SUMMARY OF SAFETY AND PROBABLE BENEFIT (SSPB)

I. GENERAL INFORMATION

Device Generic Name: nAbCyte Anti-AAVRh74var HB-FE Assay (nAbCyte Anti-AAVRh74var HB-FE Assay) for BEQVEZ (fidanacogene elaparvovec) Eligibility in Moderate to Severe Hemophilia B

Device Trade Name: nAbCyte Anti-AAVRh74var HB-FE Assay

Device Procode: QWQ

Applicant's Name and Address: Labcorp Drug Development
100 Perimeter Park Drive, Ste C
Morrisville, NC 27560

Date(s) of Panel Recommendation: None

Humanitarian Device Exemption (HDE) Number: H230005

Humanitarian Use Device (HUD) Designation Number: HUD # 20-0442

Date of HUD Designation: September 3, 2020

Date of Notice of Approval to Applicant: April 25, 2024

II. INDICATIONS FOR USE

The nAbCyte Anti-AAVRh74var HB-FE Assay for BEQVEZ™ (fidanacogene elaparvovec) Eligibility in moderate to severe Hemophilia B, or nAbCyte Anti-AAVRh74var HB-FE Assay, is a cell-based qualitative in vitro companion diagnostic device intended for the detection of neutralizing antibodies to the AAVRh74var capsid in serum specimens from adult patients previously diagnosed with moderate to severe hemophilia B to determine eligibility for treatment with the hemophilia B gene therapy, BEQVEZ (fidanacogene elaparvovec). Patients who test positive for neutralizing antibodies to the AAVRh74var capsid are not eligible for treatment with BEQVEZ (fidanacogene elaparvovec). Patients who test negative for neutralizing antibodies to the AAVRh74var capsid are eligible for treatment with BEQVEZ (fidanacogene elaparvovec).

This diagnostic device is a single-site assay for professional use only and is to be performed only at Labcorp-Monogram Biosciences by trained and qualified laboratory personnel.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the nAbCyte Anti-AAVRh74var HB-FE Assay labeling.

Follow specimen collection, sample preparation, and shipping instructions to ensure that adequate samples are sent to Labcorp-Monogram Biosciences for testing. Samples that are received hemolyzed or thawed (or partially thawed) will not be tested and another blood draw from the patient will be required.

The time interval between sample collection and subsequent testing with the nAbCyte Anti-AAVRh74var HB-FE Assay and BEQVEZ (fidanacogene elaparvovec) infusion should be as short as possible (e.g., approximately 8 weeks).

V. DEVICE DESCRIPTION

The nAbCyte Anti-AAVRh74var HB-FE Assay is a companion diagnostic (CDx) device intended for use with BEQVEZ (fidanacogene elaparvovec), a gene therapy that is a recombinant adeno-associated virus (AAV) derived vector delivering the factor IX (FIX) gene to hepatocytes and indicated for moderate to severe hemophilia B patients that do not have anitbodies to the delivery vector. The nAbCyte Anti-AAVRh74var HB-FE Assay is a cell-based antibody-mediated neutralization assay using an AAV vector (AAVRh74var CAG lucP) expressing a firefly luciferase reporter gene under control of the CAG promoter. To determine the level of neutralizing antibody (nAb) activity in serum samples provided by patients who are candidates to receive BEQVEZ, the anti-AAVRh74var nAb activity is assessed by measuring the inhibition of transduction of cell culture by AAVRh74var CAG lucP into target cells (i.e., HEK293 cells) and the subsequent expression and quantitation of luciferase via a luminometer. Serum specimens are reported as positive for anti-AAVRh74var nAb activity if the percent inhibition of luciferase signal is >50% at the cut-off dilution (1:1) and as negative if the percent inhibition is ≤50% at the 1:1 cut-off dilution. The nAbCyte Anti-AAVRh74var HB-FE Assay is performed only at Labcorp-Monogram Biosciences laboratory, a single laboratory site located at 347 Oyster Point Blvd South San Francisco, CA 94080. The Labcorp-Monogram laboratory responsible for testing and reporting results is ISO15189, CLIA, and CAP certified.

The nAbCyte Anti-AAVRh74var HB-FE Assay utilizes reagents manufactured exclusively for use with the nAbCyte Anti-AAVRh74var HB-FE Assay by Labcorp-Monogram Biosciences laboratory, as well as utilizing reagents and instrumentation which have been specifically validated for, and approved for use as part of the nAbCyte Anti-AAVRh74var HB-FE Assay (Tables 1–3, below).

Table 1: Assay Components

Item Name/Description	Purpose
AAVRh74var CAG lucP vector	AAVRh74var - luciferase reporter vector
HEK293VL Cells	Target cells
Steady-Glo Luciferase Assay System	Cell lysis and luciferase reporter reaction solution
Mouse anti-AAVRh74var monoclonal antibody (mAb) diluted in human serum	Positive Control
Human Serum	Negative Control
Fetal Bovine Serum (FBS)	Sample Diluent

Table 2: Other Critical Assay Components

Reagent, Disposable, or Equipment	Purpose
Dulbecco's Modified Eagle Medium	Cell culture medium
L-glutamine	Cell culture reagent
Trypsin-EDTA (0.05%)	Cell cuture reagent
1x Phosphate Buffered Saline (PBS)	Cell culture reagent
96-well PCR Microtiter Plate (Full Skirt)	Dilution plate
96-well White Opaque Half-well Microtiter Plate	Assay plate

