Clinical Pharmacology BLA Review

Office of Clinical Evaluation (OCE)

Office of Therapeutic Products (OTP)

Submission Number: 125786

Product Name: Fidanacogene Elaparvovec (BEQVEZ)

Proposed Indication: Treatment of hemophilia B in patients ≥18 years of age.

Applicant: Pfizer, Inc.

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Table of Contents

List of Tables

List of Figures

1. Executive Summary

BEQVEZ (fidanacogene elaparvovec) is a gene therapy that consists of a recombinant viral capsid (AAVRh74var) derived from a naturally occurring adeno-associated viral (AAV) serotype (Rh74) packaging vector containing the human coagulation Factor IX (FIX) transgene modified to a high-specific FIX activity variant (FIX-R338L). In this BLA submission, the applicant proposes BEQVEZ for the treatment of hemophilia B in patients ≥18 years of age.

The clinical pharmacology of BEQVEZ is supported by two clinical studies (Phase 1/2a # C0371005/ C0371003 and Phase 3 # C0371002). In Phase 1/2a and Phase 3 studies, a single dose of 5×10^{11} vg/kg was administered as intravenous (IV) infusion. For subjects with body mass index (BMI) >30 kg/m^2, dose was calculated based on an adjusted body weight that uses a maximum permissible BMI of 30 kg/m^2 (i.e., the actual height is used to achieve a BMI of 30kg/m^2 for dose calculation). The median dose infused (vg/kg) for subjects with BMI ≤30 kg/m^2 (n=40) was 5×10^{11} vg/kg (range: 4.9×10^{11} to 5.6×10^{11} vg/kg) and the median dose for subjects >30 kg/m^2 (n=20) was 4.9×10^{11} vg/kg (range: 3.1×10^{11} to 6.1×10^{11} vg/kg).

Vector DNA fully cleared from plasma, saliva, and semen within a mean of 1 to 4 months after BEQVEZ infusion. The pharmacodynamic activity of BEQVEZ was demonstrated by longitudinal monitoring of FIX activity. Comparative analysis of FIX activity for the two studies showed about 2-fold lower FIX activity in the Phase 3 study. In the Phase 3 study (N=45 subjects), post-treatment geometric mean FIX activity from Week 12 to Month 15 was significantly higher than the fixed threshold of 5% as evaluated by three different assays. Overall, descriptive subgroup analysis, dose-response, and population pharmacodynamic analysis of FIX activity data support the proposed dosing regimen of BEQVEZ.

2. Recommendations

From clinical pharmacology perspective, the data presented in this BLA are adequate and acceptable for approval. Labeling recommendations are provided in Section 5 of this memo.

3. Background

Hemophilia B is an X-chromosome-linked inherited bleeding disorder of a coagulation factor deficiency resulting from reduced levels or absence of factor IX (FIX). The disorder affects approximately one in 30 000 males worldwide. Traditionally, a residual baseline FIX level is used to classify patients as having severe (< 1 IU/dL), moderate (1–5 IU/dL) or mild (5–40 IU/dL) hemophilia B. Treatment of hemophilia B focuses on prophylactic intravenous (IV) infusion of either plasma-derived or recombinant FIX products. A major limitation of these conventional FIX therapies is the short half-life (18 to 22 hours), which requires every 3-4 day infusions. This short half-life of conventional FIX products is in part resolved by development of newer FIX therapies with extended half-life (86-104 hours) that allow a longer dosing interval of every 7-10 days.

More recently the FDA approved adeno-associated virus (AAV) vector-based gene therapy, HEMGENIX (etranacogene dezaparvovec-drlb), for treatment of adults with Hemophilia B who:

- Currently use Factor IX prophylaxis therapy, or
- Have current or historical life-threatening hemorrhage, or
- Have repeated, serious spontaneous bleeding episodes.

In this BLA submission, the applicant proposes BEQVEZ (fidanacogene elaparvovec) for the treatment of hemophilia B in patients ≥18 years of age. BEQVEZ is a solution of an AAV-based gene therapy for intravenous infusion. BEQVEZ is based on recombinant DNA technology that consists of a recombinant viral capsid (AAVRh74var) derived from a naturally occurring AAV serotype (Rh74) packaging vector containing the human FIX transgene modified to a high-specific FIX activity variant known as FIX-R338L.

This BLA submission is supported by the results of the following clinical studies:

- A completed 12-month Phase 1/2a dose exploration/dose expansion study of 15 subjects (Study C0371005). All 15 subjects received BEQVEZ (also previously known as SPK-9001) at a $5×10^{11}$ vg/kg, based on actual lot concentration. Fourteen out of 15 subjects were enrolled in an ongoing long-term (an additional 5 years) follow-up Phase 2a study (Study C0371003) to evaluate the long-term safety, durability of transgene expression, and effect on clinical outcomes of BEQVEZ mediated gene transfer. The data supporting clinical pharmacology analysis are based on a cut-off date of Nov 02, 2022.
- An ongoing 6-year Phase 3 efficacy/safety study (Study C0371002) in which 45 adult male hemophilia B subjects were treated with BEQVEZ. Dosing was at the $5×10^{11}$ vg/kg, based on actual lot concentration. The data supporting clinical pharmacology analysis are based on a cut-off date of Nov 16, 2022.

4. Summary of Clinical Pharmacology Findings

Pharmacokinetics (PK)

BEQVEZ vector DNA levels were quantified in blood and various shedding matrices using a quantitative polymerase chain reaction (qPCR) assay. Vector shedding after infusion with BEQVEZ was assessed in 60 patients at multiple time points in clinical studies (Phase 1/2a and Phase 3).

- The maximum vector DNA concentrations were found in plasma followed by saliva, PBMC, semen and urine.
- The mean Tmax was 1.2 days for plasma, saliva, and 1.6 days for urine. The mean Tmax was 3.8 days and 7.4 days for semen and PBMC, respectively.
- Vector DNA fully cleared from plasma, saliva, and semen within a mean of 1 to 4 months after infusion and PBMC was the slowest fluid to full clearance within a

mean of 12 months. In semen, the maximum observed time for vector DNA full clearance was 154 days.

Pharmacodynamics (PD)

In the Phase 3 study (N=45 subjects), the pharmacodynamic activity of BEQVEZ was demonstrated by longitudinal monitoring of FIX activity by one-stage assays (Actin-FSL reagent and SynthASil reagent) and chromogenic assay. The FIX activity by one-stage (Actin-FSL) and chromogenic assay were about 2-fold lower as compared to one-stage (SynthASil) assay. FIX antigen level was also monitored as part of PD assessment.

- Six subjects resumed FIX prophylaxis and FIX activity data were analyzed by imputing 1.9% for these 6 subjects. Post-treatment geometric mean FIX activity from Week 12 to Month 15 from all three assays was significantly higher than the fixed threshold of 5% for the three assays (p-value<0.0001).
- The mean (SD) FIX antigen levels were 6.6 % (5.3 %) at baseline. Following BEQVEZ administration mean FIX antigen levels were 31 % (41%) at Week 12 and 25 % (31%) at Week 52.

In Phase 1/2a study (N=15 subjects), the PD activity of BEQVEZ was demonstrated by longitudinal monitoring of FIX activity by one-stage assay (Actin-FSL reagent).

- The geometric mean for FIX activity was 22.9 % of normal, ranging from 9% to 46%. The mean peak FIX activity during the 52-week follow-up was 29.1% of normal, ranging from 11% to 58%.
- During the long-term follow-up period, geometric mean (SD) of transgene-derived FIX activity levels were 24.7% (14.4%), 22.9% (13.7%), 22.3% (13.1%), 22.5% (15.3%) and 24.5% (18.1%) during Years 2 (N=14), Year 3 (N=14), Year 4 (N=13), Year 5 (N=13), and Year 6 (N=7) post vector infusion, respectively.

- The comparative analysis of FIX activity for the two studies showed about 2-fold lower FIX activity in the Phase 3 study as compared to the Phase 1/2a. Factors such as age range, sample size, manufacturing, corticosteroid use, etc.) may have contributed to the observed discrepancies in FIX activity between the two studies.

Impact of Intrinsic and Extrinsic Factors on Pharmacodynamics

Descriptive subgroup analysis for FIX activity (using the one-stage Actin-FSL reagent for using Phase 3 data) was conducted for age, body mass index (BMI), race, and corticosteroid use.

- There is a trend of higher mean FIX activity (Week 12 to Month 15) with age, higher BMI, as well as in White race. Patients 35-62 years old (n=17) had 1.9-fold higher mean FIX activity as compared to patients 18 to <35 years old (n=28). Patients with BMI ≥25 kg/m^2 (n=29) had 1.5-fold higher mean FIX activity as compared to patients with <25 kg/m^2 (n=16). Patients in White race group (n=29) had 1.6-fold higher mean FIX activity as compared to patients in Non-white race group (n=12).
- In 28 subjects with corticosteroid treatment and 17 subjects without corticosteroid treatment, the post-treatment geometric mean for FIX:C (mean [SD]) from Week 12 to Month 15 was as 10.7 (8.0) and 15.8 (9.7), respectively.

Dose-Response and Population Pharmacodynamic Analysis

In Phase 1/2a and Phase 3 studies, a single intravenous infusion of BEQVEZ was administered at a dose of 5×10^{11} vg/kg of body weight based on actual lot concentration (nominal strength was 1×10^{13} vg/mL). For subjects with BMI >30 kg/m^2, dose was calculated based on an adjusted body weight determination that assumes a maximum permissible BMI of 30 kg/m^2 (i.e., the actual height is used to achieve a BMI of 30kg/m^2). The median dose of BEQVEZ administered on a vg/kg basis was similar for both studies and across the pooled analysis.

- The median dose infused (vg/kg) for subjects with BMI ≤30 kg/m2 (n=40) was 5×10^{11} vg/kg (range: 4.9×10^{11} to 5.6×10^{11} vg/kg) and the median dose for subjects >30 kg/m2 (n=20) was 4.9×10^{11} vg/kg (range: 3.1×10^{11} to 6.1×10^{11} vg/kg).

- The Phase 3 study is currently dosing additional (b) (4) subjects to obtain clinical experience using (b) (4) for dosage calculation as requested by the FDA during the pre-BLA meeting.

- Based on limited data, the FIX activity values for (b) (4) dosing were within the same ranges as those in subjects who were dosed with product labeled with the actual concentrations of BEQVEZ.

- Although there was a trend in increasing FIX activity with higher total number of vector genomes administered (i.e., total dose of BEQVEZ), the estimate of slope for the effect of total number of vector genomes administered on geometric mean FIX activity was not statistically significant (95% CI included zero).

A population pharmacodynamic (popPD) model was developed by pooling the FIX activity data from the Phase 1/2 & Phase 3 studies.

- The popPD model adequately described the observed FIX activity vs time data up to 2 years following BEQVEZ administration.

- The popPD model was used to screen covariates such as age, BMI, manufacturing processes and concomitant corticosteroids. Age and concomitant corticosteroids were identified as covariate affecting the FIX activity.

- Also, the popPD model was proposed to provide additional justification for (b) (4) dosing of BEQVEZ. The primary source evidence for justification of (b) (4) based dosing was obtained from CMC and limited clinical data. The popPD analysis is consistent with the results of the observed FIX activity results for both actual and limited (b) (4) dosing and provided supporting information for (b) (4) dosing.

Overall, the clinical pharmacology information is acceptable, and we agree with the applicant proposed dose of 5×10^{11} vg/kg of body weight based on nominal strength of 1×10^{13} vg/mL. For subjects with BMI >30 kg/m^2, dose was calculated based on an adjusted body weight that uses a maximum permissible BMI of 30 kg/m^2 (i.e., the actual height is used to achieve a BMI of 30kg/m^2 for dose calculation).

Immunogenicity Assessments

The administration of BEQVEZ has the potential to generate immune response in the form of neutralizing antibodies (nAb) against the vector capsid (anti-AAVRh74var), the transgene (viral-derived factor IX) and as a cellular response against the transduced cells producing FIX.

- In clinical studies, all patients receiving treatment were required to screen negative for anti-AAVRh74var antibodies and negative (< 0.6 BU) for Factor IX inhibitors in a Nijmegen modified Bethesda assay following a lifetime minimum of 50 exposure days to Factor IX replacement therapy.
- No patients developed factor IX inhibitors during the Phase 1/2a and Phase 3 clinical studies.
- In Phase 3 study, 40 of 40 (100%) subjects with nAb assessment at Week 52 (1 year) post-infusion tested positive. The nAb positivity persisted for 22 of 22 (100%) and 2 of 2 (100%) subjects with nAb assessment at 2- and 3-years post-infusion, respectively.
- In Phase 1/2a study, 14 of 14 (100%) and 15 of 15 (100%) subjects with nAb assessment at 1- and 2-weeks post-infusion, respectively, tested positive. The nAb positivity persisted for all 15 participants through the 1-year post-infusion period. As of the data cutoff date, nAb positivity persisted up to 6 years post-infusion in the LTFU study (C0371003) for 5 of 5 (100%).

For characterization of cellular immune response, reactive T-cells were measured using an enzyme-linked immunosorbent spot (ELISPOT) assay which relies on detection of cytokine secretion (in this case interferon-gamma, IFN-γ). Across all clinical studies, a tapering course of oral corticosteroids (i.e., prednisone/prednisolone) was the first consideration for suppression of presumed T-cell activation.

In Study Phase 3 study, subjects were tested for IFN-γ ELISPOT prior to BEQVEZ infusion (i.e., at screening) and when corticosteroid treatment was given for presumed T-cell response (based on transaminase increase and/or FIX:C decrease).

- 10 of 28 (35.7%) subjects with corticosteroid use for presumed T-cell response had ELISPOT assessment prior to or within 24 hours of corticosteroid initiation. Prior to corticosteroid use, 4 of 10 (40.0%) subjects with ELISPOT assessment tested positive in the overall capsid peptide pool and 3 of 10 (30.0%) subjects tested positive in the overall FIX peptide pool.
- Approximately 3 weeks after corticosteroid treatment, 4 of 8 (50.0%) subjects with ELISPOT assessment tested positive in the overall capsid peptide pool and 2 of 8 (25.0%) subjects tested positive in the overall FIX peptide pool.

