WBCT)
 - o Thromboelastography (TEG)
- Serum chemistries: Prior to injection and at approximately 2 month intervals until Month 15
- Liver biopsy: 1-2 months after injection

³ (b) (4) was used. CaCl₂ was used to initiate clotting, and clotting times of samples or standards were measured using a (b) (4). A standard curve was generated by mixing canine pooled normal plasma (b) (4) Normal Dog Colony) with pooled hemophilia B baseline dog plasma.

- (b) (4)
- Vector DNA integration site, copy number, and transgene expression by (b) (4)

Key Results:

- Clinical observations: No safety findings reported.
- Body weight and liver ultrasound:
 - In the 3-month-old cohort, a 2.2-3.4-fold increase in body weight and a 1.4-1.8-fold increase in liver size was observed at 18-24 months post-treatment, compared to baseline measurements.
 - In the 6-month-old cohort, a 1.5-1.7-fold increase in body weight and a 1.4-1.8-fold increase in liver size was observed at 18-24 months post-treatment, compared to baseline measurements.
- Hemostatic assays:
 - No dog experienced bleeding after the administration of AAV-canine cFIX-R338L.
 - Administration of AAVRh74 cFIX-R338L resulted in a dose-related shortening of aPTT in all dogs. After an initial decrease, aPTT values plateaued at approximately 30 days after administration.
 - Administration of AAVRh74 cFIX-R338L resulted in the shortening of the WBCT in all dogs administered at 3-and 6-months of age. The WBCT remained stably decreased for the time the dogs were on study out to the measurements taken at 370-547 days, with slight variations in the clotting time values observed.
- Clinical chemistry: Transient mild elevations of AST and ALT were observed in 3/6 animals administered at 3 months of age and in 1/6 animals administered at 6 months of age. The elevations in AST and ALT occurred at approximately 200 days post-administration and resolved by approximately 450 days post-administration.
- Liver biopsy:
 - (b) (4) for cFIX-R338L transgene mRNA: Hepatocytes that were (b) (4) positive were infrequently observed; these were isolated single cells commonly found in periportal areas. The (b) (4) was primarily cytoplasmic except for a few animals where it was also observed in the nucleus.
 - Vector DNA:

- Vector DNA levels in liver exhibited dose-dependency and ranged between (b) (4) vector copies/μg genomic DNA in samples obtained 1-2 months after dosing.
- A total of (b) (4) unique insertion sites (IS) were detected by (b) (4) across all samples. Among individual samples, (b) (4) unique exactly mappable IS were detected.

Reviewer's Note: *The low number of IS compared to vector copies indicates that most of the vector DNA was present in an episomal state.*

- The number of unique mappable IS was directly proportional to the level of transgene expression.
- The common integration site (CIS) analysis indicated that 20.65% of IS were clustered within a threshold of (b) (4). The most frequent CIS, Alb, consisted of (b) (4) IS and derived from 8 different samples, with dog Y19 being the highest contributor (b) (4) IS).
- IS data revealed that a total of 8.32% IS were close to predefined, well characterized genes listed in the Cancer Gene Census database. The cancer-associated gene DCC was found to be the most frequently identified cancer-associated gene, with 4 hits among all samples.

Reviewer's Conclusion:

- *This study did not evaluate the intended clinical product. Animals in this study were administered an AAVRh74var vector encoding the canine factor IX gene. However, safety and activity data resulting from administration of this surrogate would be expected to be representative of BEQVEZ.*
- *Administration of the BEQVEZ canine surrogate to dogs at dose levels up to ten-fold the recommended dose level for BEQVEZ appeared to be well tolerated and to produce hemostatic activity, as determined by aPTT shortening. Higher incidences of transient AST and ALT elevation were observed in dogs that were administered the test article at 3 months of age, compared to at 6 months.*
- *The applicant's interpretation that the vector integration profile in the liver was consistent with random integration of AAV vectors appears reasonable.*

Study #2: In Vivo Comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L Potency in Hemophilia B Mice

Report Number	11243
Date Report Signed	22-SEP-2022
Title	In vivo comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L potency in hemophilia B mice
GLP Status	No
Testing Facility	Not specified

Objective(s)		Compare (b) (4) and AAVRh74var capsid transduction and expression efficiency of human hFIX-R338L variant in a mouse model of hemophilia.
Study Animals	Strain/Breed	(b) (4)
	Species	<i>Mus musculus</i>
	Age	8-10 weeks
	Body Weight	18-20 g
	#/sex/group	10M/group
	Total #	Not specified
Test Article(s)		AAV5 hFIX-R338L, Lot: 2021-052-Mar BEQVEZ, Lot: 2021-006-Jan
Control Article(s)		PBS
Route of Administration		IV
Description of the Disease/Injury Model and Implant Procedure		These hemophilia B mice are derived from B6 background strain and exhibit curtailed clotting activity. Per applicant, this strain does not express FIX protein.
Study Groups and Dose Levels		Group 1 – PBS Group 2 – 4×10^{10} vg/kg (b) (4) hFIX-R338L Group 3 – 1×10^{11} vg/kg (b) (4) hFIX-R338L Group 4 – 4×10^{10} vg/kg BEQVEZ Group 5 – 1×10^{11} vg/kg BEQVEZ
Dosing Regimen		Single
Randomization		No
Description of Masking		Not specified
Scheduled Sacrifice Time Points		1 week and 4 weeks after injection

Key Evaluations and Assessments:

- Test article characteristics, (b) (4)
- One-stage clotting assay (aPTT)⁴, Weeks 1 and 4
- (b) (4)-Padua (b) (4) concentration in liver, Weeks 1 and 4
 - o (b) (4) concentrations were reported as a ratio of FIX to (b) (4)
- (b) (4)-Padua DNA concentration in liver, Weeks 1 and 4
 - o DNA concentrations were reported as a ratio of FIX to mouse transferrin receptor gene (mTFRC).

Key Results:

- Test article characteristics: The empty capsid percentage of (b) (4) hFIX-R338L and BEQVEZ were 39% and 38%, respectively. The percentage of (b) (4) capsid proteins were 8.6-9.0%, 21.9-25.8%, and 65.2-69.5%, respectively.

⁴(b) (4), was used. CaCl₂ was used to initiate clotting, and clotting time was measured using (b) (4). A standard curve was generated using (b) (4).