Table 3: Other Equipment and Software

Item Name/Description	Purpose
Perkin Elmer EnSight Multimode Plate Reader *†	Luminometer
Perkin Elmer Kaleido †	Off-the-shelf software for signal acquisition
Microsoft Excel Spreadsheet MG-CDx-FRMS-CF90055 †	Analysis software

* nAbCyte Anti-AAVRh74var HB-FE Assay is intended to be performed on specific serial number-controlled instruments at Labcorp-Monogram Biosciences laboratory.
† Software and cybersecurity were reviewed for in-vitro diagnostic use of the nAbCyte Anti-AAVRh74var HB-FE Assay on serial number-controlled instruments at Labcorp-Monogram Biosciences laboratory.

Specimen Collection and Handling

Collect the patient's whole blood in a glass silicone coated red top tube or a plastic clot activator, silicone coated red top tube. The collection tubes should be filled and thoroughly mixed by manual inversion to mix the blood with the clotting activation agent. After mixing, allow the blood to clot for 30 minutes with the tube standing upright. The collection tube will then be centrifuged at 1500–2000 g for 15 minutes, at which point the clot and serum are separated.

The serum should then be transferred into appropriately labeled 1.5–2.5 mL screw-top plastic vials. A minimum of 0.2 mL of serum is required for testing. The serum, while the vials are standing upright, is to be frozen immediately at -20°C or colder in a non-defrosting freezer until shipment. The frozen serum is to be shipped on dry ice to the Labcorp-Monogram Biosciences laboratory. The sample is not to be thawed after freezing at the collection site.

Assay Principle and Format

The nAbCyte Anti-AAVRh74var HB-FE Assay is a cell-based antibody-mediated neutralization assay using an AAV vector (AAVRh74var CAG lucP) expressing a firefly luciferase reporter gene under control of the CAG promoter. To determine the level of nAb activity in serum samples provided by patients who are candidates to receive BEQVEZ, anti-AAVRh74var nAb activity is assessed by measuring the inhibition of the transduction of cell cultures by AAVRh74var CAG lucP into target cells (i.e., HEK293 cells) and the subsequent expression and quantitation of luciferase via a luminometer.

Patient specimens are assayed by incubating undiluted (neat) and serial dilutions of the serum with the AAV vector prior to transduction of target cells (HEK293 cell line). The samples and controls are run in duplicate using neat serum (1:1) and serial 2-fold dilutions ranging from 1:2 to 1:16. The patient specimen is evaluated for the presence of neutralizing antibody at the designated dilution of 1:1. Additional 1:4 dilutions (e.g., 1:32 through 1: 32,768) are prepared and evaluated as assay plate quality control measures.

Each 96-well plate includes a positive control (PC), negative control (NC), transduction control (TC) and background control (BC). For run/plate acceptance and for patient results to be reported, the TC must meet the pre-defined criteria for the between-well coefficient of variation (CV) for replicate wells. The PC and NC signals at the 1:1 dilution must fall within the established acceptance range. The acceptable inhibition curves should have monotonically decreasing inhibition with increasing dilution by the pre-defined criteria.

Interpretation of Results

Determination of the anti-AAVRh74var nAb activity level is achieved via an algorithm employed to assess percent inhibition. Serum specimens are reported as anti-AAVRh74var nAb activity as calculated per the following equation:

$$\%\ Inhibition = \left(1 - \frac{RLU\ [vector + sample + diluent] - RLU[background]}{RLU[vector + diluent] - RLU[background]}\right) \times 100\%$$

The nAbCyte Anti-AAVRh74var HB-FE Assay is a qualitative assay. Results are reported back to clinicians as Positive or Negative based on the transduction of cells by an AAV vector in the presence of the serum sample relative to the transduction signal in the absence of the serum sample.

- Positive: patient is not eligible for treatment with BEQVEZ (fidanacogene elaparvovec)

- Negative: patient is eligible for treatment with BEQVEZ (fidanacogene elaparvovec) under the supervision of a physician

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for detection of anti-AAVRh74var neutralizing antibodies in human serum for the selection of patients with moderate to severe hemophilia B who are eligible for treatment with BEQVEZ (fidanacogene elaparvovec), an adeno-associated virus serotype rh74 (AAVRh74)-based gene therapy.

VII. MARKETING HISTORY

The nAbCyte Anti-AAVRh74var HB-FE Assay has not been marketed in the United States or any foreign country.

VIII. PROBABLE ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Patients with false positive results (negative for antibodies but tested positive by the assay) would continue with the current standard of care. The risk associated with a false positive result is minimal.

Patients with false negative results for the nAbCyte Anti-AAVRh74var HB-FE Assay (positive for antibodies but tested negative by the assay) would receive BEQVEZ treatment and be exposed to the risks associated with BEQVEZ treatment including the possibility of not experiencing the potential benefits of the treatment. For the specific adverse events related to the approved gene therapy, please see the approved gene therapy product label. Patients who receive treatment with BEQVEZ will have their FIX activity monitored. Based on the patient's response, they may either be tapered off FIX concentrates/hemostatic agents if BEQVEZ treatment demonstrates efficacy or continue to receive FIX treatment if response to the treatment is not achieved.