In Phase 1/2a study, subjects were tested for IFN-γ ELISPOT at prespecified time points and when corticosteroid treatment was given for presumed T-cell response (based on transaminase increase and/or FIX:C decrease).

- 3 of 15 (20.0%) subjects were treated with corticosteroid for presumed T-cell response after BEQVEZ infusion. 2 of 3 (66.7%) subjects with corticosteroid use and ELISPOT assessment tested positive in the overall capsid pool after BEQVEZ infusion.
- During LTFU (Cohort 1, Year 2 through 6), 8 subjects had ALT elevation above ULN, among which, 2 (25%) had positive ELISPOT to AAV capsid peptide. No

corticosteroids were used to treat the ALT elevations. As of the data cutoff, ALT returned to normal in 7 subjects and 1 subject completed the study with ALT above ULN.

Overall, it is difficult to determine the incidence and timing of T-cell response following BEQVEZ infusion because the test was performed only if subjects were treated with corticosteroids for presumed T-cell response. ELISpot results did not show a trend of presumed T-cell response (based on limited positive ELISpot analysis) as a function of time during the 1-year post-infusion period in either the Phase 3 or Phase 1/2a clinical studies.

5. Clinical Pharmacology Labeling Comments

Section 2.1 Dose

The recommended dose of BEQVEZ is a single-dose intravenous infusion of 5×10^{11} vector genomes per kg (vg/kg) of body weight.

To determine the patient's required dose, the following calculation steps are needed:

1. Calculation of patient's dose weight

The dosing of BEQVEZ is based on the patient's body mass index (BMI) in kg/m^2.

Patient's BMI	Patient's Dose Weight
≤ 30 kg/m^2	Dose Weight = Actual body weight
>30 kg/m^2	Determine using the following calculation: Dose Weight (kg) = 30 kg/m^2 × [Height (m)]^2

2. Calculation of patient's dose volume in milliliters (mL)

Dose weight in kilograms (kg) divided by 20 = dose in mL

The division factor 20 represents the amount of vector genomes per mL of the BEQVEZ solution (1×10^{13} vg/mL) divided by the per kilogram dose (5×10^{11} vg/kg).

Reviewer comments: The applicant proposed dose is acceptable based on the submitted clinical pharmacology information.

Section 5.4: Monitoring Laboratory Tests

Reviewer comments: The following summary of laboratory assays for FIX Assays are recommended by CMC and clinical pharmacology team.

Factor IX Assays:

When using an in vitro activated partial thromboplastin time (aPTT)-based one-stage clotting assay (OSA) for determining factor IX activity, plasma factor IX activity results can be affected by both the type of aPTT reagent, and the reference standard used in the assay. Higher inter-laboratory and inter-reagent variability in OSA results is observed at the lower factor IX activity levels (0.025 IU/mL). This is important to consider particularly when changing the laboratory and/or reagents used in the assay. Therefore, it is recommended where possible to use the same laboratory (applicable to both, chromogenic or one-stage assays) for factor IX activity monitoring over time, particularly during the timeframe for corticosteroid treatment decision making, to minimize the impact of inter-laboratory variability *[see Dosage and Administration (2.4)]*.

In the clinical study with BEQVEZ, silica-based OSA returned consistently higher values of factor IX activity compared to ellagic acid-based OSA and CSA. Generally, values of

the ellagic acid-based OSA aligned with values of CSA *[see Table 6, Clinical Pharmacology (12.2)].*

Based on clinical trials (central laboratory), the approximate conversion factor between a silica-based OSA and ellagic acid-based OSA/CSA is 2. For example, a factor IX activity level of 10 IU/dL using CSA calculates approximately to a level of 20 IU/dL using silica-based OSA. At low factor IX activity levels (0.05 IU/mL) the conversion factor is approximately 2.5.

Section 8.3 Females and Males of Reproductive Potential

Vector DNA was shed in semen but declined to undetectable levels in semen within a mean of 1 to 4 months after infusion. Male patients should refrain from donating sperm, be abstinent or use a male condom for up to 6 months after receiving TRADENAME [see Clinical Pharmacology (12.3)].

Reviewer comments: The vector   DNA shedding in semen is acceptable based on the submitted clinical pharmacology information.

Section 12.   Mechanism of Action

Reviewer comments: We have updated the MOA by including data supported statements.

Section 12.2 Pharmacodynamics

Reviewer comments: The following are clinical pharmacology labeling recommendation for section 12.2:

- Include only data supported statements.

- Remove information referring to percent subjects achieving 5% FIX activity since the clinical implication of a FIX threshold of 5% for AAV-based hemophilia gene therapy products including BEQVEZ is not fully understood.

- Include FIX activity at Week 4 in the longitudinal description of FIX activity over time up to Month 24.

- Include subsection for "Specific population" for summarizing FIX activity data.

- The FIX activity results from Phase 1/2a study were summarized without including information on durability and efficacy since the results are not consistent with the Phase 3 study. We noted about 2-fold lower FIX activity in Phase 3 study. The Phase 1/2a study results are also included for viral kinetic assessment and for population pharmacodynamic analysis of FIX activity to inform dosing.

Section 12.3 Pharmacokinetics

Reviewer comments: The following are clinical pharmacology labeling recommendation for section 12.3:

- Include a subsection for "Biodistribution (within the body) and Vector Shedding (excretion/secretion)"

- Remove unnecessary technical laboratory terms used for describing the bioassay.

- Include estimate of peak level and Tmax based in sub-group of patients that provided a more frequent sampling at early timepoints.

Section 12.6. Immunogenicity

Reviewer comments: Requested to provide a summary on anti-AAVRh74var antibodies and cellular immune response.

6. Comprehensive Clinical Pharmacology Review

6.1.   General Pharmacology and Pharmacokinetics

BEQVEZ is based on recombinant DNA technology that consists of a recombinant viral capsid (AAVRh74var) derived from a naturally occurring AAV serotype (Rh74) packaging vector containing the human coagulation factor IX (FIX) transgene modified to a high-specific factor IX activity variant known as FIX-R338L. The AAVRh74var capsid transduces hepatocytes, the natural site of factor IX synthesis. Single intravenous infusion of BEQVEZ results in cell transduction and increase in circulating Factor IX activity in patients with Hemophilia B.

The clinical pharmacology assessment includes pharmacokinetics (i.e., viral vector biodistribution and shedding), pharmacodynamics (i.e., FIX expression and FIX activity), as well as immunogenicity assessment (i.e. humoral and cellular immune responses against the AAVRh74var capsid or FIX protein).

6.1.1. Dosing

The dose selection for the Phase 1/2a was supported by safety and FIX activity levels from non-clinical studies in nonhuman primate (NHP). Doses up to 5×10^{12} vg/kg of the predecessor product (AAVRh74var-hFIX19-R338) was infused in NHP studies with acceptable safety. This dose level was approximately 10-fold higher than the selected clinical dose of 5×10^{11} vg/kg in Phase 1/2a study (C0371005).

In Phase 1/2a (C0371005) and Phase 3 (C0371002) studies, a single intravenous infusion of BEQVEZ was administered on Day 1 at a dose of 5×10^{11} vg/kg of body weight based on actual lot concentration (nominal strength is 1×10^{13} vg/mL). For subjects with BMI >30 kg/m^2, dose was calculated based on an adjusted body weight determination that assumes a maximum permissible BMI of 30 kg/m^2 (i.e., the actual height is used to achieve a BMI of 30kg/m^2). For example, for 187.96 cm (6'2") height and 167.8 kg weight

(BMI 47.5 kg/m^2) dose will be based on 106.1 kg, which is the weight associated with a BMI of 30 kg/m^2 for a 187.96 cm (6'2") tall individual.

- Phase 1/2a (C0371005): Out of 15 participants, 11 had BMI ≤30 kg/m2 and 4 had BMI >30 kg/m^2
- Phase 3 (C0371002): Out of 45 participants, 29 had BMI ≤30 kg/m2 and 16 had BMI >30 kg/m^2

The median dose of BEQVEZ administered on a vg/kg basis was similar for both studies and across the pooled analysis.

- Study C0371002/C0371005: The median dose infused (vg/kg) for subjects with BMI ≤30 kg/m2 and was $5×10^{11}$ vg/kg (range: $4.91×10^{11}$ to $5.6×10^{11}$ vg/kg) and for subjects>30 kg/m2 it was $4.93×10^{11}$ vg/kg (range: $3.09×10^{11}$ to $6.13×10^{11}$ vg/kg).
- Median total dose infused (vg) for C0371002/C0371005 subjects with BMI ≤30 kg/m^2 and BMI >30 kg/m^2 was $3.8 ×10^{13}$ vg (range: $2.67×10^{13}$ to $4.86×10^{13}$ vg) and $4.78 ×10^{13}$ vg (range: $3.99×10^{13}$ to $5.9×10^{13}$ vg), respectively.

Reviewer comments: In the USPI the applicant proposed a single-dose intravenous infusion of $5 × 10^{11}$ vg/kg with dose adjustment for subjects with BMI >30. The BMI based dose adjustment is acceptable and the dose for the two BMI group were comparable. To justify (b) (4) **-based dosing the applicant provided additional CMC, clinical and clinical pharmacology analysis. The applicant analysis and justification for** (b) (4) **-based dosing is acceptable from clinical pharmacology perspective (section 6.2.4 and 7.3).**

6.1.2. Viral vector biodistribution and shedding

In Phase 1/2a study(C0371005), the first 10 subjects were dosed with manufacturing Process (b) (4) material while a subsequent 5 subjects were dosed with manufacturing

Process^{(b) (4)} material. Biodistribution and viral vector shedding after a single IV infusion of BEQVEZ at a dose of 5×10^{11} vg/kg was assessed in PBMC, saliva, urine, semen, and serum. Samples were collected at multiple timepoints, and full clearance of vector DNA was defined as having 3 consecutive negative results (i.e., below quantification level, BQL).

The levels of vector shedding were generally highest during the first 2 weeks after vector infusion. The median (min, max) values during this 2-week period were:

- 3.16×10^4 (4.80×10^3, 4.03×10^5) copies/ug in PBMC,
- 7.13×10^4 (1.63×10^4, 7.13×10^5) copies/mL in saliva,
- 1.44×10^4 (NA, 3.61×10^5) copies/mL in semen
- 9.04×10^5 (6.55×10^4, 2.91×10^6) copies/mL in serum.

The levels gradually declined in all types of specimens over time. The median level in urine was below quantification limit (BQL) with maximum urine level of 3.57×10^3. In general, PBMCs were the slowest specimen type to achieve vector clearance ranging from 119 days to not fully cleared by the end of study. This was due to 3 of 15 subjects not having 3 consecutives negative PBMC sample results that were required within the study duration to be declared as fully cleared. For the remaining 12 participants, the mean (SD) time to undetectable vector in PBMCs was 201.5 (55.4) days, ranging from 119 to 343 days. Of note, for all three participants, either Week 26 or Week 32 was the last timepoint in the study with a result > BQL. Subsequently, results from Week 78 and a longer-term unscheduled visit also were BQL; however, these timepoints were not protocol defined to confirm 3 consecutives measurements below BQL. All the 3 subjects subsequently met criteria for full clearance in PBMC when tested in the long-term follow-up study #C0371003. In study #C0371003, the median [Min, Max] time to last negative result for PBMC (n=14) was 330.5 days [175, 644]. One out of 15 subjects were not included in the long-term follow-up study.

The vector was fully cleared by the end of study, with the range from 21 to 154 days for serum, 21 to 54 days for saliva, 10 to 84 days for semen and 5 to 49 days for urine. All but 3 subjects reached full clearance in all specimen types by the end of 52 weeks in the study #C0371005, but all subjects had reached the first BQL result by the end of the study.

In Phase 3 study (C0371002), Process $^{(b)}_{(4)}$ material was administered to the first 3 participants. Additionally, Process$^{(b)\ (4)}$ material, which is the intended commercial process was administered to the subsequent 42 subjects in the study. Viral vector shedding after a single IV infusion of BEQVEZ at a dose of 5×10^{11} vg/kg was assessed for PBMC, saliva, urine, semen, and plasma at multiple time points and full clearance of vector DNA was defined as having 3 consecutive negative results (i.e., BQL) for the given sample type confirmed using the validated qPCR assay. A subgroup of subjects(n=17) had additional early samples collected (2-, 24-, and 72-hours post-infusion) to further characterize the kinetics of vector shedding. This more detailed analysis (i.e., optional more frequent sample collection at early time points) was conducted in 17 subjects in the Vector Shedding sub-study Analysis Set to better define parameters such as peak vector DNA concentrations (Cmax) and time to peak concentrations (Tmax). The mean (SD) of Cmax, and Tmax were summarized as follows:

- Plasma: Cmax = 5.3×10^9 (4.2×10^9) and Tmax = 1.2 (0.4) days
- PBMC: Cmax= 7.3×10^5 (1.1×10^6) and Tmax = 7.4 (22) days
- Semen: Cmax= 4.5×10^5 (6.3×10^5), and Tmax= 3.8 (3.7) days
- Saliva: Cmax= 1.5×10^7(1.9×10^7) and Tmax = 1.2 (0.4) days
- Urine: Cmax= 4.8×10^4 (9.2×10^4) and Tmax = 1.6 (0.5) days

Reviewer comments: The early frequent sampling obtained from 17 subjects allowed to obtain more reasonable values for Cmax and Tmax. It is important to

note high viral vector transfer to saliva and semen within 4 days following intravenous administration of BEQVEZ.