- One-stage clotting assay (aPTT):
 - FIX activity in mice that received (b) (4) hFIX-R338L was under 0.1 FIX IU/mL at both dose levels and time points.
 - The FIX activity in mice that received BEQVEZ was under 0.5 FIX IU/mL at the low dose level of 4×10^{10} vg/kg, and on average 1.0-1.5 FIX IU/mL at the dose level of 1×10^{11} vg/kg at both Week 1 and 4.
- hFIX-R338L (b) (4) concentration in liver:
 - (b) (4) expression following (b) (4) hFIX-R338L administration was negligible.
 - Following BEQVEZ administration, hFIX/HPRT ratios were approximately 0.1 and 0.5 for low and high dose levels, respectively, at both 1 and 4 weeks after administration.
- hFIX-R338L DNA concentration in liver:
 - At week 1 after administration of (b) (4) hFIX-R338L and BEQVEZ, hFIX/(b) (4) ratios were similar at approximately 0.2 and 0.3 for low and high dose levels, respectively.
 - At week 4, hFIX(b) (4) ratios were negligible in mice that received (b) (4) hFIX-R338L. In mice that received BEQVEZ, hFIX/(b) (4) ratios were approximately 0.01 and 0.05.

Reviewer's Conclusion: *Mice with a hemophilia B phenotype that were administered BEQVEZ at the recommended clinical dose level expressed the hFIX-R338L transgene in the liver and exhibited hemostatic FIX activity, supporting the expected activity and mechanism of action of the product.*

Overview of Supplementary Pharmacology Studies

Overview of Supplementary In Vitro Studies

Study #3: In Vitro Comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L Potency in a (b) (4)



Title: In Vitro Comparison of AAVRh74var FIX-R338L and (b) (4) FIX-R338L Potency in a (b) (4)

Report No: 125358

Study Performed by: Pfizer

Objective: To assess any potency differences between AAVRh74 and (b) (4) vectors encoding the (b) (4) with the hFIX-R338L transgene.

Methods: (b) (4)




Study #4: In Vitro Comparison of the Principal Molecular Structures of AAVrh74var and (b) (4) Capsids

Title: In Vitro Comparison of the Principal Molecular Structures of AAVrh74var and (b) (4) Capsids

Report No: 123704

Study Performed by: Pfizer

Objective: To compare the deduced primary acid sequences, key structural features and physical properties of AAVRh74var and (b) (4) capsids using homology modeling and (b) (4)



Methods: (b) (4)



Study #5: T Cell Response to (b) (4)

Title: T Cell Response to (b) (4)

Report No: 082003

Study Performed by: Pfizer

Objective: To compare susceptibility of human hepatocytes that had been infected with different AAV variants, including BEQVEZ, to (b) (4)

Methods: (b) (4)

Study #6: Analytical Investigation of the Root-Causes for the Observed Variability in the Range of FIX Activity in Different FIX Assays

Title: Analytical Investigation of the Root-Causes for the Observed Variability in the Range of FIX Activity in Different FIX Assays

Report No: 044149

Study Performed by: Pfizer

Objective: To investigate the potential causes for differences in (b) (4)-Padua activity ranges in one-stage assays.

Methods: (b) (4)

(b) (4)

Study #7: Field Study to Assess Variability of Factor IX Assays in Determining Activity Levels in Plasma From Participants Treated with Fidanacogene Elaparvovec

Title: Field Study to Assess Variability of Factor IX Assays in Determining Activity Levels in Plasma From Participants Treated with Fidanacogene Elaparvovec

Report No: 041307

Study Performed by: Pfizer

Objective: To assess the variability in values for FIX activity determined by laboratories at different sites, which employ different assay methods to measure FIX activity.

Methods: (b) (4)

Study #8: Measurement of (b) (4) Levels in Plasma Treated with Fidanacogene Elaparvovec

Title: Measurement of (b) (4) Levels in Plasma Treated with Fidanacogene Elaparvovec

Report No: 043814

Study Performed by: Pfizer

Objective: To assess the potential presence of (b) (4) in plasma from BEQVEZ study participants.

Methods: (b) (4)

(b) (4)

Study #9: Analysis of FIX Activity Following Addition of Exogenous FIX to Plasma from Patients Treated with Fidanacogene Elaparvovec

Title: Analysis of FIX Activity Following Addition of Exogenous FIX to Plasma from Patients Treated with Fidanacogene Elaparvovec

Report No: 041438

Study Performed by: Pfizer

Objective: To determine whether the presence of endogenously expressed (b) (4)-Padua provided by BEQVEZ will alter the determination of FIX activity when exogenous FIX products are added to plasma from BEQVEZ-treated patients.

Methods: (b) (4)

Reviewer's comment:

These data support the applicant's conclusion that the presence (b) (4)-Padua in plasma is unlikely to interfere with measurement of FIX replacement products.

Overview of Supplementary In Vivo Studies

Study #10: Studies in male (b) (4) mice to evaluate the potency of (b) (4) expressing human factor IX in the presence or absence of circulating anti-AAV neutralizing antibodies

Title: Appendix L: Studies in male (b) (4) mice to evaluate the potency of (b) (4) expressing human factor IX in the presence or absence of circulating anti-AAV neutralizing antibodies

Report No: 014530

Study Performed by: Pfizer

Note: The transgene expressed in this study, (b) (4), is a closely related predecessor product that (b) (4)

Objective:

- To compare the expression level of the (b) (4) in plasma of healthy male (b) (4) mice that were administered either (b) (4) (AAVRh74var) (b) (4).
- To assess the ability of the vectors to transduce cells, or vector potency, in the presence of neutralizing antibodies (NABs).

Methods: (b) (4)

Study #11: Studies in male (b) (4) mice to evaluate the potency of (b) (4) for liver-specific expression of human FIX

Title: Appendix M: Studies in male (b) (4) mice to evaluate the potency of (b) (4) for liver-specific expression of human FIX

Report No: 015802

Study Performed by: Pfizer

Objective:

- To compare the expression level of AAVRh74var encoding (b) (4)-Padua and (b) (4)-Padua after injection into mice.
- To compare the activity level of the expressed (b) (4)-Padua and (b) (4)-Padua.

Note: The two transgenes expressed in this assay are (b) (4) -Padua (b) (4) -Padua is the transgene expressed in BEQVEZ.

Methods: (b) (4)

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies with BEQVEZ were conducted.

PHARMACOKINETIC STUDIES (BD)

Summary List of Pharmacokinetic Studies

The biodistribution (BD) profile following administration of BEQVEZ in mice and NHPs was evaluated in toxicology Study Nos. 12 and 14 and within a select panel of tissues in Study No. 16. These data are summarized with the respective toxicology studies.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of BEQVEZ following IV administration in various animal species.

Toxicology Studies:

Study Number	Study Title	Report Number
12	A GLP Toxicity Study of (b) (4) -hFIX-Padua by Intravenous Infusion in (b) (4) Monkeys with a 30- or 90- day Recovery Period	20059026
13	12-Month Single Dose Intravenous Bolus Injection Toxicity Mouse Study in Males Using (b) (4) or (b) (4) -Padua	8320414

⁵ Human FIX-deficient plasma (b) (4)) and aPTT (b) (4)) were (b) (4) with sample plasma. CaCl₂ was used to induce clotting, and time to clot formation was measured using a (b) (4)

Study Number	Study Title	Report Number
14	A Pilot Single Dose 8 Week Toxicity Study of (b) (4) by Intravenous Infusion in Non-Human Primates	20049402

Shedding Studies:

Study Number	Study Title	Report Number
15	Germline Transmission Analysis of Two AAV Vectors in (b) (4) Rabbits	60216

Note: Per applicant, hemophilia B is almost exclusively limited to male subjects, and BEQVEZ is not intended for administration in women. This reviewer agrees that the omission of dedicated embryo-fetal development studies and pre- and postnatal development studies is acceptable.