Procedure-related complications for the assay are limited to obtaining the serum specimen via a blood draw. The risks for the nAbCyte Anti-AAVRh74var HB-FE Assay are equivalent to risks of sample collection for other in vitro diagnostic tests and not unique to the nAbCyte Anti-AAVRh74var HB-FE Assay.

For the specific adverse events that occurred in the clinical studies, please see Section X below.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

1. Establishment of Assay Cut-off

The cut-off for the nAbCyte Anti-AAVRh74var HB-FE Assay was established prior to its use in the clinical study 1 (NCT03861273) (see Section X below). Once established, the cut-off for the device was locked and remain unchanged.

One hundred screening samples comprising serum obtained from healthy human donors and Factor IX deficient (Hemophilia B) were tested with the nAbCyte Anti-AAVRh74var HB-FE Assay. All patient samples assessed had the percent inhibition < 50% at the 1:1 dilution, except one sample. Given the favorable safety profile from the previous clinical studies and the rarity of hemophilia B disease, the cut-off was determined at the neat dilution (1:1) with the percent inhibition as 50%.

2. Anti-AAVRh74var Neutralizing Antibody Detection

The nAbCyte Anti-AAVRh74var HB-FE Assay is intended to detect anti-AAVRh74var neutralizing antibodies. No reference methods exist to detect anti-AAVRh74var neutralizing antibodies, and human derived anti-AAVRh74var neutralizing antibody reference material is not available. As such, it is important that there is empirical evidence to demonstrate that the nAbCyte Anti-AAVRh74var HB-FE Assay detects anti-AAVRh74var antibodies. The following information, studies, and approaches demonstrate that the nAbCyte Anti-AAVRh74var HB-FE Assay detect anti-AAVRh74var neutralizing antibodies:

Design of the nAbCyte Anti-AAVRh74var HB-FE Assay:
The nAbCyte Anti-AAVRh74var HB-FE Assay uses an AAV vector (AAVRh74var CAG lucP) expressing a firefly luciferase reporter gene under control of the CAG promoter. The anti-AAVRh74var nAb activity in serum samples taken from patients is assessed by measuring the inhibition of transduction in cell cultures containing AAVRh74var CAG lucP (i.e., HEK293 cells) and the subsequent expression and quantitation of luciferase via a luminometer. In the presence of anti-AAVRh74var nAb activity, the expression of luciferase is inhibited.

Anti-AAVRh74var neutralizing antibody detection:
Capsid inhibition was conducted to demonstrate that the nAbCyte Anti-AAVRh74var HB-FE Assay detects anti-AAVRh74var nAbs. In the capsid inhibition study, four test sera samples (positive control, samples with 60%, 70% and 90% inhibition) were treated with empty capsids which compete for capsid binding. The results showed a reduction of anti-AAVRh74 nAb activity in all four samples, indicating the nAbCyte Anti-AAVRh74var HB-FE Assay detects anti-AAVRh74var neutralizing antibodies.

Immunoglobulin (IgG) depletion was also conducted to demonstrate that the nAbCyte Anti-AAVRh74var HB-FE Assay detects anti-AAVRh74var nAbs. In the study, four test sera samples (positive control, samples with 60%, 70% and 90% inhibition) were pre-incubated with Protein G beads, which bind to any free immunoglobulin and thus rendering them unavailable to contribute to neutralizing antibody activity. The significant reduction of the inhibition activity indicates that the nAbCyte Anti-AAVRh74var HB-FE Assay does detect anti-AAVRh74var neutralizing antibodies.

Anti-AAVRh74var mouse antibody (mAb) recognition:
Due to the high percent similarity of sequence alignment between the AAVRh74 capsid and AAV6 as well as AAV9 capsids, the ability of the nAbCyte Anti-AAVRh74var HB-FE Assay to distinguish AAVRh74var directed nAb activity was demonstrated by evaluating the anti-AAVRh74var mAb reactivity against AAV6-CMVIVS-fLuc and AAV9-CMVIVS-fLuc infection. The study also evaluated anti-AAV6 and anti-AAV9 mAb control sera to neutralize AAVRh74var CAG lucP vector infection. Test results indicate that the nAbCyte Anti-AAVRh74var HB-FE Assay is specific for detection of anti-AAVRh74var neutralizing antibodies.

Assay response for clinical samples pre- and post-BEQVEZ dose:
Pre- and post-BEQVEZ dose samples from subjects enrolled in the clinical study 1 (NCT03861273) were evaluated. All 42 subjects who were negative as determined by the assay at baseline (pre-dose) were shown to convert to positive (with 100% inhibition), as determined by the assay, after 52 weeks post-dose with BEQVEZ, at which time patients would be expected to have anti-AAVRh74var neutralizing antibodies detectable by the assay.

AAVRh74var seroprevalence as determined by the nAbCyte Anti-AAVRh74var HB-FE Assay:

The C0371004 study (NCT03587116) was a non-investigational product, multi-center, lead-in study designed to evaluate current Factor IX (FIX) prophylaxis replacement therapy, in the usual care setting of moderately severe to severe adult hemophilia B participants (FIX:C≤2%) who are negative for neutralizing antibodies (nAb) to adeno associated virus vector AAVRh74var. The study consisted of two phases: the screening phase, which included a laboratory blood draw to determine nAb status to AAVRh74var for the group of hemophilia B participants, and the second phase consisted of the data collection phase for those hemophilia B participants who were below the established threshold for nAb to AAVRh74var. The study was designed to provide prospective efficacy and selected safety data of FIX prophylaxis replacement therapy in the usual setting of those hemophilia B participants. Upon successfully completing the C0371004 study, participants were consented and screened for entry into the therapeutic Phase 3 hemophilia B gene therapy study described in the Clinical Performance section below.