In the Dosed analysis (n=45 subjects), peak levels of vector DNA occurred within the first two weeks after infusion. High vector DNA concentrations were found in plasma. Vector DNA declined to undetectable levels in plasma, saliva, and semen within 1-4 months after infusion and PBMC was slowest to clear to undetectable levels within 7 months. In urine, peak vector DNA concentration was very low relative to plasma and declined to undetectable levels within 3 weeks after infusion. Full clearance, defined as 3 consecutive negative results (i.e., below quantification limit) in a particular matrix, was observed in all participants. In general, PBMCs were the slowest to clear with a mean (SD) time to last undetectable vector of 163.3 (109.4) days in the 45 subjects included in the Dosed Analysis Set. The mean (SD) time to vector clearance for other matrix is as follows:

- Plasma: 98.6 (54.7) days

- Semen: 41.1 (20.1) days

- Saliva: 42.4 (17.2) days

- Urine: 20.4 (13.3) days

Overall based on the combined data (C0371005/C0371002) the mean time to reach full vector DNA clearance was longest in PBMC (364 Days). The vector DNA clearance from plasma was the second longest with a full clearance reached by mean of 98.6 days.

Reviewer comments: The applicant provided scientific literatures-based evidence to support longer persistence of AAV in whole blood. These published studies suggest that the observed longer persistence in PBMCs could be due to the potential of AAV to persist in human leukocytes and other cellular fractions.

6.2.   Pharmacodynamic Assessments

6.2.1. FIX Activity and Protein Expression

FIX antigen was measured in studies C0371002, C0371005, and C0371003 using the FIX Antigen (b) (4)   kit. One-stage clotting assay using Actin-FSL reagent was used to measure FIX in Phase 1/2a study and in the Phase 3 study. Two additional FIX activity assays (one-stage assay using the (b) (4)   SynthASil reagent and chromogenic assay) were also used for Phase 3 study (#C0371002).

Reviewer comments: Per the CMC assay reviewer the validation of these assays is acceptable for characterizing FIX activity following administration of BEQVEZ (See CMC memo on the validation of the FIX assay). From clinical pharmacology perspective FIX activity results were described separately for each of the three assays.

In Phase 1/2a study (C0371005), FIX activity was monitored twice weekly or thrice-weekly in Weeks 1-8, and once in Weeks 10, 12, 14, 16, 18, 22, 26, 32, 42 and 52 using a one-stage clotting assay with Actin-FSL. Steady-state was nominally defined as Week 12 to 12 months after infusion, and steady-state FIX:C levels were calculated as a geometric mean of individual geometric mean transgene-derived FIX activity levels during this time, considering adequate washout from FIX replacement product. Washout was 96 hours for plasma-derived or recombinant FIX, or up to 168 hours washout for extended half-life recombinant FIX. Following a single infusion, transgene-derived FIX levels of 5% were achieved in all subjects around Week 1. The steady-state FIX activity was above 5% of normal in all subjects and the steady-state FIX activity levels were reached by approximately Week 12 (Table 1). The geometric mean for FIX:C was 22.9% of normal, ranging from 9% to 46%. One [6.7%]) participant reached geometric mean for FIX:C

activity level ≥40% of normal. The mean peak FIX:C activity during the 52-week follow-up was 29.1% of normal, ranging from 11% to 58%.

During long-term follow-up period (Study #C0371003), FIX activity was monitored every 13 weeks to Week 156 and every 26 weeks to Week 312 using the central one-stage clotting assay with Actin-FSL. As of data cut, geometric mean (SD) of transgene-derived FIX activity levels were 24.7% (14.4%), 22.9% (13.7%), 22.3% (13.1%), 22.5% (15.3%) and 24.5% (18.1%) during Years 2 (N=14), Year 3 (N=14), Year 4 (N=13), Year 5 (N=13), and Year 6 (N=7) post vector infusion, respectively.

The FIX antigen expression appears higher than the FIX activity, but it is important to note the higher variability of FIX antigen level. The mean ratio of FIX antigen to FIX activity range from 1.1 to 4.7 (Table 1).

Reviewer comments: **Exogenous treatment with FIX replacement therapy and the proposed washout period (96- hours for plasma -derived or recombinant FIX, or up to 168 hours washout for extended half-life recombinant) may not be adequate for some FIX products with extended half-life. We requested to re-evaluate FIX activity data with updated washout period (i.e., by excluding FIX activity data within 5 times the half-life of the exogenous FIX products). As shown in Table 1, the longitudinal FIX activity per protocol (i.e., original analysis) and updated analysis (i.e., by excluding FIX activity data within 5 times the half-life of exogenous products) were essentially identical indicating that the proposed washout period is adequate.**

In Phase 3 study (C0371002), at baseline, mean (±SD) FIX antigen levels were 6.6 (5.3). At Week 12 post-BEQVEZ infusion, mean (±SD) FIX antigen levels were 31 (41). At Week 52 post-BEQVEZ infusion, mean (±SD) FIX antigen levels remained stable at 25 ±31. The overall FIX antigen level along with FIX activity is displayed in Figure 1. The underlying

hemophilia mutations may confound interpretation of these results as some patients can have detectable levels of antigen at baseline due to the nature of the mutation.

Reviewer comments: It is difficult to interpret the results of FIX antigen level due to the high variability and limited baseline levels (n=5). In general, the FIX expression appears to peak within 3-6 weeks and remain stable with high variability during the follow-up period like the results in Phase 1 study.

In Phase 3 study (C0371002), FIX activity data were analyzed by imputing 1.9% for six subjects (13%) that resumed FIX prophylaxis per the primary analysis plan. Samples taken within 7 days or 14 days if EHL product is used as exogeneous FIX replacement therapy are not included in the assessment of FIX:C post IP-infusion. Additional analysis was performed excluding FIX activity data within 5xhalf-life of the exogenous FIX replacement therapy and showed comparable FIX activity between the protocol defined washout and updated analysis with excluding values within 5xhalf-life of the exogenous FIX replacement therapy. Post-treatment geometric mean for FIX activity from Week 12 to Month 15 from all three assays was significantly higher than the fixed threshold of 5% for the three assays (p-value<0.0001). FIX activity values were also significantly higher than the fixed threshold of 5% for all assays during Year 2 and Year 3 in the Dosed Analysis Set. The data after three years were limited as only 2 subjects were followed beyond Year 3. The mean (SD) FIX activity by the three assay methods is summarized in Table 2.

Table 1: FIX Activity by One-stage Assay (Actin-FSL) Observation for Study C0371005/1003 (Dosed Analysis Set)

	FIX Activity (Original Analysis[a])	FIX Activity (Excluding 5 half-lives)	FIX Antigen (Excluding 5 half-lives[b])
Week 4 (Month 1), n	15	14	14
Mean (SD)	14.73 (7.742)	13.61 (4.442)	26.82 (30.252)
Median (Min, Max)	12.40 (7.4, 38.6)	12.39 (7.0, 22.0)	13.00 (1.0, 93.5)
Week 12 (Month 3), n	15	15	15
Mean (SD)	21.58 (9.761)	21.45 (9.515)	32.32 (36.194)
Median (Min, Max)	20.60 (5.0, 42.2)	20.25 (5.6, 42.2)	12.50 (1.0, 103.5)
Week 26 (Month 6), n	15	14	14
Mean (SD)	23.56 (10.527)	23.83 (10.844)	30.46 (33.093)
Median (Min, Max)	20.10 (9.2, 50.6)	20.55 (9.2, 50.6)	13.00 (0.9, 89.0)
Month 12, n	15	15	15
Mean (SD)	25.70 (12.786)	25.75 (12.733)	29.53 (31.518)
Median (Min, Max)	21.70 (8.4, 54.8)	21.70 (8.4, 54.8)	12.00 (0.9, 93.0)
Month 15, n	9	9	9
Mean (SD)	27.88 (13.518)	27.88 (13.518)	33.78 (41.185)
Median (Min, Max)	23.50 (11.7, 49.5)	23.50 (11.7, 49.5)	15.00 (2.0, 110.0)
Month 18, n	13	13	13
Mean (SD)	25.97 (13.958)	25.97 (13.958)	34.23 (31.594)
Median (Min, Max)	20.40 (8.4, 55.4)	20.40 (8.4, 55.4)	32.00 (1.0, 85.0)
Month 24, n	14	14	14
Mean (SD)	24.93 (14.982)	24.93 (14.982)	37.92 (40.556)
Median (Min, Max)	22.05 (2.1, 52.8)	22.05 (2.1, 52.8)	24.50 (0.9, 119.0)
Month 36, n	13	13	13
Mean (SD)	22.59 (13.525)	22.59 (13.525)	35.46 (34.454)
Median (Min, Max)	16.20 (8.0, 46.7)	16.20 (8.0, 46.7)	25.00 (1.0, 92.0)
Month 48, n	11	11	11
Mean (SD)	21.46 (13.522)	21.46 (13.522)	40.64 (37.879)
Median (Min, Max)	13.30 (9.6, 44.0)	13.30 (9.6, 44.0)	30.00 (2.0, 92.0)
Month 60, n	8	8	8
Mean (SD)	22.38 (17.245)	22.38 (17.245)	47.00 (47.482)
Median (Min, Max)	17.00 (4.6, 51.8)	17.00 (4.6, 51.8)	34.00 (2.0, 126.0)
Month 72, n	5	5	4
Mean (SD)	21.46 (18.778)	21.46 (18.778)	61.50 (54.114)
Median (Min, Max)	11.10 (6.7, 52.1)	11.10 (6.7, 52.1)	55.50 (2.0, 133.0)

Note: a. For C0371005/C0371003, any samples taken within 4 days (7 days if EHL product is used) of exogeneous FIX replacement therapy are not included in the summary of FIX:C post IP-infusion. b. Any samples taken within 5 half-lives of exogeneous FIX replacement product are not included in the summary of FIX:C or FIX antigen. Source: Response to clin pharm IR#1; Table 2

Figure 1: Mean ± SE Plot of FIX Activity and FIX Antigen Over Time in C0371002 – Dosed Analysis Set
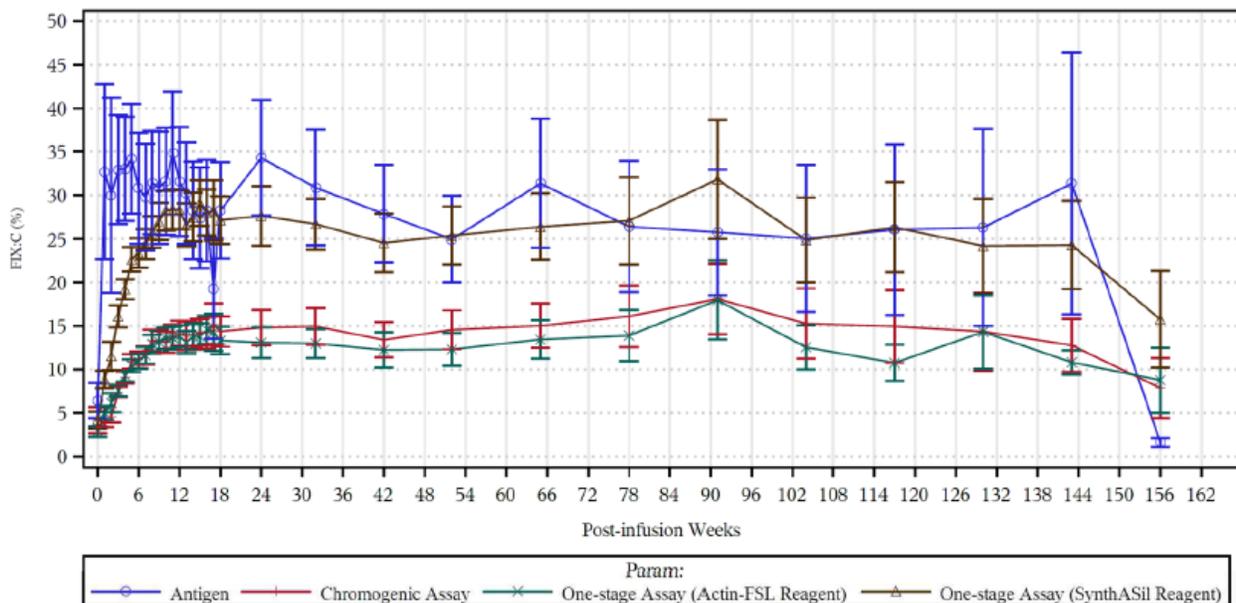
Source: Response to clin pharm IR#1; Figure 1

Table 2: Summary Mean (SD) FIX Activity by Assay for Study C0371002

|	Actin-FSL reagent	SynthASil reagent	Chromogenic assay
Week 4	9.25 (4.4), n=42	19.18 (7.5), n=42	9.08 (4.5), n=43
Week 12	13.52 (8.1), n=43	27.79 (15.2), n=44	13.91 (9.3), n=44
Month 6	13.1(11.1), n=41	27.67 (21.3), n=39	14.83 (13), n=40
Month 15	13.10 (12.8), n=34	27.57(21.3), n=35	15.82 (17), n=35
Month 24	12.67 (11.9), n=22	25.00 (22.6), n=22	15.40 (18.8), n=22

Source: Table 8; Module 2.5

Reviewer comments: In Phase 3 study, the FIX activity by one-stage (actin-FSL) and chromogenic assay were about 2-fold lower as compared to one-stage (SynthASil) assay (Table 2). In the USPI, we recommended including a conversion factor between the assays. The clinical implication of establishing a FIX threshold of 5% for AAV-based hemophilia gene therapy products including BEQVEZ is not fully understood. Also, the applicant assumed a "stable-level" was achieved within 3-12 months, but we noted a high variability in FIX activity and in some subjects FIX activity continues to increase after 1-year. Considering the high variability in summary statistics of FIX activity (Table 2) we closely looked at longitudinal plots using the one-stage assay (Actin-FSL) for individual subjects and grouped the results into the following three scenarios:

- FIX activity in some subjects was below 10% and the level can vary between 1 to 10% (Figure 2).
- A stable FIX activity ranging between 15-30% was achieved in some subjects (Figure 3).
- A FIX activity ranging between 40-80% was achieved in some subjects. In some subjects a stable level may not be achieved as FIX activity continue to increase for over 2-years of follow-up period (Figure 4).

Overall, the high inter-individual variability in FIX activity with a potential for <2 % or in some cases with continuous increase warrant monitoring of FIX activity and bleeding outcome. The USPI recommend monitoring of FIX activity.