Genotoxicity Studies:

Study Number	Study Title	Report Number
16	Single-Dose Intravenous Study of PF 06838435 in (b) (4) Monkeys with a 13- or 104-Week Observation Phase	20GR049

Overview of Toxicology Studies
Study #12: A GLP Toxicity Study of (b) (4) -hFIX-Padua by Intravenous Infusion in (b) (4) Monkeys with a 30- or 90- day Recovery Period

Report Number		20059026
Date Report Signed		22-DEC-2016
Title		A GLP Toxicity Study of (b) (4) -hFIX-Padua by Intravenous Infusion in (b) (4) Monkeys with a 30- or 90-day Recovery Period
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To determine acute and chronic toxicity and vector genome biodistribution of an adeno-associated virus (AAV) vector (b) (4) human FIX-Padua transgene (AAVRh74var-hFIX-Padua) when administered to (b) (4) monkeys via intravenous injection.
Study Animals	Strain/Breed	(b) (4)
	Species	(b) (4)
	Age	2.6 – 5.6 years old
	Body Weight	2.2 – 5.6 kg
	#/sex/group	Only males were evaluated. 2/group in the vehicle control group for both Phase I and Phase II. 5 – 7/group in the group receiving AAVRh74var (b) (4)-Padua in both Phase I and Phase II.
Total #		39
Test Article(s)		AAVRh74var (b) (4)-Padua; Lot No: ARHM4FP1-C1404N <i>Note: The AAVRh74var (b) (4)-Padua vector used in this study is identical to the clinical product except the (b) (4).</i>

Control Article(s)	(b) (4) NaCl, (b) (4) NaPO ₄ , (b) (4) /Poloxamer 188, (b) (4) Lot No: EXFP1-C1406N and EXFP1-C1411N
Route of Administration	IV infusion (30 minute) via saphenous vein
Study Groups and Dose Levels	<p>Phase I</p> <p>Group 1 – Vehicle</p> <p>Group 2 – 1 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p>Group 3 – 2 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p>Group 4 – 5 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p>Phase II</p> <p>Group 1 – Vehicle</p> <p>Group 2 – 1 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p>Group 3 – 2 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p>Group 4 – 5 x 10¹² vg/kg AAVRh74var (b) (4)-Padua</p> <p><i>Reviewer's Note: Per the study report, the two phases were temporally staggered to provide sufficient time for screening of animals for neutralizing AAV-antibodies.</i></p>
Dosing Regimen	Single
Randomization	Yes
Description of Masking	Not specified
Scheduled Sacrifice Time Points	<p>Phase I:</p> <p>Day 92 (all of Group 1; 3 animals without anti-hFIX antibodies that were randomly selected from each group in Groups 2-4), Day 542 (2-3 of remaining animals from each group in Groups 2-4)</p> <p>Phase II:</p> <p>Day 30 (all of Group 1; 3 animals without anti-hFIX antibodies that were randomly selected from each group in Groups 2-4), Day 457 (2 animals from Group 3)</p>

Key Evaluations and Assessments:

In-life assessments:

- Cage-side observation: Animals were checked once daily for mortality and moribundity.
- Body weights (BW): Baseline (pre-dose) and weekly
- Food consumption: Daily (qualitative)
- Clinical pathology (hematology and coagulation, including aPTT and PT; and clinical chemistry):
 - Phase I: Baseline and weeks 2, 4, 6, 8, 10, 12, 13 (all animals), 36 (Groups 2-4), and 78 (Groups 2, 3, and 4).
 - Phase II: Baseline and weeks 2, 4 (all animals), 24 (Groups 2-4), and 66 (Group 3) in Phase 2.
- Urinalysis:

- Phase I: Week 78 in Groups 2, 3, and 4
- Phase II: Week 66 in Group 3
- Pharmacodynamics (Phase I: blood was drawn pre-dose, weekly to Week 13, every other week; Phase II: twice pre-dose, weekly to Week 4, and every other week):
 - hFIX protein levels in plasma by a (b) (4). The (b) (4) was specific for human FIX (b) (4)
 - hFIX activity level by aPTT assay⁶.
Note: The aPTT assay does not differentiate between (b) (4) and human FIX activity.
 - Thrombin-antithrombin (TAT) complex levels (b) (4)
 - Fibrinogen D-dimer level ((b) (4))
- Immune response:
 - T cell response against AAVRh74var capsid or hFIX peptides by Enzyme-linked immunosorbent spot (ELISpot):
 - Phase I: pre-dose, Weeks 2, 4, 8, 12, and Day 92 (terminal)
 - Phase II: pre-dose, Week 2, and Day 30 (terminal)
 - Antibody levels and titers (Phase I: pre-dose, weekly to Week 13, and every other week until necropsy; Phase II: twice pre-dose, weekly to Week 4, and every other week until necropsy):
 - Anti-hFIX antibody levels by (b) (4).
 - Anti-hFIX neutralizing antibody titer (Bethesda titer):
 - Anti-AAV IgG antibody levels in plasma by (b) (4).
 - Anti-AAV neutralizing antibody levels in plasma with a functional test¹⁰.

⁶ The aPTT assay was performed by (b) (4) and an aPTT (b) (4). The time to clot was measured using a (b) (4). A standard curve was generated with pooled normal human plasma (b) (4) and used to calculate the activity of each sample. The baseline FIX activity value before vector administration was subtracted from measurements following treatment in reported percentage values.

⁷ A sample positive for D-dimer was assigned to values that were at least 0.54 µg/mL and 20% above baseline levels.

⁸ Recombinant hFIX (b) (4) was used to capture anti-hFIX antibodies in samples, and (b) (4) was used for detection.

⁹ AAVRh74var capsids (b) (4) was used to capture anti-AAV antibodies, and (b) (4) was used for detection.