All patients were tested for presence of anti-AAVRh74var nAbs in C0371004 study that included 314 hemophilia B participants. Of the 314 hemophilia B patients, 193 tested positive for anti-AAVRh74var nAbs while 121 tested negative for anti-AAVRh74var nAbs with the nAbCyte Anti-AAVRh74var HB-FE Assay. Based on this data, sixty-one percent (193/314) were found to be positive for the anti-AAVRh74var nAb activity.

3. Precision Studies

Description of samples in the precision studies.
Samples evaluated in the precision studies were made from single or pooled human sera to achieve different inhibition levels at the 1:1 dilution. Aliquots of each sample type were stored in frozen storage (-70°C or colder) until use in the precision studies.

- Sample 1: Negative sample targeting approximately 30% inhibition.
- Sample 2: Negative sample targeting approximately 40% inhibition.
- Sample 3: Low positive sample targeting approximately 60% inhibition.
- Sample 4: Positive sample targeting approximately 70% inhibition.
- Sample 5: High positive sample targeting approximately >90% inhibition.

Precision Study #1: Repeatability

Design: The repeatability study evaluated each of the five sample types in 20–40 replicates using a single lot of reagents, and run on a single instrument system by a single operator. A replicate is the mean of the measurements from two duplicate wells on the plate.

Table 4: Repeatability Study Results

Sample #	N	Mean % Inhibition	%CV	Anticipated Result	Result As Anticipated/ Total
1	20	21.7	20%	Negative	20/20
2	40	32.7	13%	Negative	40/40
3	40	67.6	6%	Positive	40/40
4	20	63.6	7%	Positive	20/20
5	20	100	0%	Positive	20/20

Precision Study #2: Within-laboratory precision (repeatabilityand between-day components)

Design: The within-laboratory precision study was based on the single-site precision evaluation study described in *Clinical and Laboratory Standards Institute (CLSI) EP05-A3 – Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.* The study was performed over 20 days, with two runs (plates) per day, and two replicate measurements per sample type (a replicate measurement is an average of two replicates of the same sample on the same plate). A

single lot of critical reagents was used in the study, and the study was run on a single instrument system by a single operator. A total of 80 replicates were collected per sample (20 days x 2 runs/per day) x 2 replicates = 80 replicates per sample).

Table 5: 20-Day Precision Study Results

Sample #	N	Mean % Inhibition	Repeatability		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV
1	80	23.6	5.0	23%	1.2	5%	5.2	23%
2	80	33.2	3.4	15%	3.6	16%	5.0	22%
3	80	66.5	2.7	13%	1.8	8%	3.3	15%
4	80	66.1	3.5	15%	0.7	3%	3.6	15%
5	80	100.0	0.0	0%	0.0	0%	0.1	0%

Table 6: 20-Day Precision Study – Qualitative Results

Sample #	Anticipated Result	Result As Anticipated/Total
1	Negative	80/80
2	Negative	80/80
3	Positive	80/80
4	Positive	80/80
5	Positive	80/80

Precision Study #3: Within-Laboratory Precision (Operator-to-Operator Variability)

Design: The study to evaluate operator-to-operator variability was based on CLSI EP05-A3. Each sample type was evaluated by each of three operators, over ten (non-consecutive) days, with two runs (plates) per day, and with one replicate on each plate. The study was conducted using a single lot of critical reagents and was performed on a single instrument system. A total of 60 determinations (3 operators x 10 days x 2 determination/day) were collected per serum sample.

Table 7: Operator Precision Study Results

Sample #	N	Mean % Inhibition	Repeatability		Between-Day		Between-Operator		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	60	22.8	5.5	24%	5.6	24%	3.2	14%	8.5	37%
2	60	33.8	4.5	13%	4.8	14%	3.9	12%	7.7	23%
3	60	63.1	3.9	6%	4.4	7%	4.0	6%	7.1	11%
4	60	63.9	5.2	8%	0.3	0%	2.7	4%	5.8	9%
5	60	100.0	0.0	0%	0.0	0%	0.0	0%	0.0	0%

Table 8: Operator Precision Study – Qualitative Results

Sample #	Anticipated Result	Result As Anticipated/Total
1	Negative	60/60
2	Negative	59/60
3	Positive	58/60
4	Positive	59/60

5	Positive	59/60

Operator-to-Operator Variability (Manual pipetting method)

Design: The study to evaluate operator-to-operator variability using manual pipetting method was based on CLSI EP05-A3. Two sample type (Sample 2 and Sample 3) was evaluated by each of three operators, over ten (non-consecutive) days, with two runs (plates) per day, and with one replicate on each plate. The study was conducted using a single lot of critical reagents and was performed on a single instrument system. A total of 60 determinations (3 operators x 10 days x 2 determination/day) were collected per serum sample.