Figure 2: Factor IX Activity Plots vs. Time for Representative Subjects with Factor IX Activity below 10% (Study C0371002; One-stage Actin-FSL)
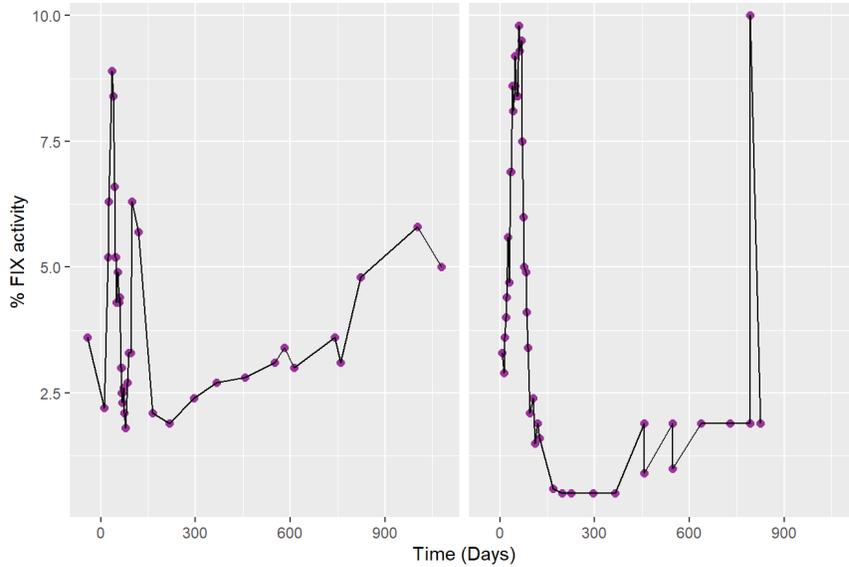
Source: FDA Reviewer analysis

Figure 3: Factor IX Activity Plots vs. Time for Representative Subjects with a Stable FIX Activity in the range of 15- 30% (Study C0371002; One-stage Actin-FSL)
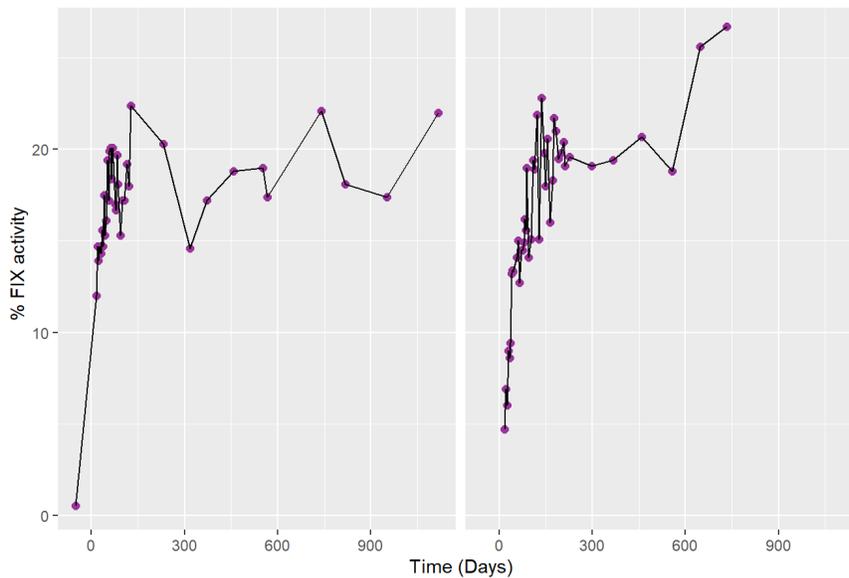
Source: Reviewer analysis

Figure 4: Factor IX Activity Plots vs. Time for Representative Subjects with a FIX Activity above 40% (Study C0371002; One-stage Actin-FSL)
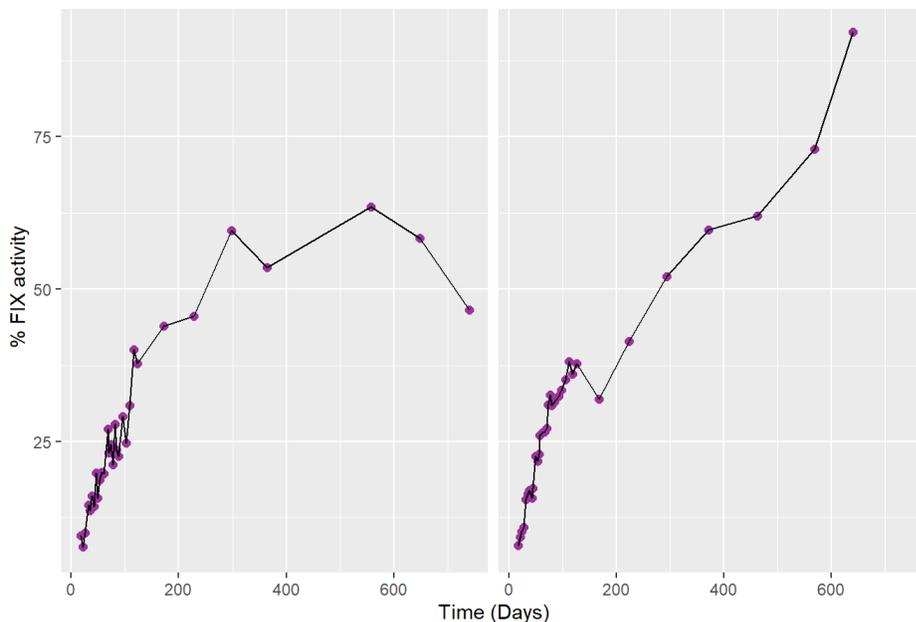
Source: Reviewer analysis

6.2.2. Comparative FIX activity Across Studies

The one-stage assay with Actin-FSL is used for comparative analysis since this is the only assay used for study C0371005/C0371003). The Mean (SD) FIX activity (%) at Week 12 and Month 24 are summarized below and displayed graphically in Figure 5:

- Week 12: 13.5 (8.13) (C0371002; n=43) and 21.4 (9.51) (C0371005/C0371003; n=15)
- Month 24: 12.7 (11.88) (C0371002; n=22) and 24.9 (14.98) (C0371005/C0371003; n=14)

Reviewer comments: The comparative analysis of FIX activity for the two studies showed about 2-fold lower FIX activity in the Phase 3 study as compared to the results obtained from the Phase 1/2a. We requested the applicant to provide

justification for the observed the difference in FIX activity for the two studies. Based on the applicant response combination of factors (e.g., age, sample size, manufacturing, cellular immune response, etc.) may have contributed to the observed discrepancies in FIX activity. The applicant response is summarized below.

The observed post-treatment FIX activity values based on one-stage Actin-FSL assay for Study C0371002 were lower than those observed in Study C0371005/C0371003. Identifying a single root cause for this observed difference is confounded by several differences between the two studies. Discussion of some aspects that were different between these two studies is provided below.

- Age of study population: The median (min, max) age of subjects in Study C0371002 were 29 (18, 62) years. The median (min, max) age of subjects in Study C0371005 were 42 (18, 61) years, respectively. A trend of higher mean FIX activity was observed with increased age (See subgroup analysis for details).

- Sample size: A larger participant pool (n=45) was dosed in the Phase 3 Study C0371002 compared to a smaller one (n=15) in Study C0371005.

- (b) (4) manufacturing process: Study C0371005 used Process (b) (4) and Process (b) (4) whereas the intended commercial Process (b) (4) was primarily used in Study C0371002.

- Presumed cellular immune response: In Studies C0371002 and C0371005/C0371003, 31 subjects received corticosteroids due to suspected cellular response to AAVRh74var capsid manifesting as elevations in transaminases and/or decline in FIX activity level. Elevation in liver transaminases resolved with corticosteroids within 120 days of BEQVEZ infusion. Additionally, comparison of time to corticosteroid initiation, duration of corticosteroid use and

time to corticosteroid wean did not show clinically significant differences between data of subjects in Study C0371002 and subjects in Study C0371005/C0371003.

- Proportion of subjects resuming prophylaxis: In the Dosed population, 6 (13.3%) subjects in Study C0371002 subjects resumed prophylactic treatment. No clear trend has emerged that would suggest a population/characteristic that would increase the probability of a participant to resume prophylaxis; however, the small sample size precludes a definitive conclusion. Mean (SD) time to treatment resumption was 337.7 (169.82) days, with the earliest resumption of exogenous FIX at Day 155 post BEQVEZ infusion. No subjects in C0371005/C0371003 resumed prophylaxis treatment.

Figure 5: Box-Whisker Plot of FIX:C One-stage Assay (Actin-FSL Reagent)
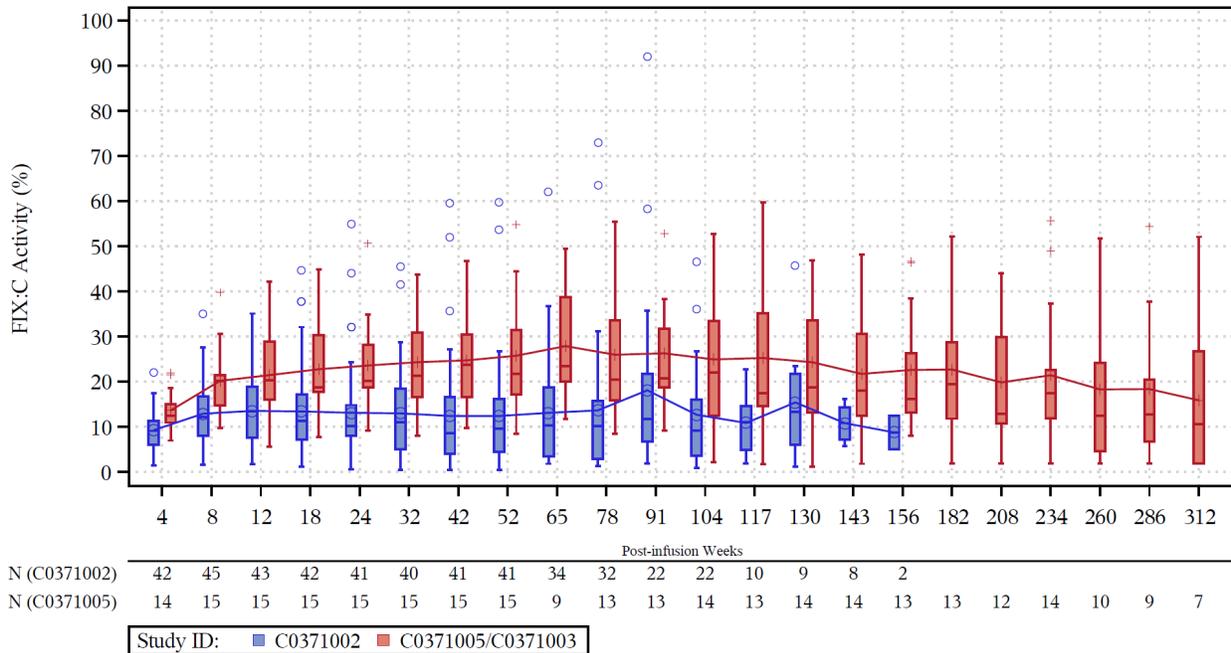
Source: Figure 14.2.2.1; appendix-integrated-tables-figures

6.2.3. FIX activity in Specific Population

Descriptive subgroup analysis for FIX activity (using the one-stage Actin-FSL reagent) was conducted for age, BMI, region, race, ethnicity, country, and corticosteroid use. Additionally, exploratory statistically analysis for intrinsic and extrinsic factors were evaluated using population pharmacodynamic model (See Section 6.2.4).

Age: In analysis of post-treatment geometric mean of FIX:C with imputation from Week 12 to Month 15 by age across the studies (C0371002 and C0371005/C0371003, and C0371002 +C0371005/C0371003), a trend of higher mean FIX:C was observed with increased age.

- In the pooled analysis (C0371002 + C0371005/C0371003), the mean (SD; 95% CI) FIX:C in subjects <35 years (n=33) was 11.4 (6.4; 95% CI 9.1, 13.6) compared to 20.1 (12.3; 95% CI 15.2, 24.9) in subjects ≥35 years of age (n=27). The mean (SD; 95% CI) FIX:C in subjects<45 years (n=47) was 13.2 (8.9; 95% CI 10.6,15.8) compared to 22.9 (11.9; 95% CI 15.7, 30.1) in subjects ≥45 years of age (n=13).
- In C0371002, the mean (SD; 95% CI) FIX:C in subjects <35 years (n=28) was 10.27 (5.98; 95% CI 7.95, 12.59) compared to 16.47 (11.53; 95% CI 10.55, 22.40). in subjects ≥35 years of age (n=17). The mean (SD;95% CI) FIX: in subjects <45 years (n=38) was 10.98 (7.06; 95% CI 8.66, 13.30) compared to 21.51 (12.93; 95% CI 9.55, 33.46) in subjects ≥45 years of age (n=7).

BMI: In analysis of post-treatment geometric mean for FIX activity from Week 12 to Month 15 by BMI across the studies (C0371002 and C0371002 +C0371005/C0371003), a trend of higher mean FIX:C was observed with increased BMI.

- In the pooled analysis (C0371002+C0371005/C0371003), the mean (SD; 95% CI) FIX:C in subjects >30 kg/m2 (n=20) was 16.1 (9.7; 95% CI 11.6, 20.7) compared to 14.9 (10.8; 95% CI 11.4, 18.3) in subjects ≤30 kg/m2 (n=40). The mean (SD;

95% CI) FIX:C in subjects ≥25 kg/m2 (n=39) was 17.2 (11.30; 95% CI 13.5, 20.9) compared to 11.7 (7.3; 95% CI 8.4, 15.1) in subjects<25 kg/m2 (n=21).

- In C0371002, the mean (SD; 95% CI) FIX:C in subjects >30 kg/m2 (n=16) was 14.9 (10.30; 9% CI 9.4, 20.4) compared to 11.4 (8.0; 95% CI 8.3, 14.4) in subjects ≤30 kg/m2 (n=29). The mean (SD) FIX:C in subjects ≥25 kg/m2 (n=29) was 14.20 (9.6; 95% CI 10.5, 17.9) compared to 9.7 (6.8; 95% CI 6.1, 13.3) in subjects <25 kg/m2 (n=16).