¹⁰ (b) (4)

Post-mortem assessments:

- Gross pathology performed by a veterinary pathologist.
- Organ weights: brain, epididymis, adrenal gland, pituitary gland, prostate, thyroid, heart, kidney, liver, lung, spleen, testis, and thymus
- Histopathology:
 - Unscheduled deaths, Day 30 and 92 time points: aorta, bone marrow, bone (femur, sternum), brain, epididymis, esophagus, eye, gallbladder, adrenal gland, mammary gland, parathyroid gland, pituitary gland, prostate, salivary gland, seminal vesicle, thyroid gland, gross lesions, gut-associated lymphoid tissue, heart, kidney, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mandibular, mesenteric, and inguinal), muscle (skeletal), nerves (optic, sciatic), pancreas, administration site, skin, small intestine (duodenum, ileum, jejunum), spinal cord, stomach, testis, thymus, tongue, trachea, and urinary bladder by a board-certified Veterinary Pathologist
 - Day 457 and Day 542: liver and spleen
- BD of vector as performed by qPCR on heart, lung, thymus, spleen, lymph nodes (hepatic, inguinal, and mesenteric), pancreas, urinary bladder, stomach, large intestines, colon, brain, kidneys, testes, gross lesions, skin, skeletal muscle (psoas and diaphragm), peripheral blood mononuclear cell (PBMC) samples, bone marrow, and bone
 - Time points were Day 30 (Groups 1, 3, and 4), Day 92 (Group 1 and 4), Day 457 (liver and spleen only; Group 3), and Day 542 (liver and spleen only; Groups 2-4)

*Key Results:*In-life assessments:

- Clinical observations:
 - One animal, 4005 in Group 4, was euthanized on Day 57 due to chronic clinical signs of severe, watery feces. Observations for watery feces were present pre-study at Day -6. Clinical pathology changes noted minimally prolonged aPTT on Day 51 with partial recovery by Day 57, and fibrinogen levels that were higher than other animals on study but consistent with pre-study values. Gross pathological findings included fluid in pericardial cavity, dilation of cecum and colon with gas and fluid content, and a small thymus. Major microscopic findings included a focal ulcer, multifocal erosion, as well as decreased cellularity in lymph nodes. Fecal culture isolated a few colonies of *Campylobacter spp.*

Reviewer's Note: The pathologist's conclusion that the cause of death in Animal 4005 was unlikely to be related to AAVRh74var (b) (4)-Padua is reasonable, as watery feces were present pre-dose and the clinical and gross pathological findings in Animal 4005 were absent in other animals that received AAVRh74var (b) (4)-Padua.

- BW, food consumption: There was no apparent effect of AAVRh74var -hFIX-Padua on BW or food consumption.
- Hematology, coagulation:
 - There were no apparent AAVRh74var (b) (4)-Padua-related changes in hematology.
 - There was a minimally shortened aPTT at all dose regimens relative to baseline, mostly on Days 9 and 23 (Phases I and II), relative to baseline values and compared to the control dose for Group 4 only.
- Clinical chemistry, urinalysis: There were no apparent AAVRh74var-(b) (4)-Padua-related changes in the clinical chemistry.
- Pharmacodynamics:
 - All animals in the study expressed hFIX following vector delivery. hFIX protein levels were variable and ranged from 2.1% (Animal 2001) to 25.9% (Animal 4004) of normal antigen level on Week 3, corresponding to concentrations of 103.2 ng/mL to 3702.9 ng/mL. In 8/20 animals in Phase I, hFIX expression was lost by Week 13. By Day 457 or 542, hFIX protein level had declined to <40 ng/mL in all animals, except for Animal 3012 in Phase II.
 - hFIX activity level generally correlated with hFIX levels. An increase to 2-4x normal human activity was observed in all groups, peaking around Week 3, before declining.
 - Thrombin-antithrombin complex levels at baseline varied widely from 2.9 µg/L to 118.2 µg/L. Per the applicant, this variability was due to improper procedures for blood collection and plasma preparation. The applicant concludes that data for TAT data is uninterpretable.
 - D-dimer levels in Phase I animals that received AAVRh74var (b) (4)-Padua experienced intermittent increases above baseline levels that rarely lasted over two consecutive time points. In Phase II, D-dimer levels generally declined from baseline values in all groups.
- Immune response:
 - T cell response:
 - In Phase I, there was no detectable response of significance against FIX peptide pools in any of the 11 test animals at any of the time points evaluated. In 4/11 animals, positive T cell activation was detected against AAVRh74var capsid.
 - In Phase II, there was no detectable response of significance against AAVRh74var capsid or FIX peptides.

- Antibody levels and titers:
 - In Phase I, all animals developed anti-hFIX IgG to levels greater than 1000 ng/mL. The anti-hFIX IgG concentration increased over time, with a notable increase around day 300. In Phase II, Group 4 animals developed anti-hFIX IgG levels at higher than 1000 ng/mL. However, Group 3 and half of Group 2 animals developed anti-hFIX IgG levels at <500 ng/mL.

Reviewer's Note: *Development of anti-hFIX antibodies likely contributed to loss of hFIX expression.*

- In Phase I, no animals in Groups 1 or 2 and approximately a third of animals in Groups 3 and 4 developed neutralizing anti-hFIX antibodies to Bethesda titer above 0.4. Phase II data were not provided.
- Administration of AAVRh74var (b) (4)-Padua led to development of anti-AAV antibodies in most of the recipient animals. Anti-AAV antibodies levels generally increased with time, through Day 457 or 542 endpoints.
- Anti-AAV neutralizing antibodies were detected in all Phase I animals, except Animal 3011, that received AAVRh74var (b) (4)-Padua. Phase II data were not provided.

Postmortem assessment:

- Gross pathology, organ weights: There were no AAVRh74var (b) (4)-Padua-related macroscopic changes.
- Histopathology: There were no AAVRh74var (b) (4)-Padua-related histopathologic findings, including evidence of thrombosis.
- BD:
 - Phase I animals: On Day 92, Group 1 samples were negative for hFIX DNA. Human FIX DNA was detected in samples of all tissues from Group 4 animals, with highest levels at $>1 \times 10^5$ copies/ μ g detected in liver, spleen, and inguinal lymph node. On Day 542, the highest levels of hFIX DNA were detected in liver samples ($2.5 - 13 \times 10^6$ copies/ μ g) and correlated with dose level.
 - Phase II animals: On Day 30: Group 1 samples were negative for hFIX DNA. As in Phase I animals, hFIX DNA was detected in samples of all tissues, with highest levels at $>1 \times 10^5$ copies/ μ g in liver, spleen, and inguinal lymph node. On Day 457, the highest levels of hFIX DNA were observed in liver samples (approximately 3×10^6 copies/ μ g), while levels in the spleen were $6 - 10 \times 10^3$ copies/ μ g.

Reviewer's Conclusion:

- Administration of AAVRh74var (b) (4)-Padua appeared generally well tolerated in animals and induced hFIX expression in all animals. Vector DNA was detected in all assessed tissues, including the testes, with highest levels being observed in liver, spleen, and inguinal lymph node.
- The clinical product was not used in this study and the vector differs from BEQVEZ in the (b) (4). However, the AAVRh74var (b) (4)-Padua predecessor product uses the (b) (4) as BEQVEZ, and long-term transgene expression and specific activity are expected to be similar to BEQVEZ when administered at the recommended clinical dose level (see Study 11).
- The development of anti-hFIX antibodies might be due to species-specific differences in FIX sequence. No FIX inhibitors were detected in any clinical trial participant who received BEQVEZ, up to 6 years post-infusion.