Table 9: Operator Precision Study Results (Manual pipetting method)

Sample #	N	Mean % Inhibition	Repeatability		Between-Day		Between-Operator		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	60	47.1	4.2	9%	4.8	10%	3.7	8%	4.2	9%
3	60	67.2	3.4	5%	4.4	7%	3.3	5%	3.7	6%

Precision Study #4: Within-Laboratory Precision (Instrument-to-Instrument Variability)

Design: Instrument-to-instrument variability was evaluated using data collected from all plates from both Operator-to-Operator and Lot-to-Lot studies using three luminometers. A replicate is the mean of the measurements from two duplicate wells on the plate. Samples were tested on each instrument on discrete plates, as independent runs. A total of 120 replicates per sample were collected.

Table 10: Instrument Precision Study Results

Sample	N	Mean % Inhibition	Repeatability		Between-Day		Between-Luminometer		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	120	22.5	7.3	33%	2.4	11%	0.0	0%	7.7	34%
2	120	33.2	4.2	13%	5.1	15%	0.0	0%	6.6	20%
3	120	62.9	3.6	6%	4.6	7%	0.0	0%	5.8	9%
4	120	63.8	4.8	8%	1.9	3%	0.0	0%	5.2	8%
5	120	100.0	0.0	0%	0.0	0%	0.0	0%	0.0	0%

Table 11: Instrument Precision Study – Qualitative Results

Sample #	Anticipated Result	Result As Anticipated/ Total
1	Negative	120/120
2	Negative	120/120
3	Positive	119/120
4	Positive	120/120
5	Positive	120/120

Precision Study #5: Within-Laboratory Precision (Lot-to-Lot Variability)

Design: The study to evaluate critical reagent lot-to-lot variability was based on CLSI EP05-A3. Each sample type was run with three unique reagent lots, over ten (non-consecutive) days, with two runs (plates) per day, and with one replicate on each run. A replicate is the mean of the measurements from two duplicate wells on the plate. The study was run on a single instrument system by a single operator. A total of 60 replicates per sample were collected (10 days x 3 Lots x 2 run/day x 1 replicates = 60 replicates per sample).

Table 12: Critical Reagent Lot Precision Study

Sample	N	Mean % Inhibition	Repeatability		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	60	20.3	6.0	30%	3.0	15%	3.2	16%	7.4	37%
2	60	32.6	4.9	15%	2.4	7%	2.6	8%	6.1	19%
3	60	66.3	3.1	5%	0.5	1%	0.7	1%	3.3	5%
4	60	62.5	3.7	6%	0.0	0%	2.6	4%	4.5	7%
5	60	100.0	0.0	0%	0.0	0%	0.0	0%	0.0	0%

Table 13: Critical Reagent Lot Precision Study – Qualitative Results

Sample #	Anticipated Result	Result As Anticipated/ Total
1	Negative	60/60
2	Negative	59/60
3	Positive	60/60
4	Positive	60/60
5	Positive	59/60

nAbCyte Anti-AAVRh74var HB-FE Assay Overall Precision

The tables below present estimates of the repeatability, between-run, between-day, between-operator, between-lot and between-instrument components of precision using data from the studies described above.

Table 14: Overall Precision

Sample	N	Mean % Inhibition	Repeatability †		Between-Day *		Between-Operator **		Between-Lot #		Between-Luminometer ‡		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	579	21.5	6.1	28%	3.3	15%	2.3	11%	3.4	16%	0.0	0%	8.0	37%
2	600	32.7	4.4	13%	3.9	12%	4.0	12%	2.7	8%	0.1	0%	7.6	23%
3	600	64.9	3.3	5%	3.1	5%	4.1	6%	0.9	1%	0.0	0%	6.1	9%
4	580	63.4	4.2	7%	1.2	2%	2.9	5%	2.8	4%	0.0	0%	5.9	9%
5	580	100.0	0.0	0%	0.0	0%	0.0	0%	0.0	0%	0.0	0%	0.0	0%

†Repeatability was estimated with pooling study 1 and study 2.
*Between-day variation was estimated with pooling study1 to study 5.
**Between-operator variation was estimated using study 3.

Between-Lot variation was estimated using study 5.
‡ Between-instrument variation was estimated using study 4.

Table 15: Overall Precision Study Qualitative Result

Sample #	Anticipated Result	Number of Anticipated/Total
1	Negative	579/579
2	Negative	600/600
3	Positive	598/600
4	Positive	580/580
5	Positive	580/580

4. Linearity study

Not applicable.

5. Analytical Sensitivity

The C5-C95 interval were calculated based on *CLSI EP12-A2 – User Protocol for Evaluation of Qualitative Test Performance*. The C5-C95 interval, which is the range of analyte concentration around the cut-off such that observed results at concentrations outside this interval are consistently negative (concentrations <C5) or consistently positive (concentrations>C95), is 43–57% inhibition at the 1:1 dilution.

6. Endogenous Interfering Substances (Analytical Specificity)

A study was performed based on *CLSI EP07 A3 – Interference Testing in Clinical Chemistry.* This study was to evaluate endogenous substances typically found in serum samples and potential interferents to the assay. Five sera samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition) and the Negative Control were used in this study. Five (5) replicates of each sample were tested at each concentration of each endogenous substance as recommended in Table 2 of *CLSI EP37 - Ed. 1 Supplemental Tables for Interference Testing in Clinical Chemistry*. An endogenous substance is not considered an interferent if addition of the test substance did not change the qualitative output of any of the treated samples compared to the control sample or the percent difference between treated samples and control sample was <10% for Samples 2–5 and <15% for Sample 1.