It is important to note that continuous factors such as body weight, BMI and total number of vector genomes administered (i.e. dose) were correlated with each other inherent to weight-based dosing of AAV-based gene therapies. An exploratory linear regression analysis of geometric mean FIX activity vs age, bodyweight, BMI and vector dose are graphically displayed in Figure 6, and summarized as follows:

- FIX activity increased with increasing age. The estimate of slope for the effect of age on geometric mean FIX activity suggested a statistically significant relationship (95% CI did not include zero).

- Although there was a trend with FIX activity increasing with increasing body weight and BMI, the estimate of slope for the effect of body weight or BMI on geometric mean FIX activity was not statistically significant (95% CI included zero).

- Although there was a trend with FIX activity increasing with increasing total number of vector genomes administered, the estimate of slope for the effect of total number of vector genomes administered on geometric mean FIX activity was not statistically significant (95% CI included zero).

Race and Ethnicity: In C0371002, the mean (SD) FIX:C at 15 months in White subjects (n=29) was 15.1 (15.8), and it was 9.4 (7.6) in Non-white subjects(n=12). The mean (SD) FIX:C at 15 months in Non-Hispanic or Latino subjects in (n=33) was 13.91 (14.6), and it was 11.3 (12.3) in other subjects(n=12).

Corticosteroid use: In 28 subjects in C0371002 with corticosteroid treatment and 17 subjects without corticosteroid treatment, the post-treatment geometric mean for FIX:C (mean [SD]) from Week 12 to Month 15 by assay was as follows:

- Actin-FSL reagent: 10.7 (8.0) and 15.8 (9.7)
- SynthASil reagent: 22.2 (14.7) and 32.0 (18.8)
- Chromogenic assay: 11.5 (9.3) and 16.7 (11.5)

It should be noted that in C0371002, the 28 subjects with corticosteroid treatment include the 6 subjects who resumed prophylaxis. FIX levels post-resumption for these subjects were imputed as 1.9%. In the pooled analysis (C0371002 and C0371005/C0371003), 31 subjects were treated with corticosteroid and 29 subjects were not. The post-treatment geometric mean for FIX:C (mean [SD]) from Week 12 to Month 15 (one-stage assay with Actin-FSL reagent) for subjects treated with corticosteroids was 12.1(10.1) and it was 18.7(9.7) for those who did not use corticosteroids.

6.2.4. Population Modeling of FIX Activity

A population pharmacodynamic (popPD) model was developed by pooling the FIX activity data from the Phase 1/2 & Phase 3 studies (see section 7.3 for details). The model integrates gene and protein expression dynamics using Hargrove-Schmidt model[1] (Figure 7):

- The model is a 2-compartment model that assumes information flow from deoxyribonucleic acid (DNA) to FIX via a translation constant, kt , and a first-order rate constant of synthesis, ksyn.
- Loss or degradation of the transgene is accounted for by a first-order degradation rate constant, kdeg.

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[1] Hargrove JL and Schmidt FH (1989). The role of mRNA and protein stability in gene expression. The FASEB Journal vol. 3: 2360–2370.

The popPD model was used to screen covariates such as age, BMI, manufacturing processes and concomitant corticosteroids. The following covariates were identified to significantly improve the popPD model prediction at a p-value <0.01 according to the likelihood ratio test (LRT):

- Age on *kdeg and ksyn*
- Concomitant corticosteroids on *ktr,d (see the appendix section 7.3 for details)*

In the clinical studies subjects were administered corticosteroids in response to suspected immune-related increases in ALT and AST. Such that, this covariate was considered descriptive and consistent with the study design with limited predictive utility and was not included in multivariate analyses. Following forward inclusion procedures, the effects of age on *kdeg* and *ksyn* were retained in the popPD model.

Reviewer comments: The final model reasonably described the observed FIX activity, but it systematically deviates from the observed data at later time-points. The proposed application of the modeling and simulation analysis was to provide additional justification for (b) (4) -based dosing of BEQVEZ. It should be noted that the primary evidence for justification of (b) (4) dosing was based on submitted CMC data and supporting clinical study from patients dosed with (b) (4) . Study C0371002 is currently dosing additional (b) (4) subjects to obtain clinical experience using (b) (4) for dosage calculation as requested by the FDA during the pre-BLA meeting. From the ongoing study, the FIX activity values for (b) (4) dosing were within the same ranges as those in subjects who were treated with product labeled with the actual concentrations of BEQVEZ. The applicant also provided popPD model-based analysis as supportive evidence for (b) (4) dosing. The popPD analysis is consistent with the results of the observed FIX activity results for both actual and (b) (4) dosing. Overall, the

popPD analysis is acceptable for covariate screening, and it provides supportive evidence for (b) (4) **-based dosing of BEQVEZ.**

Figure 6: Individual Geometric Mean FIX Activity (Week 12 to Month 15) versus Intrinsic and Extrinsic Factors (pooled analysis-C0371002+C0371005/C0371003)
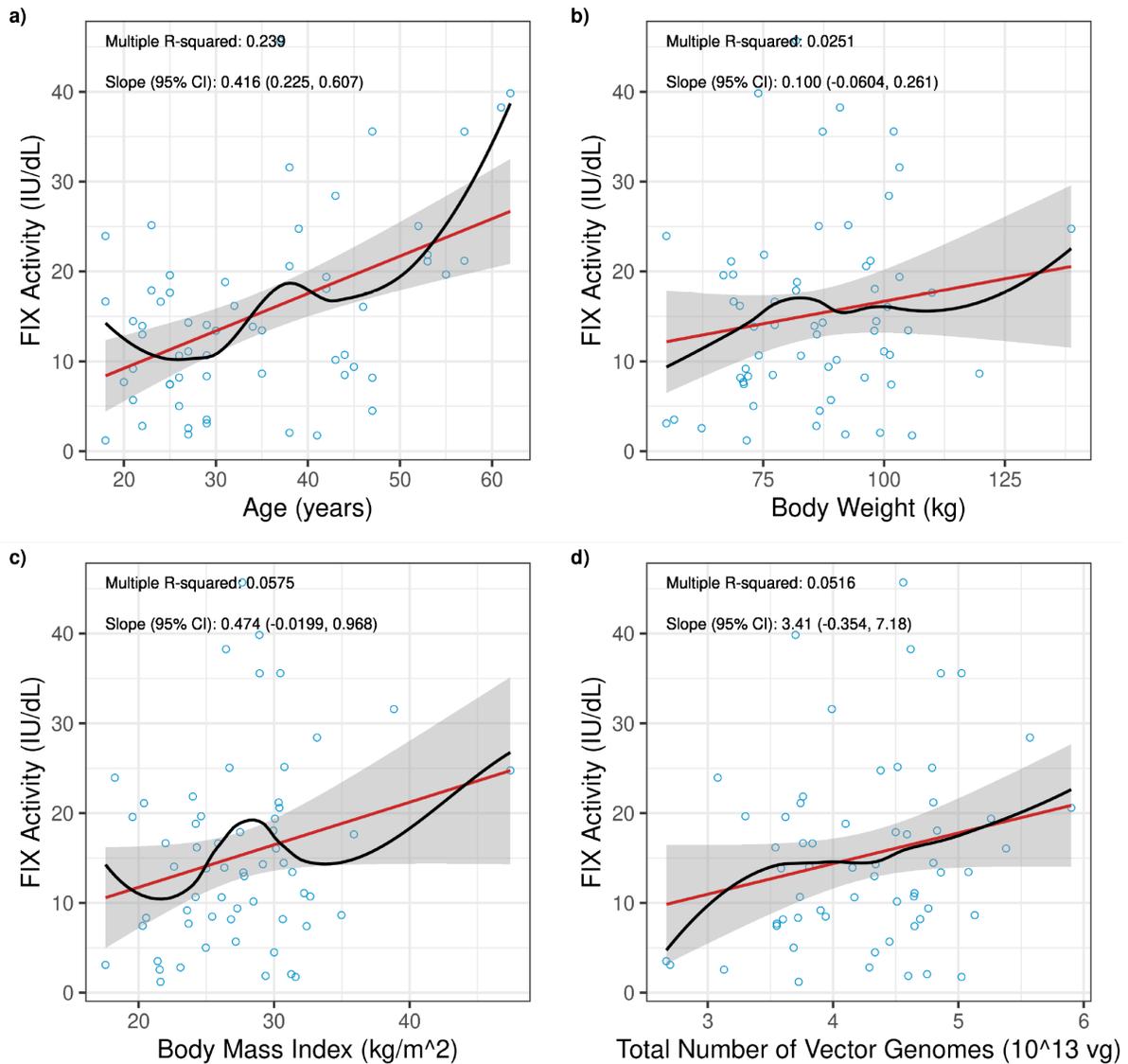
Source: Population modeling analysis report-add2; Figure 2

6.3. Immunogenicity Assessments

Prior to AAV-vector based gene therapy administration, some subjects can be exposed to wild-type AAV virus and may have circulating baseline neutralizing antibodies (nAb) that may jeopardize gene transduction and transgene synthesis. After AAV-vector based gene therapy administration, nAb are produced and expected to persist for several years. Also, the viral capsid and transgene product can activate T-cell response. Thus, clinical immunogenicity evaluation included post-treatment nAb against FIX protein (FIX inhibitors), anti-drug antibodies (ADA) to AAVRh74var capsid, nAb to AAVRh74var capsid, and potential T-cell response(s) to capsid and/or transgene product. The following immunogenicity summary is based the following 3 clinical studies:

- Registrational Phase 3 Study C0371002 (ongoing, data cutoff 16 Nov 2022)
- Phase 1/2a Study C0371005 (completed; also referred to as SPK-9001-101)
- LTFU Phase 2a Study C0371003 (ongoing, data cutoff 02 Nov 2022; also referred to as SPK-9001-LTFU-101)

FIX Inhibitors:

The development of FIX inhibitors, which are nAb against the FIX protein, is a serious complication that can arise in hemophilia B patients treated with FIX replacement therapies. The BEQVEZ clinical development program enrolled adult subjects at low risk of FIX inhibitor formation (i.e., with ≥50 exposure days of prior experience with FIX product, negative Bethesda assay at screening, and no prior history of FIX inhibitors). FIX inhibitors (nAb to native FIX or FIX replacement products) were measured in human plasma in Studies C0371002, C0371003, and C0371005 using Nijmegen-Bethesda

assay. Positivity for FIX inhibitor was defined as titer ≥0.6 BU/mL. No FIX inhibitor has been detected in any participant who received BEQVEZ infusion in Study C0371002 or C0371005 and its LTFU study (C0371003, Cohort 1) where subjects were monitored up to 6 years post-infusion.

Reviewer comments: These clinical studies included subjects with prior exposure with exogenous FIX products (≥50 exposure days). Thus, the development of FIX inhibitor following BEQVEZ infusion in subjects naïve to FIX replacement product is unknown.

ADA and nAb to AAVRh74var Capsid:

ADA against the AAVRh74var capsid was measured in serum in study C0371002 using (b) (4). At baseline, no subjects tested positive for ADA against AAVRh74var capsid. At 1-year following infusion of BEQVEZ, 39 (95.1%) subjects tested positive for ADA against AAVRh74var capsid with a median (min, max) titer of 68267.00 (9531.0, 546133.0). As of the data cutoff date, ADA positivity persisted for 2 of 2 (100%) subjects with ADA assessment at 3 years post-infusion. The median ADA titer at 3 years post-infusion was 56449 (range: 18042, 94855). FIX activities with the one-stage and chromogenic assays in the subjects positive for ADA against AAVRh74var capsid were comparable to the overall population at Week 52.

Neutralizing antibodies (nAb) activity against the AAVRh74var capsid was measured in human serum in Studies C0371002, C0371005, and C0371003. An initial antigen-specific (b) (4) assay was developed as a clinical grade assay for the determination of nAb titer in serum. Subsequently, (b) (4) anti-AAVRh74var nAb assay using recombinant AAV transduction to evaluate anti-AAVRh74var nAb activity in human serum was developed. Studies C0371002 and C0371005/C0371003 both had predefined

38

eligibility thresholds for nAb against AAVRh74var capsid (<1:1 in C0371002 and <1:5 in C0371005). All subjects had nAb titer below eligibility threshold at study entry. High nAb titers (up to 32768) were observed in all study subjects with nAb assessments following BEQVEZ infusion and it persisted up to 6 years post-infusion.

- In Study C0371002, 40 of 40 (100%) subjects with nAb assessment at Week 52 (1 year) post-infusion tested positive. Additionally, 2 subjects with samples collected at unplanned visits approximately 1-year post-infusion had positive nAb assessment, and the sample from Week 52 post-infusion visit was missing for 1 participant. nAb positivity persisted for 22 of 22 (100%) and 2 of 2 (100%) subjects with nAb assessment at 2- and 3-years post-infusion, respectively.

- In Study C0371005, 14 of 14 (100%) and 15 of 15 (100%) subjects with nAb assessment at 1- and 2-weeks post-infusion, respectively, tested positive. nAb positivity persisted for all 15 subjects through the 1-year post-infusion period. As of the data cutoff date, nAb positivity persisted up to 6 years post-infusion in the LTFU study (C0371003) for 5 of 5 (100%).

- Despite all study subjects developed high nAb post-infusion of after BEQVEZ the FIX activity from Week 12 to Month 15 was significantly higher than the proposed threshold of 5%.

T-Cell Responses

Potential T-cell responses directed against the transgene FIX protein, or the capsid may contribute to immunogenicity of BEQVEZ. For characterization of cellular immune response, reactive T-cells were measured using an enzyme-linked immunosorbent spot (ELISPOT) assay which relies on detection of cytokine secretion (in this case interferon-gamma).

Across all clinical studies, a tapering course of oral corticosteroids (i.e., prednisone/prednisolone) was the first consideration for suppression of presumed T-cell activation. Prednisolone/prednisone taper should not be started until ALT and/or AST have declined in at least 2 consecutive laboratory draws or have returned to approximately baseline (pre-infusion) levels and any decline in FIX:C activity has plateaued. Combined oral corticosteroids and IV corticosteroids (methylprednisolone) was recommended if there was no evidence of resolution of transaminase elevation while on oral corticosteroids treatment.