Study #13: 12-Month Single Dose Intravenous Bolus Injection Toxicity Mouse Study in Males Using (b) (4)-Padua

Report Number		8320414
Date Report Signed		07-DEC-2016
Title		12-Month Single Dose Intravenous Bolus Injection Toxicity Mouse Study in Males Using (b) (4)-Padua
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To evaluate the toxicity of the test articles, AAVRh74var (b) (4)-Padua (PD) and AAVRh74var (b) (4) when administered as a single dose via intravenous bolus injection to male mice.
Study Animals	Strain/Breed	(b) (4)
	Species	(b) (4)
	Age	At least 6 to 7 weeks
	Body Weight	20.1 – 24.8 g
	#/sex/group	20/group; all animals were male
	Total #	110
Test Article(s)		AAVRh74var (b) (4)-Padua, Lot No. (b) (4)-1030 AAVRh74var (b) (4)-WT, Lot No. (b) (4)-904 <i>Note: AAVRh74var (b) (4)-Padua is identical to the clinical product except (b) (4). hFIX protein is functional in the mouse coagulation system¹¹.</i>
Control Article(s)		(b) (4) Sodium Chloride (NaCl), (b) (4) Sodium Phosphate, (b) (4) /Poloxamer 188, (b) (4)
Route of Administration		IV bolus injection into tail vein
Description of the Disease/Injury Model and Implant Procedure		None

¹¹ Kung SH, Hagstrom JN, Cass D, Tai SJ, Lin HF, Stafford DW, High KA. Human factor IX corrects the bleeding diathesis of mice with hemophilia B. Blood (1998). Feb 1;91(3):784-90.

Study Groups and Dose Levels	Group 1 – 0 vg/animal Group 2 – 1.04×10^9 vg/animal AAVRh74var (b) (4)-Padua Group 3 – 1.93×10^9 vg/animal AAVRh74var (b) (4)-Padua Group 4 – 3.45×10^9 vg/animal AAVRh74var (b) (4) Group 5 – 6.44×10^9 vg/animal AAVRh74var (b) (4) Reviewer's Note: The applicant intended for the dose levels of PD and (b) (4) groups to be identical, but the initial determination of vector titers was inaccurate. The values above reflect the actual dose levels administered.
Dosing Regimen	Single
Randomization	Yes
Description of Masking	Not specified
Scheduled Sacrifice Time Points	Day 365

Key Evaluations and Assessments:

In-life assessments:

- Health monitoring: Animals were checked for mortality and moribundity, twice daily
- Clinical examinations/physical exams:
 - Cage-side observations were conducted once daily;
 - Detailed observations were conducted pre-dose, prior to dosing on Day 1, weekly, and on the day of scheduled sacrifice
- BW were recorded once pre-dose, before dosing on Day 1, and weekly
- Food consumption was recorded weekly
- hFIX antigen and hFIX activity were analyzed in blood plasma collected from unfasted animals, once pre-dose, on Days 14 and 29, Weeks 13 and 26, Days 288, 318, and 346, and once in the last 7 days before sacrifice
 - hFIX antigen levels were analyzed with an (b) (4)
 - hFIX activity was measured by aPTT in a subset of samples, where enough blood volume was available, using human FIX-deficient plasma (b) (4) and an aPTT (b) (4) (see method in Study 12).

Reviewer's Note: Blood was collected using a submandibular blood collection procedure.

Postmortem assessments:

- Gross pathology including examination of external features, body orifices, abdominal and cranial cavities, organs, and tissues
- Organ weights of adrenal glands, brain, epididymis, gall bladder, heart, kidney, liver, spleen, and testes

- Histopathology of adrenal glands, aorta, brain, cecum, colon, duodenum, epididymis, esophagus, eye, femur, gall bladder, Harderian gland, heart, ileum, jejunum, kidney, lesions, liver, lung, lymph nodes (mandibular and mesenteric), muscle (biceps and femoris), optic nerve, pancreas, pituitary gland, prostate, rectum, salivary gland, sciatic nerve, seminal vesicle, skin, spinal cord, spleen, sternum, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder

Key Results:

- Health monitoring: 20 animals were found dead or euthanized in moribund condition during the study. 14 deaths were related to test article administration. These were 5 animals in Group 2, 1 animal in Group 3, 3 animals in Group 4, and 5 animals in Group 5.

***Note:** Per the applicant, frequent submandibular blood collection procedures may have induced local tissue and vascular injury, including hemorrhage, thrombosis, necrosis, and inadvertent puncture of adjacent vasculature, whose sequelae were exacerbated by increased FIX activity and contributed to the early mortalities.*

- Clinical observations:
 - Skin findings of scabs, thinning hair, rough haircoat, and alopecia at multiple sites and localized to dorsal/cervical regions were observed in 11 test article-related early death animals. There was no clear dose level correlation. In three animals, ears were missing, likely related to skin findings.
 - Missing or small right eye was observed in two early death animals in Group 2 (A23660 and A23664).
 - Barrel rolling and irregular respiration was observed in an early death Group 2 animal A23661.
- BW: Intermittent BW losses were secondary to ongoing clinical observations or were unrelated to the test article.
- Food consumption: Intermittent fluctuations were secondary to ongoing clinical observations or unrelated to test article.
- hFIX antigen and hFIX activity:
 - hFIX antigen levels increased and plateaued around day 29, with levels of 108 ng/mL in Group 2, 667 ng/mL in Group 3, 5,111 ng/mL in Group 4, and 15,111 ng/mL in Group 5. hFIX levels in the vehicle control group were negligible.
 - hFIX activity increased and plateaued around day 29, correlating with hFIX antigen levels. hFIX activity level was approximately 78% normal in Group 2, 74% normal in Group 3, 132% normal in Group 4, and 146% normal in Group 5.

- Gross pathology:
 - See Clinical observations for skin findings.
- Organ weights: No test article-related organ weight changes were observed.
- Histopathology:
 - Skin/subcutis findings correlated with macroscopic observations of skin, and included mixed inflammation with crust, epidermal hyperplasia, and ulcer. These findings were more frequent in animals that received test article administration, but without dose level dependency.
 - Brain findings:
 - Slight or minimal acute hemorrhage, necrosis, fibroplasia, gliosis, degeneration, vascular thickening, parenchymal loss, and pigmentation were observed. These were not correlated with dose level but occurred with greater frequency in Groups 2 (8 animals), 4 (3 animals), and 5 (2 animals) than in Group 1 and 3.
 - Moderate hemorrhage was observed in 2 early mortality animals from Group 2 (A23659 and A23661).