The following endogenous substances were not found to interfere with the nAbCyte Anti-AAVRh74var HB-FE Assay results at the indicated concentration(s).

Table 16: Non-interfering endogenous substances

Endogenous Components	Test Concentration
Hemoglobin	1000 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL

Endogenous Components	Test Concentration
Triglyceride	1500 mg/dL
Albumin	6 g/dL
Rheumatoid Factor	20 IU/mL
Cholesterol*	400 mg/dL

*Cholesterol was only tested with two samples (Sample 3 and 4, with five replicates per sample) that were made to be approximately 10% above and 10% below the assay cut-off, respectively.

7. Exogenous Interfering Substances (Analytical Specificity)

A study was performed based on *CLSI EP07 A3 – Interference Testing in Clinical Chemistry* and evaluated exogenous substances to include anticoagulants, and concomitant medications commonly used by the patient population. Five sera samples exhibiting anti-AAVRh74var nAb activity throughout the assay range (0 to 100% inhibition) and Negative Control were used in this study. Five (5) replicates of each sample were tested at each concentration of each exogenous substance as recommended in Table 1 of *CLSI EP37 - Ed. 1 Supplemental Tables for Interference Testing in Clinical Chemistry* or, for concomitant medications not listed in CLSI EP37, at levels based on the reported Cmax values (3X Cmax as highest concentration tested). An exogenous substance is not considered an interferent if addition of the test substance did not change the qualitative output of any of the treated samples compared to the control sample or the percent difference between treated samples and control sample was <10% for Samples 2–5 and <15% for Sample 1. No exogenous substances were found to interfere with the assay at the concentration tested.

The following exogenous substances were not found to interfere with the nAbCyte Anti-AAVRh74var HB-FE Assay results at the indicated concentration(s).

Table 17: Non-interfering exogenous substances

Substance	Test Concentration
Factor IX Raplacement Therapy (e.g., BeneFix)	210 IU/dL
Factor IX Raplacement Therapy (e.g., AlphaNine)	300 IU/dL
Celecoxib	0.879 mg/dL
Acetaminophen	30 µg/dL
Darunavir	1.59 mg/dL
Efavirenz	1.2 mg/dL
Dolutegravir	0.0064 mg/dL
Tenofovir	0.0978 mg/dL
Tranexamic Acid	49.23 µg/mL
Amino Caproic Acid	0.900 mg/dL
Fetal Bovine Serum (FBS)	10%
Oxycodone	0.0342 mg/dL

Substance	Test Concentration
Harvoni (ledipasvir, sofosbuvir)	969 ng/mL
	1854 ng/mL
Zepatier (elbasvir, grazoprevir)	363 ng/mL
	495 ng/mL

8. Cross-reactivity study (Analytical Specificity)

A study was performed to determine whether anti-viral antibodies potentially contained in a patient serum sample can result in false positive or false negative measurements of anti-AAVRh74var nAb activity. Five sera samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition) and Negative Control were used in this study. Five (5) replicates of each sample were tested at each concentration of each antibody. An antibody is not considered an interferent if addition of the test substance did not change the qualitative output of any of the treated samples compared to the control sample or the percent difference between treated samples and control sample was <10% for Samples 2–5 and <15% for Sample 1. The following anti-viral antibodies were not found to interfere with the nAbCyte Anti-AAVRh74var HB-FE Assay results at the indicated concentration(s).

Table 18: Cross-Reactivity Antibodies

Anti-Viral Antibody	Non-interfering Test Concentration (µg/mL)
Polio, type 1,2,3	200
Adenovirus (all 41 types)	200
HBV	200
Chicken Pox	200
Mumps	200
Measles	200
HCV	200
HIV	200

The nAbCyte Anti-AAVRh74var HB-FE Assay was evaluated for potential interference from, or cross-reactivity to, adeno-associated (AAV) serotypes AAV6 and AAV9. The results demonstrated that there is no reactivity for AAV6 and AAV9 to the nAbCyte Anti-AAVRh74var HB-FE Assay.

9. Prozone/High-dose hook effect

A high-dose hook effect study was performed to characterize the performance of the nAbCyte Anti-AAVRh74var HB-FE Assay when used to test a dilution series of specimens containing very high levels of AAVRh74var neutralizing antibodies that have the potential to cause a high- dose hook effect. The study utilized one mouse monoclonal anti-AAVRh74var neutralizing antibody contrived human serum sample at 200 µg/mL and two post-dose patient serum samples. The samples were diluted in six-fold series for

at least 10 dilutions by using the negative serum as a diluent. Each dilution step was tested in three replicates using one lot of reagents. The results from this study indicated that there were no false negative results observed for tested samples with high anti-AAVRh74var neutralizing antibody, and that anti-AAVRh74var neutralizing antibody at the elevated concentrations tested does not produce a prozone (hook) effect for the nAbCyte Anti-AAVRh74var HB-FE Assay.

10. Carryover study

A study was performed to evaluate the susceptibility of the nAbCyte Anti-AAVRh74var HB-FE Assay to within-assay sample carryover. The samples used in the study were composed of a negative sample and three sera samples exhibiting anti-AAVRh74var nAb across the assay range (around 40%, 60% and 90% inhibition). The samples were evaluated with ten replicates/determinations per plate. All (100%) negative and positive replicates on the plates provided the expected results, demonstrating that the nAbCyte Anti-AAVRh74var HB-FE Assay is not susceptible to within-assay plate carryover.