In Study C0371002, subjects were tested for IFN-γ ELISPOT prior to BEQVEZ infusion (i.e., at screening) and when corticosteroid treatment was given for presumed T-cell response (based on transaminase increase and/or FIX:C decrease).

- Twenty-eight of 45 (62.2%) subjects were treated with corticosteroids for presumed cellular immune response. The median time to corticosteroid initiation was 37.5 days (range, 11-123).

- At baseline, 12 of 23 (52.2%) subjects with corticosteroid use and ELISPOT assessment tested positive in the overall capsid pool and 11 of 23 (47.8%) tested positive in the overall FIX pool (includes WT, FIX polymorphism, and Padua).

- 10 of 28 (35.7%) subjects with corticosteroid use for presumed T-cell response had ELISPOT assessment prior to or within 24 hours of corticosteroid initiation. Prior to corticosteroid use, 4 of 10 (40.0%) subjects with ELISPOT assessment tested positive in the overall capsid pool and 3 of 10 (30.0%) subjects tested positive in the overall FIX pool. Approximately 3 weeks after corticosteroid treatment, 4 of 8 (50.0%) subjects with ELISPOT assessment tested positive in the overall capsid pool and 2 of 8 (25.0%) subjects tested positive in the overall FIX pool.

In Study C0371005/C0371003, subjects were tested for IFN-γ ELISPOT at prespecified time points and when corticosteroid treatment was given for presumed T-cell response (based on transaminase increase and/or FIX:C decrease). At baseline, none of the subjects with corticosteroid use tested positive in neither the overall capsid pool nor the overall FIX pool.

- 3 of 15 (20.0%) subjects were treated with corticosteroid for presumed T-cell response after BEQVEZ infusion. The median time to corticosteroid initiation was 57 (range; 35., 71) days post-infusion.

- 2 of 3 (66.7%) subjects with corticosteroid use and ELISPOT assessment tested positive in the overall capsid pool after BEQVEZ infusion. In 1 subject, ELISPOT values fell below the defined cutoff for positive following treatment with oral corticosteroids. The other subject had ELISPOT values normalize by the time corticosteroid treatment was initiated. The significance of these findings is unknown.

- During LTFU (Cohort 1, Year 2 through 6), 8 subjects had ALT elevation above ULN, among which, 2 (25%) had positive ELISPOT to AAV capsid peptide. No corticosteroids were used to treat the ALT elevations. As of the data cutoff, ALT returned to normal in 7 subjects and 1 subject completed the study with ALT above ULN.

In Studies C0371002 and C0371005/C0371003, some subjects experienced a presumed T-cell response to the AAVRh74var capsid, manifesting as transaminase elevation or FIX:C decrease, after BEQVEZ infusion. These cases (N=31 [51.7%]) were treated with corticosteroids (per study protocol) due to the potential immunologic etiology of these events. Six subjects treated with corticosteroid ultimately resumed FIX prophylaxis at which point the FIX activity was imputed as 1.9%. In studyC0371002, post-treatment geometric mean of FIX:C activity from Week 12 to Month 15 post-infusion was slightly

lower in subjects treated with corticosteroids compared to those not treated with corticosteroids (Actin-FSL reagent 1-stage assay: 10.69% versus 15.79%; SynthASil 1-stage assay: 22.16% versus 32.04%; chromogenic assay: 11.50% versus 16.75%).

Reviewer comment: **It is difficult to determine the incidence and timing of T-cell response following BEQVEZ infusion because the test was performed only if subjects were treated with corticosteroids for presumed T-cell response. About 50% of with evaluable ELISPOT had detectable circulating T cells that responded to capsid peptides. These data suggest that many cases of elevation in transaminases may be associated with an active immune response to capsid proteins. However, the limited data available for ELISPOT assay preclude a definitive conclusion.**

7. Appendix

7.1. Study#1- C0371005 (Phase 1) and C0371003 (Phase 2a)

Title: Gene Therapy, Open-Label, Dose-Escalation Study of SPK-9001 (Adeno-Associated Viral Vector With Human Factor IX Gene) in Subjects With Hemophilia B

Objectives: The primary objective of the study is to evaluate the safety and tolerability of a single IV infusion of SPK-9001in patients with hemophilia B 18 years of age with ≤2 IU/dL (≤2%) endogenous FIX.

The secondary objective of the study is to characterize the kinetics of SPK-9001 by quantification of FIX antigen levels and FIX activity.

Clinical pharmacology relevant exploratory objectives include assessment of :

- Vector shedding
- Number of bleeding events
- Number of factor IX infusion and annualized FIX consumption

Methodology/Study Design:

This was a Phase 1/2a, open-label, non-randomized, dose-escalation and multi-center study to evaluate the safety, tolerability, and kinetics of a single IV infusion of SPK-9001 in hemophilia B subjects with ≤2 IU/dL (≤2%) endogenous FIX levels. Approximately 15 evaluable subjects were initially planned to be dosed with a single IV infusion of SPK-9001 at one of 3 different dose levels:

- 5×10^{11} vg/kg
- 1×10^{12} vg/kg
- 2×10^{12} vg/kg

For subjects with body mass index (BMI) exceeding 30 kg/m2, the study dose will be calculated based on an adjusted body weight determination that assumes a maximum permissible BMI of 30 kg/m2. For example, a subject who is 6'2" and weighs 370

pounds (BMI 47.5 kg/m2) would receive a vector dose based on an adjusted body weight of 234 pounds (which is the body weight associated with a BMI of 30 kg/m2 for a 6'2" individual).

Subjects were required to have washout of at least 96 hours (4 days) for FIX protein product, or up to approximately 168 hours (7 days) of washout for extended half-life FIX protein product, prior to any blood draw and infusion at Day 0 visit. At Day 0 visit, subjects were infused with 100 IU/kg of their usual FIX protein product over 10 (±2) minutes, under the supervision from the site staff and/or investigator(s) to assess FIX incremental recovery. Number of vials, total volume, and total dosage were monitored and recorded by the site staff. Following the bolus infusion of the usual FIX protein product, the participant was infused with SPK-9001 for approximately 60 minutes via infusion pump. The rate of administration was determined based on the total volume of the SPK-9001 required for the participant to infuse for approximately 60 minutes. (b) (4) manufacturing processes, Process (b) (4) and Process (b) (4), were used in this study. Of the 15 subjects who received the SPK-9001 infusion, 10 subjects received Process (b) (4) test drug, and 5 subjects received Process (b) (4) test drug.

Study Disposition:

All 15 subjects were male with the mean age of 38.6 years, ranging from 18 to 61 years. Most subjects were ≥35 years (10 [66.7%]) and White or Caucasian (12 [80.0%]). The mean bodyweight was 83 ± 14 kg (range 55-103). The mean body mass index (BMI) was 26 ± 4.4 kg/m^2 (range 18-33). Among the 15 treated participants, there were 11 (73.3%) prophylaxis subjects and 4 (26.7%) on-demand subjects at baseline. Most subjects had no family history of FIX inhibitor (12 [80.0%]) and had hemophilia B with FIX:C level less than 1% (10 [66.7%]).

The median duration of SPK-9001 infusion was 60.0 minutes, ranging from 60 to 78 minutes. All 15 subjects received 5x10^11 vg/kg. The median total dose of SPK-9001 infused was 4.620×10^{13} vg (range 3.080×10^{13} to 5.900×10^{13} vg).

Pharmacokinetic/Pharmacodynamic Analysis

    1. **Vector biodistribution and shedding analysis:**

Polymerase chain reaction (PCR) analysis was performed on serum, peripheral blood mononuclear cells (PBMCs), saliva, and urine at Screening or Day 0 visit prior to vector infusion and starting from Week 1 post-vector infusion and continuing at every scheduled visit until 3 consecutive samples were negative (at or below the limit of detection of the assay) for the given sample type. PCR analysis was performed on semen at Screening or Day 0 visit prior to vector infusion, Week 1 and every 4 weeks starting from Week 4 post-vector infusion until 3 consecutive samples were negative (at or below the limit of detection of the assay). After Week 16, semen samples collection occurred at every scheduled visit (as opposed to every 4 weeks) if 3 consecutive negatives had not yet been obtained.

    2. **FIX transgene expression and FIX activity:**

FIX transgene expression (plasma FIX activity) was monitored twice weekly or thrice weekly in Weeks 1-8, and once in Weeks 10, 12, 14, 16, 18, 22, 26, 32, 42 and 52.

Plasma FIX activity levels were analyzed and used to determine peak and steady-state vector-derived circulating FIX activity levels.

Immunogenicity Assessments: Neutralizing antibodies activity against AAVRh74var capsid. For characterization of cellular immune response, reactive T-cells were measured using an enzyme-linked immunosorbent spot (ELISPOT) assay which relies on detection of cytokine secretion (in this case interferon-gamma).

7.2.   Study#2- C0371002 (Phase 3)

Title: Phase 3, Open Label, Single Arm Study to Evaluate Efficacy and Safety of FIX Gene Transfer With PF-06838435 (rAAV-Spark100-hFIX-Padua) in Adult Male Subjects With Moderately Severe to Severe Hemophilia B (FIX:C≤2%) (BeneGene-2; #C0371005)

Objectives: The primary objective of the study is to demonstrate the efficacy of a single infusion of BEQVEZ in male subjects≥18 years of age with moderately severe to severe hemophilia B (FIX:C ≤2%). The primary endpoint is non-inferiority on ABR for total bleeds (treated and untreated) from Week 12 to Month 15 versus standard of care FIX prophylaxis replacement regimen, comparing pre- and post-IP infusion.

From clinical pharmacology perspective the following are included as key secondary objectives:

- Vector-derived FIX:C level at steady state (from Week 12 to 15 months) demonstrated to be greater than 5%. FIX:C will also be summarized descriptively by study visit.
- Annualized infusion rate (AIR) of exogenous FIX from Week 12 to Month 15 versus AIR of FIX with standard of care FIX replacement regimen pre-IP infusion.

The clinical pharmacology relevant safety objectives include assessments of:

- FIX inhibitors

- Drug related elevated hepatic transaminases

- Other immunogenicity-based laboratory data including nAb to AAV capsid, immune response (presumed T-cell activation) to AAV capsid protein and/or FIX transgene

The clinical pharmacology relevant exploratory objectives include assessment of:

- Vector shedding of BEQVEZ as measured by qPCR in plasma, saliva, PBMC, urine, and semen until 3 consecutive specimens test negative for the given specimen type.

- FIX antigen levels.

- Correlation of FIX activity between one stage assay and chromogenic assay.

Methodology/Study Design: This Phase 3, open-label, single arm, multi-site study compared the efficacy of a single IV infusion of BEQVEZ with routine FIX prophylaxis in adult male subjects from the lead-in study (C0371004) with moderately severe to severe hemophilia B (FIX:C ≤2%). Study C0371004 prospectively collected efficacy data and selected safety data on subjects for at least 6 months and these data are utilized as the FIX prophylaxis control for comparison with data post-BEQVEZ infusion in Study C0371002. Eligible study subjects completed a minimum 6 months of routine FIX prophylaxis therapy during the lead in study (C0371004). The planned study duration for each participant in this study was 312 weeks.

A single intravenous infusion of BEQVEZ was administered on Day 1 at a dose of 5×10^{11} vg/kg of body weight based on actual lot concentration (nominal strength is 1×10^{13} vg/mL). For a participant with BMI >30 kg/m2, dose will be calculated based on an adjusted body weight determination that assumes a maximum permissible BMI of 30 kg/m2, eg, for 187.96 cms (6'2") height and 167.8 kg weight (BMI 47.5 kg/m2) dose

will be based on 106.1 kg, which is the weight associated with a BMI of 30 kg/m2 for a 187.96 cms (6'2") tall individual.

Study Disposition: A total of 51 subjects who completed the lead-in study (C0371004) were screened in this study and 45 subjects received a single dose of BEQVEZ and included in the Dosed/Safety Analysis Set. Of these, 41 (80.4%) had 15 months of follow-up and   4 out of 45 subjects had not completed 15 months of follow-up at the time of data cutoff, and thus were not included in the Evaluable Analysis Set. The median (range) age was 29 years (18-62). The median (range) bodyweight was 86.2 kg (53.4-141.6), and the median(range) BMI was 27.7 kg/m^2(17.6-48.4).

Pharmacokinetic/Pharmacodynamic Analysis:

1. **Vector biodistribution and shedding analysis:** Viral vector shedding was assessed for PBMC, saliva, urine, semen, and plasma at multiple time points and full clearance of vector DNA was defined as having 3 consecutive negative results (i.e., BQL) for the given sample type confirmed using the validated qPCR assay.

2. **FIX transgene and FIX activity:**   Blood samples were analyzed for FIX expression and FIX activity by the one-stage assay (Actin reagent and SynthAsil reagent) and chromogenic assay. FIX activity from week 12 (day 82) to month 15 (day 469) after PF-06838435 infusion is considered key secondary endpoint and the analysis includes:

- Comparison with a fixed threshold of FIX activity of 5%
- Geometric mean of all valid measurements of FIX:C following steady state (defined as 12 weeks [Day 82]) to Month 15 [Day 469] inclusive post PF-06838435 infusion)

- Population mean of the individual participant geometric mean FIX:C and summary of absolute value of FIX:C over time by study visit.

- Data from all three assays (one-stage assay with Actin reagent, one-stage assay with SynthAsil reagent, and chromogenic assay).

- During the study, subjects are requested to suspend their prophylaxis FIX replacement regimen. Only on-demand FIX replacement is permissible for the treatment of bleeding events. Any sample taken within 7 days after (14 days if Extended half-life [EHL] product is used) exogenous FIX replacement therapy administered for any purpose (including treatment of bleeding, preventive purpose, or resumption of FIX prophylaxis regimen) will be excluded from the analysis of FIX: C. Once prophylaxis FIX replacement is re-established, FIX:C data at the visits following resumption of FIX prophylaxis regimen will be imputed as 1.9%, slightly below the baseline value (2%) to be conservative.