Reviewer's Conclusion: Administration of AAVRh74var (b) (4)-WT and AAVRh74var (b) (4) Padua led to an increase in FIX antigen and corresponding increase in FIX activity. This was associated with early deaths in wild type mice. In both early deaths and surviving mice, adverse findings were present in the brain and skin. No analysis was performed on the skin or in the brain to determine if the AAV products were present at these sites.

Study #14: A Pilot Single Dose 8 Week Toxicity Study of (b) (4)
by Intravenous Infusion in Non-Human Primates

Report Number	20049402
Date Report Signed	14-NOV-2016
Title	A Pilot Single Dose 8 Week Toxicity Study of (b) (4) by Intravenous Infusion in Non-Human Primates
GLP Status	No
Testing Facility	(b) (4)

Objective(s)		<p>The objectives of this study were to:</p> <ul style="list-style-type: none"> Compare FIX levels derived from (b) (4) Compare FIX levels derived from (b) (4) via intravenous injection on Day 1, Determine human factor IX (hFIX) antigen levels, hFIX inhibitory antibodies and AAV capsid antibody (Ab) titers, and Study vector genome biodistribution after systemic delivery of AAV vectors.
Study Animals	Strain/Breed	(b) (4) monkeys
	Species	(b) (4)
	Age	3.1 – 4.4 years
	Body Weight	2.7 – 3.9 kg
	#/sex/group	2 (only males used)
	Total #	22
Test Article(s)		See Study Groups and Dose Levels
Control Article(s)		None used
Route of Administration		Intravenous
Description of the Disease/Injury Model and Implant Procedure		None
Study Groups and Dose Levels		<p>Phase I</p> <p>Group 1 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1032</p> <p>Group 2 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1031</p> <p>Group 3 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1030</p> <p>Phase II</p> <p>Group 4 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1089</p> <p>Group 5 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1097</p> <p>Group 6 – (b) (4), 3 x 10¹² vg/kg; Lot: (b) (4) -1098</p> <p>Phase III</p> <p>Group 7 – (b) (4), 1 x 10¹² vg/kg; Lot: (b) (4) 1030</p> <p>Group 8 – (b) (4), 1 x 10¹² vg/kg; Lot: (b) (4) 0034</p> <p>Group 9 – (b) (4), 1 x 10¹² vg/kg; Lot: (b) (4) 0043</p> <p><i>Note: The BEQVEZ predecessor products used in Groups 3 and 7-9 have the (b) (4) as BEQVEZ. The transgene used in Group 8 is (b) (4) in BEQVEZ.</i></p>
Dosing Regimen		Single dose
Randomization		<p>No</p> <p>Animals were assigned to groups based on results of a screen for the levels of anti-AAV capsid neutralizing antibodies.</p>
Description of Masking		Not specified
Scheduled Sacrifice Time Points		Day 56

Key Evaluations and Assessments:

In-life assessments:

- Mortality and moribundity: Once daily from prior to dosing and throughout study
- Clinical observations, cage side observations: Once daily from prior to dosing and throughout study
- BW: Measured prior to dose initiation weekly throughout the study
- Food consumption: Measured once daily prior to dose initiation and continuing throughout the study
- Clinical pathology: Hematology, coagulation, and clinical chemistry were evaluated at Week -1, Days 15, 29, 43, and 55.
- hFIX antigen, anti-hFIX antibody, and anti-AAV neutralizing antibody titer analysis: Measured once prior to dose initiation and weekly throughout the study. Methods are described under Study 12.
- PBMC collection for T cell activation: Measured at Weeks 2 and 8

Postmortem assessments:

- Vector biodistribution with qPCR on Phase I and Phase II (but not Phase III) animals for the heart, liver, thymus, spleen, hepatic and inguinal lymph nodes, skeletal muscle (psoas and diaphragm), brain, kidneys, testes, lung, and any gross lesions.
- Gross necropsy
- Histopathology of spleen, kidney, liver, and lung samples

Key Results:

- Mortalities and morbidity: No unscheduled deaths were reported.
- Clinical signs and local observations: Non-sustained convulsions were observed in a Group 2 male (Animal No. 2001) on Day 2. The pathologist considered it unrelated to test article due to the transient nature of the finding and because it was not observed in any other animal or dose group.
- There were no test article related findings for BW, water consumption, food consumption, and clinical pathology.

- There were no test article-related findings for macroscopic examinations, organ weights, or histopathology examinations.
- Biodistribution: The highest levels of hFIX DNA were detected in samples of liver (b) (4) copies/μg) and spleen (b) (4) copies/μg) in all groups at 56 days following vector administration. Detection of hFIX DNA was considered negative or present up to (b) (4) copies/μg in samples of brain, thymus, and psoas muscle. hFIX DNA was considered either negative or up to (b) (4) copies/μg in testes. Diaphragm, heart, lung, and kidney samples were positive for hFIX DNA in all groups, but not as high as levels seen in liver and spleen.
- hFIX antigen concentration, hFIX antibody levels, and anti-AAV neutralizing antibody titer analysis:
 - All animals in the study expressed hFIX following AAV vector delivery. One animal in Group 3 (AAVRh74var) had a peak hFIX expression of approximately 407 ng/ml but lost circulating hFIX expression due to the development of an anti-hFIX antibody. The anti-hFIX inhibitory antibody did not cross react with endogenous (b) (4) FIX since there was no prolongation of clotting time.
 - Neutralizing antibodies against the administered capsid increased in all animals although four animals in the following groups: Group 1 (2 animals), Group 4 (1 animal) and Group 6 (1 animal), showed modest anti-AAV titers eight weeks following vector administration.

Reviewer's Conclusion:

- *This study did not evaluate the intended clinical product. The Group 3 test article, (b) (4), is identical to AAVRh74var(b) (4)-Padua.*
- *All 6 serotypes tested were able to drive expression of human FIX following administration of 3×10^{12} vg/kg.*
- *Test article-related toxicities were not observed for any of the serotypes evaluated at the dose levels evaluated (1×10^{12} and 3×10^{12} vg/kg).*
- *Neutralizing antibody titers were present in most of the AAV-treated animals over the duration of the study.*

Study #15: Germline Transmission Analysis of Two AAV Vectors in (b) (4) Rabbits

Report Number	060216
Date Report Signed	20-MAY-2016
Title	Preliminary sponsor study report on the administration of (b) (4) hFIX and (b) (4) -hFIX in (b) (4) rabbits
GLP Status	Yes

Testing Facility		(b) (4)
Objective(s)		The objectives of this study are: (1) to evaluate dissemination of AAVRh74var and AAV-Spark3 capsids to semen and to determine the kinetics of vector clearance (2) to determine human factor IX (hFIX) antigen and anti-FIX antibody levels.
Study Animals	Strain/Breed	(b) (4) rabbit
	Species	(b) (4)
	Age	6 months
	Body Weight	3-4 kg
	#/sex/group	2-5
	Total #	22
Test Article(s)		AAVRh74var (b) (4); Lot: (b) (4) 0046 AAVRh74var (b) (4)-Padua; Lot: (b) (4) 1030 (b) (4); Lot: (b) (4) 0047
Control Article(s)		Phosphate buffered saline
Route of Administration		IV
Description of the Disease/Injury Model and Implant Procedure		None
Study Groups and Dose Levels		Group 1 – AAVRh74var (b) (4), 1 x 10 ¹² vg/kg Group 2A – AAVRh74var (b) (4), 1 x 10 ¹³ vg/kg Group 2B – AAVRh74var (b) (4)-Padua, 1 x 10 ¹³ vg/kg Group 3 – (b) (4), 1 x 10 ¹² vg/kg Group 4 – (b) (4), 1 x 10 ¹³ vg/kg Group 5 – Vehicle <i>NOTE: Group 2A and 2B were combined in subsequent analysis as the AAVRh74var high dose group. Groups 1 and 2 used BEQVEZ predecessor products that (b) (4) with BEQVEZ. The transgene expression in Group 2 (b) (4) to that of BEQVEZ.</i>
Dosing Regimen		Single
Randomization		Yes
Description of Masking		Not specified
Scheduled Sacrifice Time Points		Key assessments were all in-life procedures

Key Evaluations and Assessments:

- Vector dissemination in semen. Samples were collected prior to dosing and at 1, 2, 4, 6, 8, and 10 weeks and 3-8 months after dosing.
 - o Genomic DNA purified from semen samples was analyzed for hFIX sequence using qPCR (b) (4). The Lower limit of quantitation (LLoQ) of the qPCR assay was determined to be (b) (4) copies/μg. Samples were considered positive with readings above the LLoQ. Semen samples from rabbits that were negative for hFIX vector for at least 3 consecutive time points were not analyzed further.

- hFIX antigen and anti-hFIX antibody levels in plasma, before dosing, 7, 28, 56, 94, 112, 147, and 175 days after administration. See Study 12 for description of quantification of hFIX antigen and anti-hFIX antibodies.

Key Results:

- Vector dissemination into semen:
 - Semen DNA from animals at pre-dose and from vehicle-injected animals were negative for hFIX sequences.
 - Positive samples were found in up to 2/5 animals receiving AAV-Spark3 vector at both dose levels (Groups 3 and 4) up to Week 2. Samples from later time points were negative.
 - Positive samples were found in up to 3/5 animals receiving a low dose level of AAVRh74var up to Week 4 (Group 1). Samples at later time points were negative. Positive samples were found in 4/5 or higher animals receiving a high dose level of AAVRh74var (Groups 2A and 2B) up to week 4. Positive samples continued to be found until Month 4, after which samples were negative.
- hFIX antigen levels:
 - In animals that received AAVRh74var, hFIX antigen was detected in all animals in Groups 1 and 2 and exhibited dose level dependency. There was no decline in antigen levels within the study duration.
 - In animals that received (b) (4), hFIX antigen was detected in all animals in Groups 3 and 4 and was maintained throughout the study duration.
- Anti-hFIX antibodies:
 - In animals that received AAVRh74var, Anti-hFIX antibodies were present in 2/5 animals and 0/5 animals in Group 1 on days 28 and 175, respectively. Anti-hFIX antibodies were present in 1/5 animals on day 28 and increased to 4/5 animals on day 175 in Group 2.
 - In animals that received (b) (4), Anti-hFIX antibodies were present in 3/5 and 0/5 animals in Groups 3 and 4, respectively, on day 28. The number of positive animals in Group 3 declined to 1/5 animals by day 175.

Reviewer's Note: The clinical product was not used in this study. However, the test articles used in Groups 1, 2A, and 2B contained (b) (4)

in Group 2B is (b) (4)

The presence and eventual clearance of vector in semen is consistent with existing literature of AAV8 vectors. However, no analysis of potential impact on fertility or transmission to germline cells or offspring was performed.

Study #16: Single-Dose Intravenous Study of PF 06838435 in (b) (4) Monkeys with a 13- or 104-Week Observation Phase

Report Number		20GR049
Date Report Signed		26-JUN-2023
Title		Single-Dose Intravenous Study of PF-06838435 in (b) (4) Monkeys with a 13- or 104-Week Observation Phase
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		The objectives of this study were to assess the vector DNA integration into liver host cell genome and evaluate potential neoplastic transformation following a single intravenous (IV) infusion of 5×10^{12} vg/kg of BEQVEZ administered to (b) (4) monkey.
Study Animals	Strain/Breed	(b) (4) monkeys, Mauritian origin
	Species	(b) (4)
	Age	40-43 months
	Body Weight	3.9-5.4 kg for males, 2.8-4.0 kg for females
	#/sex/group	3-7 animals; see Study Groups and Dose Levels
	Total #	19
Test Article(s)		BEQVEZ; Lot: H000017659-CM0088
Control Article(s)		(b) (4) sodium chloride, (b) (4) sodium phosphate, (b) (4) poloxamer 188, at (b) (4)
Route of Administration		IV slow bolus injection
Description of the Disease/Injury Model and Implant Procedure		None. Animals administered BEQVEZ had AAV neutralizing antibodies with a titer below 7.
Study Groups and Dose Levels		Group 1 – vehicle control Group 2 – 5×10^{12} vg/kg BEQVEZ
Dosing Regimen		Single
Randomization		Yes
Description of Masking		None specified
Scheduled Sacrifice Time Points		Day 92 interim necropsy; day 742, 749, 751, 756, or 757 terminal necropsy

Key Evaluations and Assessments:

In-life assessments:

- Health monitoring: Animals were checked for mortality and moribundity twice daily
- Clinical examinations/physical exams:
 - Cage-side observations were conducted once daily
 - Detailed observations were conducted pre-dose, on the day before dosing, 2 and 4 hours post-dose, weekly, and on the day of scheduled sacrifice
- BWs were recorded pre-dose, on the day before dosing, and weekly
- Food consumption was recorded weekly

- Physical examination on animals were conducted once pre-dose, Week 13, and Week 104
- Liver evaluations via ultrasound were conducted once on Weeks 2, 13, 52, and 104
- (b) (4)-Padua expression from blood plasma collected on Days 30, 92, 181, 360, 540, and 741 of the dosing phase from unfasted animals
 - Samples were analyzed for (b) (4)-Padua expression in (b) (4) method. This sample monitored the (b) (4), which is specific to (b) (4)-Padua.
- Clinical pathology:
 - Hematology; coagulation, including aPTT, prothrombin time, and fibrinogen; and clinical chemistry on Days 30, 181, 360, 540, and 741
 - Urinalysis on days of scheduled necropsy
- Immune response to PF-06838435 by PBMC isolated on Days 15, 30, 56, 92, and 741
 - (b) (4) by PBMC in response to AAVRh74var or (b) (4)-Padua peptides was assessed with an ELISpot assay