Immunogenicity assessment: Samples were collected for assessment of anti-AAV capsid protein antibodies (ADA) and neutralizing antibodies (NAb). Samples determined to be positive for ADA further characterized for NAb. Samples were collected for measurement of :
- FIX inhibitor
- Cellular Immune Response by ELISPOT (enzyme–linked immuno–spot)

7.3.   Study#3: Population Modeling of Factor IX Activity Following the Administration of Factor IX Replacement Therapy and/or BEQVEZ in Patients with Hemophilia B

The objectives of the population modeling analysis were to:

- Describe the time-course of FIX activity in patients with hemophilia B following the administration of FIX replacement therapy and/or BEQVEZ.

- Identify and quantify the impact of intrinsic and extrinsic factors on FIX activity.

- Provide a model-based estimate of peak FIX activity, time to peak FIX activity, and characterization of longer-term FIX activity following the administration of BEQVEZ.

- Predict the impact of dosing based on (b) (4) on FIX activity relative to dosing based on actual release titer in a virtual population administered BEQVEZ.

Analysis Data and Model Development:

FIX activity data from previous clinical studies of recombinant human Factor IX (rFIX) and the current studies for BEQVEZ were pooled for the population analysis. A previous population model for FIX activity was developed based on pooled data from 8 clinical studies of nonacog alfa replacement therapy in patients with hemophilia B. Two additional studies in hemophilia B patients administered nonacog alfa were used to validate the model. The final model was a 2-compartment model, with inter-individual variability (IIV) on clearance (CL), volume of the central compartment (V1), inter-compartmental clearance (Q), volume of the peripheral compartment (V2), inter-occasion variability (IOV) on CL and V1, and a combined random unexplained variability (RUV) model. Model development used (b) (4) . Population parameter estimation used (b) (4) and individual parameters were obtained from empirical (b) (4) The (b) (4) subroutine with TOL = 9 was used for solving differential equations. (b) (4) was used for sampling importance resampling (SIR). Statistical and graphical output were generated using the R programming and statistical language.

Development of the structural model for combined nonacog alfa and BEQVEZ data was conducted in a 2-stage process:

50

1. Development of structural model for nonacog alfa data to quantitatively describe FIX disposition and clearance.

2. Pooling of data obtained from BEQVEZ studies with nonacog alfa data to describe the FIX synthesis from BEQVEZ and degradation of the

transgene.

Since FIX activity data was not baseline-corrected and data from BEQVEZ was to be included in the population modeling dataset compared to the previous analysis, differences on FIX activity characteristics under these conditions were investigated:

1. Models accommodating gene and protein expression dynamics (Hargrove-Schmidt model) as described in Figure 1:

- The Hargrove-Schmidt model is a 2-compartment model that assumes information flow from deoxyribonucleic acid (DNA) to FIX via a translation constant, k_t, and a first-order rate constant of synthesis, k_{syn}.

- Loss or degradation of the transgene is accounted for by a first-order degradation rate constant, k_{deg}.

2. Fixed allometric scaling (referenced to a 70 kg individual with fixed exponents of 0.75 for clearance, 1 for volume, and -0.25 for rate-constant parameters).

3. Alternative compartment models, such as 3-compartment kinetics, were considered based on trends observed in diagnostic plots.

4. Accounting for baseline FIX activity via endogenous production or residual FIX activity due to inadequate washout of prior FIX replacement therapy.

5. It was assumed that the population PK model for Alprolix® adequately accounted for each EHL product's contribution to FIX activity. Population-typical parameters for individuals administered EHL products were used to describe their contributions to FIX activity.

Reviewer comments: The pooled analysis of exogenous FIX products and AAV is questionable considering the complexity of the model, limited data for the AAV therapy (63 subjects for AAV vs 274 for FIX replacement therapy). The model has also several assumptions. For example, some of the structural model for replacement FIX products assume model parameters from published studies, and we have not reviewed these models. Also, the applicant stated that "It was assumed that the population PK model for Alprolix® adequately accounted for each EHL product's contribution to FIX activity". These assumptions are questionable considering essential differences in the replacement FIX products characteristics (e.g., dose range, PK variability, assay discrepancies, and sample size). Thus, we requested to re-estimate the popPD model parameters only for AAV with inclusion of FIX activity data per the study protocol (i.e., excluding FIX activity values from exogenous administration). The following is summary of the updated simplified popPD analysis. The original popPD model is briefly summarized toward the end of this section.

Updated Simplified Modeling for FIX Activity:

Several structural changes have been implemented in the updated model following truncation of the analysis dataset (i.e., exclude exogenous FIX PK data):

- Information regarding FIX replacement therapy dosing events (i.e., time and amount administered) was not implemented as model input.

- Characterization of FIX disposition and elimination was modified from a 3- to a 1-compartment model as FIX activity observations informing the estimation of parameters for peripheral and extravascular compartments (i.e., inter-compartmental clearances and volumes) are now excluded.

- Thus, as depicted in Figure 7 only parameters reflecting disposition of AAV was estimated.

Figure 7: Figure 1. Schematic View of FIX Disposition Model
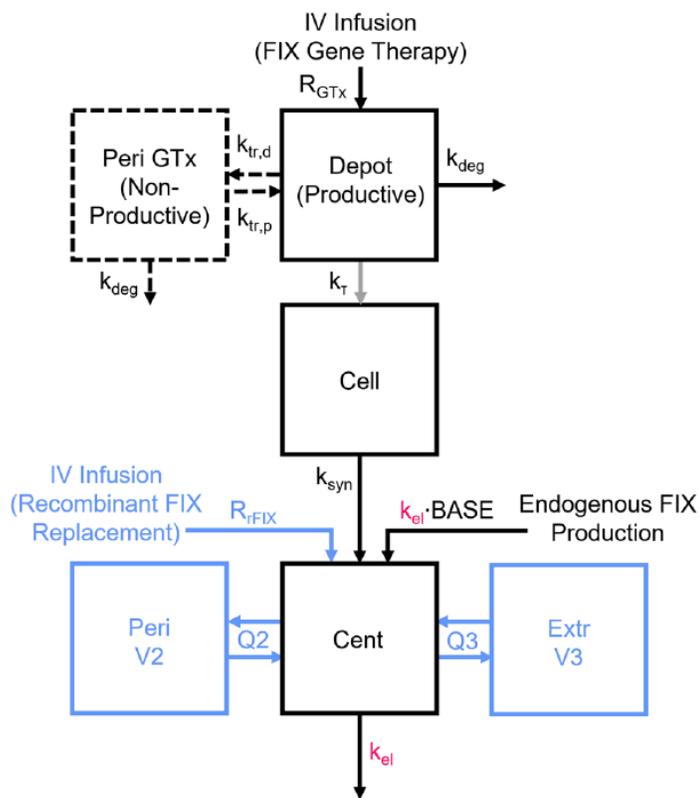
Note: RGTx is the zero-order infusion rate of BEQVEZ(vg/hr), DEPOT is the amount of productive transgene (vg), PERIG is the amount of non-productive transgene for subpopulation 2, CELL is the concentration of FIX in the site of FIX synthesis (IU/dL), kdeg is the first-order rate constant for degradation of transgene (hr-1), ktr,d is the first-order rate constant for transgene transition from the productive to non-productive state (hr-1), ktr,p is the first-order rate constant for transgene transition from the non-productive to productive state (hr-1), ksyn is a first-order rate constant for turnover of FIX at the site of action (hr-1), and kT is a translation constant for the ratio of gene to expressed protein (IU/dL/vg hr-1). BASE is the baseline FIX activity (IU/dL), CENT is the concentration of FIX in the central compartment, and kel is the first-order elimination rate constant for FIX from the central compartment (hr-1). Parameters and compartments highlighted in blue are model components in the original model that no longer exist in the present model. Parameters highlighted in red are new model.parameters. Source: Figure 1; Applicant response clin pharm IR#2.

Covariate Analysis:

A summary of the covariates univariately tested on the structural model and their effect on the model fit as determined by changes in objective function value (OFV) and variability in parameters is presented in Table 3.The following covariates were carried forward for multivariate analyses as their inclusion improved the fit of the model at a p-value of less than 0.01 according to the likelihood ratio test (LRT):

- Effect of age on *kdeg*
- Effect of age on *ksyn*

The effect of concomitant corticosteroids on *ktr,d* was statistically significant at a p-value of less than 0.01 (Table 3). However, in the clinical studies subjects were administered corticosteroids in response to suspected immune-related increases in ALT and AST. Such that, this covariate was considered descriptive and consistent with the study design with limited predictive utility and was not included in multivariate analyses. Covariates previously included in the combined model (i.e., AAV and FIX replacement therapies) that were not carried forward for present multivariate analyses were the effect of manufacturing process on *ksyn,* the effect of age on relative FIX activity (*FIXA*), and the effect of body weight on *ktr*. These covariates were not considered statistically significant at a p-value of less than 0.01 in the updated model (Table 3). Following forward inclusion procedures, the effects of age on *kdeg* and *ksyn* were retained in the model.

Parameter estimates and sampling importance resampling (SIR) results for the final model are presented in Table 4. A prediction-corrected visual predictive check of the final model is presented in Figure 8. The final model's predictions overlay the observed FIX activity with generally good agreement but depicts systematic deviation of the representation of the median response at later time-points. The impact of age and body weight on peak FIX activity, time to peak FIX activity, and area under the curve for the

follow-up interval (AUC$_T$) over a 15-year period were simulated and the summary of geometric mean values of these metrics (Actin FSL assay) for each covariate scenario are presented in Table 5.

Table 3: Univariate Analysis Results Compared to Final Structural Model

| Description	OFV	ΔOFV	df		p-value
Effect of age on k_{deg} (referenced to 35 years)	-2540.46	-59.8	1		< 0.01
Effect of age on k_{syn} (referenced to 35 years)	-2530.13	-49.4	1		< 0.01
Effect of concomitant corticosteroids on k_{syn}	-2525.95	-45.3	1		< 0.01
Effect of concomitant corticosteroids on $k_{tr,d}$	-2524.11	-43.4	1		< 0.01
Effect of age on $BASE$ (referenced to 35 years)	-2486.77	-6.07	1		0.0137
Effect of age on $FIXA$ (referenced to 35 years)	-2483.62	-2.92	1		0.0874
Effect of age on $BASEPNRS$ (referenced to 35 years)	-2482	-1.3	1		0.254
Effect of concomitant corticosteroids on k_T	-2481.74	-1.04	1		0.307
Effect of age on k_{el} (referenced to 35 years)	-2481.23	-0.531	1		0.466
Effect of age on k_T (referenced to 35 years)	-2480.76	-0.059	1		0.808
Effect of BMI on k_T (referenced to 25 kg/m^2)	-2480.72	-0.0203	1		0.887
Effect of body weight on k_T (referenced to 70 kg)	-2480.7	-0.00093	1		0.976
Effect of manufacturing processes on k_T	-2475.97	4.73	2	1	
Effect of concomitant corticosteroids on k_{deg}	-2469.39	11.3	1	1	
Effect of manufacturing processes on k_{syn}	-2256.84	224	2	1	

Note: p-value based on the likelihood ratio test (LRT). ΔOFV is referenced to OFV of the final structural model. Abbreviations: degrees of freedom (df), relative FIX activity *(FIXA)*, objective function value (OFV). Source: Table 1; Applicant response clin pharm IR#2.

Table 4: Parameter Estimates for Final Model

Parameter	Value	95% CI	SIR Median	SIR 95% CI	SHR (%)
Objective Function Value	-2557.3	-	-	-	-
Condition Number	15.9	-	-	-	-
Population Parameter					
Baseline (*BASE*; IU/dL)	1.77	(1.69, 1.84)	1.77	(1.64, 1.89)	-
Half-life of FIX elimination (k_{el}; hours)	31.3	(10.0, 52.5)	31.8	(16.2, 55.1)	-
Half-life of FIX synthesis (k_{syn}; days)	26.5	(24.3, 28.6)	26.5	(23.9, 29.0)	-
Translation constant (k_τ; IU/dL/vg·10^{-13} hr^{-1})	0.0915	(0.0224, 0.161)	0.0907	(0.0491, 0.180)	-
Half-life of transgene degradation (k_{deg}; weeks)	451	(355, 548)	450	(350, 646)	-
Half-life of productive to non-productive transition ($k_{tr,d}$; weeks)	4.57	(4.22, 4.92)	4.57	(4.10, 5.23)	-
Half-life of non-productive to productive transition ($k_{tr,p}$; weeks)	28.9	(26.8, 30.9)	28.8	(26.1, 32.6)	-
Box-Cox transformation parameter on k_τ (*BXCXK*)	-0.639	(-1.19, -0.090)	-0.619	(-0.963, -0.289)	-
Standard deviation of residuals (*RUVADD*; SD)	0.326	(0.323, 0.329)	0.326	(0.322, 0.331)	-
Log-odds of being assigned to constant FIX production (*P_NRSt*)	0.931	(0.172, 1.69)	0.929	(0.420, 1.51)	-
Effect of age on k_{syn} (referenced to 35 years)	-1.82	(-2.29, -1.36)	-1.83	(-2.36, -1.24)	-
Effect of age on k_{deg} (referenced to 35 years)	-0.416	(-0.514, -0.318)	-0.415	(-0.569, -0.274)	-
Inter-Individual Variability					
$\omega^2_{k\tau}$	0.504	(0.234, 0.775)	0.511	(0.345, 0.815)	1.64
Random Unexplained Variability					
σ^2_{res}	1	Fixed	1	Fixed	1.28

Note: Allometric scaling was applied to all rate constant parameters with an exponent of -0.25 referenced to a 70 kg individual. Abbreviations: confidence interval (CI), sampling importance resampling (SIR), shrinkage (SHR), standard deviation (SD). Source: Table 2; Applicant response clin pharm IR#2.