Postmortem assessments:

- Gross pathology including examination of external features; body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues
- Organ weights of adrenal glands, brain, gall bladder, heart, kidney, liver, spleen, testes, and thyroid
- Microscopic histopathology of lesions and liver
- Immune response to BEQVEZ by splenocytes, harvested from 0.5 g (interim) or 2.5 g (terminal) sample of spleen
 - (b) (4) in response to AAVRh74var or FIX peptides was assessed with ELISpot analysis
- Dorsal root ganglia (DRG; cervical, thoracic, and lumbar) DNA (b) (4) collection for qPCR analysis of (b) (4) and transgene expression, performed by (b) (4)
- Liver (lateral, median, and caudate lobes of at least 100 mg per sample; actual weights were not recorded) DNA (b) (4) collection and analysis (non-GLP)
 - (b) (4)

- (b) (4), performed by the applicant
- (b) (4)
- Heart and spleen tissue collection; frozen, not analyzed

Key Results:

- No mortalities or morbidities were observed. No test article-related findings were observed in clinical and physical examinations or BW.
- Liver evaluations via ultrasound: No test article-related findings were observed. One male (Animal P0107) administered 5×10^{12} vg/kg was noted to have a small module on the superior margin of the liver during Week 2. However, this nodule remained homogeneous and was not identified at the 1 or 2-year evaluations. Per the applicant, this finding was likely a benign self-resolving myelolipoma.
- (b) (4)-Padua expression in plasma: (b) (4)-Padua was detected in plasma on Day 30 at 190 – 739 ng/mL in all animals that received BEQVEZ. Levels declined with each subsequent time point to 23.4 – 161 ng/mL by Day 540. In 3 animals, however, this decline was followed by an increase in (b) (4)-Padua levels at the Day 741 time point.
- Clinical pathology:
 - Hematology: No test article-related findings were noted.
 - Coagulation: A minor, test article-related shortening of aPTT in all animals was noted on Day 30 ($0.81x - 0.91x$ of baseline aPTT). The number of animals with shortened aPTT decreased over time throughout subsequent time points. No animals exhibited shortened aPTT at Day 741 except for Animal P0103, which exhibited $0.93x$ aPTT compared to baseline. This animal also had the highest level of (b) (4)-Padua expression at Day 741.
- Immune response (PBMC):
 - On Day 56, a female (Animal P0302) administered 5×10^{12} vg/kg of BEQVEZ had an increased number of (b) (4) PBMC in response to AAVRh74var peptides, compared to prior to initiation of dosing. This was not conclusively attributed to test article administration due to a low number of (b) (4) PBMC observed and absence of response in the two other animals in that group.
 - Analysis was not performed on remaining animals or time points because, per the applicant, there was no indication of a possible cell mediated immune response in microscopic examinations of liver or spleen at terminal necropsy.

- Gross pathology: No test article-related changes were observed.
- Organ weights: An increase in spleen absolute and relative (to body and brain weight) weights were observed BEQVEZ-dosed females compared to concurrent controls. BEQVEZ-related effects on organ weight were noted at terminal necropsy.
- Microscopic histopathology: Mild mononuclear cell infiltrates were noted in the liver of both sexes in BEQVEZ-dosed animals, and was considered test article-related. No BEQVEZ-related findings were present at terminal necropsy.
- Immune response (splenocytes): No test article-related (b) (4) from AAVRh74var or FIX peptides were observed.
- DRG analysis for (b) (4)
 - An average of (b) (4) per µg DNA at the interim time point and (b) (4) per µg DNA at the final time point in animals that received BEQVEZ. This was approximately 3 log lower than what was detected for liver samples.
 - Transgene expression was very low or not detected in DRG.
- Liver analysis for (b) (4) and IS (performed by (b) (4) :
 - Negative controls had no findings of (b) (4) or IS, except one IS that was deemed background.
 - In animals that received BEQVEZ, the (b) (4) decreased from an average of (b) (4) per section at the interim time point to average of (b) (4) per section at the terminal time point.
 - IS decreased from an average of (b) (4) IS per section at the interim time point to (b) (4) IS per section at the terminal time point.
 - 37.2% IS were considered to fall in common integration sites (CIS) at the interim time point, and 41.91% of IS were considered CIS at the terminal time point. The CIS with the most IS were (b) (4) .
 - 9.5% IS at the interim time point and 8.92% of IS at the terminal time point were determined to be close to a cancer gene.
 - The overall integration profile was determined using (b) (4)

- Liver analysis for (b) (4), IS, and transgene expression (performed by applicant):
 - In BEQVEZ-dosed animals, (b) (4) at the interim time point was 2.72 to 6.59 (b) (4) and 0.48 to 2.76 (b) (4) at the terminal time point. Per the applicant's conversion to (b) (4) per section, the (b) (4) determined by (b) (4) analysis was 4.48 – 19.43 fold (b) (4) than Pfizer's.
 - For IS analysis, high homology regions were masked. The number of IS ranged from (b) (4) per µg DNA at the interim time point and (b) (4) per µg DNA at the terminal time point. Per the applicant, the number of IS determined by (b) (4) were within 0.84 to 4.72 fold these values.
 - CIS included (b) (4). There was no overlap in the 10 CIS with the most frequent IS as determined by (b) (4) and the applicant, respectively.
 - More (b) (4) and IS were identified in hepatocytes than non-hepatocytes, and the integrated sequences derived from the transgene and subsequent (b) (4) sequence, rather than from sequences present in (b) (4).
 - An average of 3.69% of IS at the interim time point and 2.59% of IS at the terminal time point occurred within (b) (4) of a known cancer gene.
 - Based on a ratio of IS to (b) (4), it is estimated that <0.1% of the vector integrated into the host genome, while >99.9% remained in the episome.

Reviewer's Note:

- *The number of detected IS in the liver was broadly similar or declined between interim and terminal time points, suggesting an absence of clonal expansion. Although IS were detected in the vicinity of cancer genes, these genes were not identified as vector CIS. Based on the IS profile, genotoxicity from vector integration appears to be a low risk.*
- *Per the applicant, average mutation rate in (b) (4) of liver is (b) (4). The IS from the product is 16.3×10^{-4} or 6.14×10^{-4} IS per (b) (4), based on (b) (4) and the applicant's analyses, respectively.*

APPLICANT'S PROPOSED LABEL

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

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

CONCLUSION OF NONCLINICAL STUDIES

Review of the available nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

BEQVEZ, Fidanacogene Elaparvovec, PF-06838435, (b) (4), AAVRh74var, (b) (4) Padua, hFIX-R338L, (b) (4)-Padua, FIX Padua, gene therapy, hemophilia B, hFIX, nonhuman primates, hemophilia B mice, Good Laboratory Practice, intravenous, pharmacology, toxicology, genotoxicity, germline transmission