Figure 8: Prediction-Corrected Visual Predictive Check
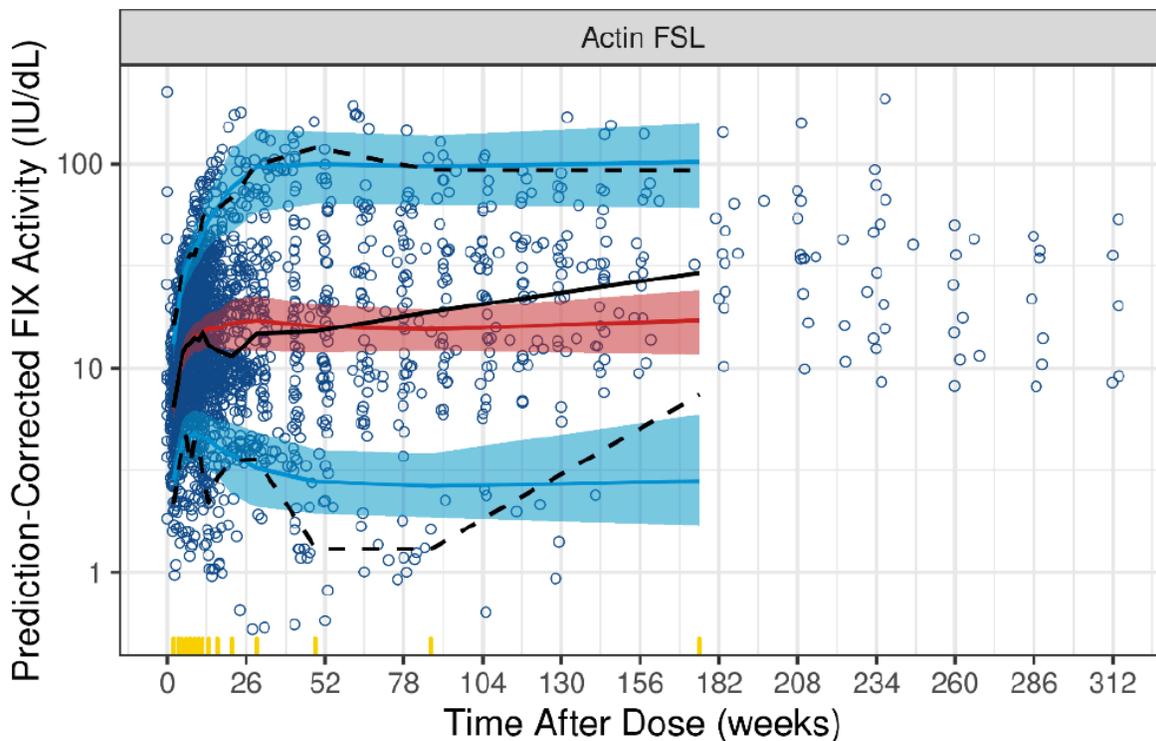
Note: The prediction-corrected observed data are represented by blue circles and black lines (median, 5th and 95th percentiles). The prediction-corrected simulated FIX activity based on the index population (n = 1000 simulations) are represented by the red line and red shaded ribbon (median and 95% PI of the median, respectively) and the blue lines and blue shaded ribbons (median and 95% PIs of the 5th and 95th percentiles, respectively). Yellow indicators on the x-axis represent the time bins for summarizing the data. Observed and simulated BLQ observed are excluded. Abbreviations: below limit of quantification (BLQ), prediction interval (PI)
Source: Figure 3; Applicant response clin pharm IR#2.

Table 5: Geometric Mean Time-Course of FIX Activity Metrics for Covariate Scenarios

	Peak FIX Activity (IU/dL)	Time to Peak FIX Activity (weeks)	AUCtau (IU.year/dL)
Reference	15.2 (13.5, 17.1)	18.9 (16.5, 21.5)	128 (11,148)
Younger age (18.8 years)	15.1 (13.5,16.8)	13.2 (11.9, 14.6)	76.7 (68.6, 85.4)
Older age (56.5 years)	15.1 (13.4, 17.0)	24.6 (21.1, 28.5)	159 (136, 185)
Low body weight(56.5kg)	9.83 (8.89, 10.9)	17.0 (14.9, 19.4)	86.4 (76.5, 98.0)
High body weight (108 kg)	19.6 (17.3, 21.9)	20.0 (17.5, 22.8)	163 (138,188)

Note: For each covariate scenario, FIX activity-time profiles for 1000 trials of 63 randomly drawn individuals administered $5 \cdot 10^{11}$ vg/kg BEQVEZ were simulated for 15 years based on the Actin FSL assay. Each trial was summarized as the geometric mean peak FIX activity, time to peak FIX activity, and AUCtau (15-year period). The geometric means of all 1000 trials are summarized as the median and 90% PI. Reference low and high values are the 5th and 95th percentiles of the analysis population. Summaries are based on a mixed population comprised of 72% and 28% of the subpopulations 1 and 2 phenotypes, respectively. The reference scenario is based on a population with weight 86.1 kg, aged 35 years. Source: Table 3; Applicant response clin pharm IR#2.

Reviewer comment: The popPD analysis reasonably quantified the median FIX activity (Actin FSL) up to 2-years post-treatment. However, the model prediction systematically deviates from the observed median FIX activity after 2-years. This deviation could be due to the inherent variability and discrepancies between of the FIX activity data between Phase 1/2a and Phase 3 studies. The FIX activity data after 2-years is mostly derived from the Phase 1/2a study. Overall, the popPD model is not appropriate to extrapolate FIX activity up to 15 years as proposed by the Applicant but is appropriate to describe longitudinal FIX activity data up to 2 years following administration of BEQVEZ. Below is a summary of the original model that combine FIX activity data from exogenous FIX replacement products and BEQVEZ.

PopPD model Analysis of Exogenous FIX Replacement Products and BEQVEZ

Reviewer comments: **It should be noted that the population PK analysis of the FIX replacement products were not reviewed as part of this BLA submission. The following were the applicant justification for conducting combined population analysis:**

- **Develop a unified modeling approach by leveraging all available FIX activity data form BEQVEZ clinical studies while being able to reasonably characterize the contribution of exogenous FIX replacement products.**

- **This combined model was developed while FDA and applicant were determining the adequacy of washout period for replacement products. The modeling approach accounts for PK of the exogenous products that preclude criteria for washout period.**

The time-course of FIX activity from 8 nonacog alfa studies and BEQVEZ studies was adequately described by a compartmental model for gene and protein expression following the administration of BEQVEZ, and 3-compartment models for FIX replacement products (SHL and EHL). Important intrinsic factors (relative to an individual with weight 86.1 kg, aged 35 years and administered $5x10^{11}$ vg/kg via manufacturing process 3) that accounted for variability in FIX activity:

- Younger age (18.8 years, representative of the 5th percentile of the analysis population) demonstrated 0.3% decrease in peak FIX activity, 36.8% earlier time to peak FIX activity, and 33.6% lower AUCt (15-year period) compared to a 35-year-old individual.

- Older age (56.5 years, representative of the 95th percentile of the analysis population) demonstrated 0.8% decrease in peak FIX activity, 43% later time to

peak FIX activity, and 21% higher AUCt (15-year period) compared to a 35-year-old individual.

- Lower body weight (56.5 kg, representative of the 5th percentile of the analysis population) demonstrated 34.8% decrease in peak FIX activity, 9.9% earlier time to peak FIX activity, and 30.8% decrease in AUCt (15-year period), when compared to an 86.1 kg individual.

- Higher body weight (108 kg, representative of the 95th percentile of the analysis population) demonstrated 28% increase in peak FIX activity, 6% later time to peak FIX activity, and 24% increase in AUCt (15-year period), when compared to an 86.1 kg individual.

- Manufacturing process (b) (4) demonstrated 3% increase in peak FIX activity, 19.1% earlier time to peak FIX activity, and no difference in AUCt (15-year period), when compared to manufacturing process (b) (4)

- Manufacturing process (b) (4) demonstrated 5% increase in peak FIX activity, 30.5% earlier time to peak FIX activity, and 0.2% decrease in AUCt (15-year period), when compared to manufacturing process (b) (4)

Overall, it was noted that the predicted variability in FIX activity as quantified by the 90% PI was lower in the modified model developed with the truncated analysis dataset compared to the combined modeling approach.

Reviewer comments: The proposed application of the popPD analysis was to provide additional justification for (b) (4) **dosing of BEQVEZ. During the pre-BLA meeting discussion, the applicant agreed to provide supportive safety and efficacy data from at least** (b) (4) **patients treated with** (b) (4) **. The following section is summary of the FIX activity data from Study C0371002 that is currently dosing additional** (b) (4) **patients to obtain clinical experience using** (b) (4) **for dosage calculation. The applicant also provided model-based assessment as**

supportive evidence for (b) (4) dosing. The data from this (b) (4) dosing was also used to further validate the popPD analysis.

Clinical Experience with (b) (4) **Dosing**

A total of (b) (4) subjects were dosed in Study C0371002 using (b) (4) , with follow up of < 9 weeks (n=11), ≥ 9 to <12 weeks (n=3) and ≥ 12 weeks (n=6). The demographics of these subjects were within overall study population of C0371002. The FIX activity results observed through the 26 Oct 2023 data cutoff in subjects dosed with (b) (4) of BEQVEZ is summarized in Table 6. These FIX activity values were within the same ranges as those in subjects who were dosed with product labeled with the actual concentrations of BEQVEZ.

<u>Reviewer comments:</u> **The FIX activity for** (b) (4) **dosing is comparable with values obtained in patients dosed with actual vector concentrations. For example, at Week 4 (n=42) and Week 12 (n=43), the one-stage FSL assay mean (SD) FIX activity levels for patients dosed with actual concentrations were 9.25 (4.4) and 13.52 (8.1), respectively. The corresponding values for** (b) (4) **dosing was 11.76 (6.1) at Week 4 (n=15) and 12.02 (12.1) at Week 12 (n=6).**

Table 6: Summary of FIX Activity in Subjects Treated with (b) (4) of BEQVEZ

Visit	One-stage Assay (Actin-FSL Reagent)	One-stage Assay (SynthASil Reagent)	Chromogenic Assay
Week 4 , n	15	15	15
Mean (SD)	11.76 (6.1)	19.23 (9.5)	9.55 (6.1)
Median (Min, Max)	10.90 (3.9, 26.0)	18.50 (6.6, 42.4)	7.70 (2.5, 24.2)
(Q1, Q3)	(7.45, 15.57)	(12.05, 25.30)	(5.50, 12.77)
Week 8, n	11	11	10
Mean (SD)	18.16 (10.4)	27.44 (14.9)	14.82 (8.7)
Median (Min, Max)	19.43 (3.2, 36.3)	28.67 (7.1, 54.3)	14.45 (1.8, 29.2)
(Q1, Q3)	(9.93, 23.70)	(16.85, 34.40)	(6.77, 19.90)
Week 12, n	6	6	6
Mean (SD)	16.02 (12.1)	23.35 (16.6)	12.24 (10.8)
Median (Min, Max)	12.40 (4.1, 37.3)	17.08 (8.3, 52.4)	7.33 (2.4, 29.9)
(Q1, Q3)	(7.65, 22.30)	(12.15, 33.13)	(5.50, 21.07)

Source: Response to FDA IR#29; Table 2.

Population PD Model-based Simulation of FIX Activity for (b) (4) Dosing

While (b) (4) participants were (b) (4) dosed by the data cut-off date (26 Oct 2023), upon application of appropriate washout rule, observed FIX activity data from 17 subjects in study C0371002 receiving (b) (4) dosing (b) (4) of BEQVEZ were used to assess the validity of model in predicting FIX activity in patients receiving (b) (4) dosing. Stochastic simulation of FIX activity in a population was preformed using the structural model parameter estimates of the combined model and updated model. The simulations utilized demographic information from the combined model's analysis dataset and a

(b) (4) dose of (b) (4) with a (b) (4) variability (based on CMC assay information). The simulation results adequately captured the observed FIX activity for patients in C0371002 that received (b) (4) dosing. Overall, the observed data with (b) (4) dosing appear to agree with model predictions, providing a limited external validation of the popPD model performance.

Simulation of FIX Activity in Virtual Subjects Using Actual and (b) (4) Dosing

Stochastic simulations were conducted to predict the impact of drug product variability on FIX activity. The simulations assumed a target product titer of 1×10^{13} vg/mL with ±15% variability. In all simulation scenarios, FIX activity time-courses were predicted for the virtual population based on the Actin FSL assay up to Week 65 (duration of C0371002 phase 3 study) following the administration of BEQVEZ.

Despite the vg doses in the BEQVEZ clinical development program being derived from actual release titers for product lots, variability in the vg doses administered was observed due to weight-based dosing (and dose-adjustment for individuals with BMI greater than 30 kg/m^2) practices. Such that the population model for FIX activity, which uses vg doses as dosing input, was developed under an adequate dose range and can be used to assess the impact of (b) (4) dosing practices by simulation.

The distribution of simulated vg doses and vg/kg doses targeting (b) (4) for the assessment of dosing practices used (b) (4) versus actual release titer is summarized in Table 7. Compared to dosing based on actual release titer, (b) (4) dosing increased the range of simulated vg doses by (b) (4). Both simulated dosing scenarios demonstrated a lower median vg dose relative to the observed dose range as the simulations encompass a wider range of body weights. The predicted FIX activity over

63

time for the virtual population following actual and (b) (4) dosing is displayed in Figure 9. The overlay of predicted FIX activity following actual and (b) (4) dosing demonstrates that the width of the 95% prediction interval for (b) (4) dosing were not appreciably wider relative to dosing based on actual release titer at Weeks 12 and 65.

In summary, the impact of (b) (4) dosing practices on the overall distribution of vg doses administered is small relative to the contribution of variability in body weight (and adjusted body weights for individuals with BMI greater than 30 kg/m^2) used to calculate BEQVEZ doses. Thus, the practice of (b) (4) dosing is not expected to impact FIX activity relative to actual dosing experience.

Table 7: Summary of Observed and Simulated BEQVEZ Doses

(b) (4)

Reviewer comments: The modeling and simulation exercise is consistent with the results of the observed FIX activity results for both actual and (b) (4) dosing. Overall, the modeling and simulation analysis is acceptable, and it provides supportive evidence for (b) (4) dosing.

Figure 9: Predicted FIX Activity Following Dosed with Actual vs. (b) (4) Dosing

(b) (4)