11. Sample Stability

A study was conducted to evaluate the effect of sample storage under various conditions and storage durations for serum samples. Five sera samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition), in addition to the Positive and Negative Control were used in this study. Each sera sample type was tested five times at each condition using a single reagent lot.

Stability of the patient sample during collection and processing for use with the nAbCyte Anti-AAVRh74var HB-FE Assay was determined. The information reviewed supported the following serum sample stability claims for the nAbCyte Anti-AAVRh74var HB-FE Assay:

Table 19: Sample Collection Stability

Storage Condition	Duration of stability
Ambient temperature (18° to 28°C)	20 hours
Refrigerated (4°C)	72 hours
Frozen (-20°C)	1 month
Frozen (-70°C)	12 months
Freeze/Thaw	3 cycles

Stability of the patient sample during transport to Labcorp Monogram Biosciences Laboratories for use with the nAbCyte Anti-AAVRh74var HB-FE Assay was determined. Samples should be stored inside dry ice boxes (using an insulated shipping container with dry ice) during transport. The information reviewed supported the following serum sample stability claims for the nAbCyte Anti-AAVRh74var HB-FE Assay:

Table 20: Sample Transport Stability

Transport Condition	Duration of stability
Room temperature/ambient	3 days
Elevated temperature (37°C)	3 days

12. Reagent stability

Reagent stability studies were performed to establish real-time shelf-life stability and in-use stability for critical reagents when used with the nAbCyte Anti-AAVRh74var HB-FE Assay. Reagent stability studies were conducted as recommended in *CLSI EP25-A – Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*, evaluating the performance of multiple vendor lots of each critical reagent using five sera samples exhibiting anti-AAVRh74var nAb throughout the assay range (0 to 100% inhibition). The information reviewed supported the following reagent stability claims for the nAbCyte Anti-AAVRh74var HB-FE Assay:

Table 21: Reagent Stability

Reagent	Storage Condition	Duration of Stability
AAVRh74var CAG LucP Vector	Frozen (-70°C)	12 months
Read Mix	Frozen (-70°C)	12 months
Fetal Bovine Serum	Frozen (-70°C)	12 months
Anti-AAVRh74var Negative Control	Frozen (-70°C)	12 months
Anti-AAVRh74var Positive Control	Frozen (-70°C)	12 months

X. SUMMARY OF CLINICAL INFORMATION

Probable benefit and safety of the nAbCyte Anti-AAVRh74var HB-FE Assay was demonstrated through testing of specimens from patients with moderate to severe hemophilia B enrolled in the clinical study 1 (study objective to evaluate the safety and efficacy of BEQVEZ; ClinicalTrials.gov Identifier NCT03861273). The results from this study were used to establish a reasonable assurance of safety of the nAbCyte Anti-AAVRh74var HB-FE Assay for the selection of patients with moderate to severe hemophilia B who are eligible for treatment with BEQVEZ treatment. Data from this clinical study support this HDE approval decision. A summary of the clinical study is presented below.

A. Study Design

The efficacy of BEQVEZ was evaluated in an ongoing, prospective, open-label, single-arm, multi-national Phase 3 study. The study enrolled 45 adult male patients with moderately severe to severe hemophilia B (factor IX activity ≤2 IU/dL) and all received a single intravenous infusion of BEQVEZ at a dose of 5×10^{11} vg/kg of body weight and entered a follow-up period of 6-years. Of the 45 patients, 41 completed at least 15 months of follow up. The median follow up of the 45 treated patients was 2 years (range: 0.4 to 3.2 years). The effectiveness of the assay was determined based on the correlation

between negative nAbCyte Anti-AAVRh74var HB-FE Assay test results and responder status post-BEQVEZ (fidanacogene elaparvovec) treatment.

1. Clinical Inclusion and Exclusion Criteria

   Key inclusion criteria for patient enrollment in clinical study 1 (NCT03861273):

   - Participants must have completed at least 6 months of routine FIX prophylaxis therapy during the lead-in study prior to providing consent at the screening visit for this study.
   - Participants who have documented moderately severe to severe hemophilia B, defined as FIX:C ≤2%.
   - Participants must agree to suspend prophylaxis therapy for hemophilia B after administration of the IP. FIX replacement therapy is allowed as needed.
   - Acceptable screening laboratory values as follows:
     • Hemoglobin ≥11 g/dL;
     • Platelets ≥100,000 cells/μL;
     • Creatinine ≤2.0 mg/dL.
   - Sexually active participants must have agreed to use an acceptable method of effective contraception.
   - Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

   Key exclusion criteria for patient enrollment in clinical study 1 (NCT03861273):

   - Anti-AAVRh74var neutralizing antibodies (nAb) titer ≥1:1 (i.e., positive for nAb), determined by nAbCyte Anti-AAVRh74var HB-FE Assay in a central laboratory during screening.
   - Prior history of inhibitor to FIX or positive inhibitor testing as measured by the central laboratory ≥0.6 Bethesda Units (BU) during screening. Clinical signs or symptoms of decreased response to FIX.
   - Known hypersensitivity to FIX replacement product or intravenous immunoglobulin administration.
   - History of chronic infection or other chronic disease that investigator deems as an unacceptable risk.
   - Any concurrent clinically significant major disease or condition that the investigator deems unsuitable for participation or other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavior (including alcoholism) or laboratory abnormality that may increase the risk associated with study participation or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the participant inappropriate for entry into this study.

- Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) >2x upper limit of normal (ULN), based on central laboratory results.
- Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%), based on central laboratory results.
- Current unstable liver or biliary disease per investigator assessment defined by the presence of ascites, hepatic encephalopathy, coagulopathy, hypoalbuminemia, esophageal or gastric varices, persistent jaundice, or cirrhosis.
- Currently on antiviral therapy for hepatitis B or C.
- Any participant with a planned surgical procedure requiring FIX surgical prophylactic factor treatment in the next 15 months.
- Participants using therapies that are restricted.
- Previously dosed in a gene therapy research trial at any time or in an interventional clinical study within the last 12 weeks, excluding participation in study C0371004.
- Active hepatitis B or C; HBsAg, HBV-DNA positivity, or HCV-RNA positivity.
- Significant liver disease, as defined by pre-existing diagnosis of portal hypertension, splenomegaly or hepatic encephalopathy. Additionally, during screening, a serum albumin level below normal limits and/or significant liver fibrosis by any of the following diagnostic modalities: FibroScan score >8 kPa units, Fibro Test/FibroSURE >0.48* or AST-to-Platelet Ratio Index (APRI) >1. In the investigator's opinion, if there is concern regarding the FibroTest results due to a confounding medical history (e.g., proteinuria can impact FibroTest result), the investigator can perform a different assessment of liver fibrosis (e.g., FibroScan or APRI) during the screening period.
- Serological evidence of HIV-1 or HIV-2 infection with either CD4+ cell count ≤200 mm or viral load >20 copies/mL.
- Investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or participants who are Pfizer employees, including their family members, directly involved in the conduct of the study.
- Unable to comply with scheduled visits, treatment plan, laboratory tests and other study procedures for up to six years post infusion of BEQVEZ in the investigator's judgement.
- Sensitivity to heparin or heparin-induced thrombocytopenia.
- Sensitivity to any of the study interventions, or components thereof, or drug or other allergy that, in the opinion of the investigator or the sponsor's Medical Monitor, contraindicates participation in the study.

2. Follow-up Schedule

Disease assessment and other clinical assessments were conducted according to the protocol during the trial. Post-infusion and safety follow-up was conducted through 52-weeks post-infusion. Additional safety follow-up is conducted throughout the 6-year study period post-infusion.

3. Clinical Endpoints

The primary endpoint was a non-inferiority (NI) test of annualized bleeding rate (ABR) during the efficacy evaluation period (EEP, Week 12 (Day 82) to the last visit prior to data cutoff following BEQVEZ treatment) compared with baseline ABR during the lead-in period.

B. Accountability of HDE Cohort

A total of 45 participants subjects were enrolled in the clinical study 1 (NCT03861273). All subjects were screened with the nAbCyte Anti-AAVRh74var HB-FE Assay and received a single dose of BEQVEZ. Since no more than a single dose of study treatment on Day 1 was administered during the study, there were no treatment discontinuations. Of the 45 participants infused with a single dose of BEQVEZ, 41 participants had completed 15 months of follow-up. All 45 participants were included in the Dosed Analysis Set that was used for the efficacy evaluation.

C. Study Population Demographics and Baseline Parameters

In clinical study 1 (NCT03861273), 45 subjects, aged 18 to 62 years (median: 29 years), were selected to receive BEQVEZ, in part based on results from the nAbCyte Anti-AAVRh74var HB-FE Assay. The population was 73.3% White (33 patients), 15.6% Asian (7 patients), and 2.2% Black (1 patient). A total of 13 (29%) and 15 (33%) patients had a history of hepatitis B and C, respectively. One (2%) patient was HIV positive and two (4%) other patients had positive HIV antibodies but undetectable viral load and no history of HIV. Subjects were previously treated only with FIX prophylaxis therapy.

Table 22: Demographics of clinical study 1 (NCT03861273) population	
Age at enrollment, years	
Mean (SD)	33.2 (10.9)
Median (Range)	29.0 (18, 62)
Sex, n (%)	
Male	45 (100)
Race, n (%)	
Asian	7 (15.6)
Black or African American	1 (2.2)
Native Hawaiian or other Pacific Islander	0
White	33 (73.3)
Not provided due to patient privacy	4 (8.9)
Ethnicity, n (%)	
Hispanic or Latino	2 (4.4)
Not Hispanic or Latino	35 (77.8)
Not provided due to patient privacy	8 (17.8)
Type of FIX treatment for hemophilia B, n (%)	
Prophylaxis	45 (100)

D. Safety and Probable Benefit Results

1. Safety Results

The nAbCyte Anti-AAVRh74var HB-FE Assay involves the testing of serum processed from blood samples. Blood samples are routinely collected as part of the management of hemophilia B. Sample collection for this test presents no additional safety hazard to the patient being tested.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The safety of BEQVEZ was evaluated in 60 (45 subjects in clinical study 1 (NCT03861273) and 15 subjects in clinical study 2 (NCT02484092/NCT03307980)) patients who received the recommended dose (5×10^{11} vg/kg) in two open-label clinical studies. No serious adverse reactions were reported in patients treated with BEQVEZ. The most common adverse reactions observed in ≥5% of subjects post-dose are listed in Table 23: Safety data collected in clinical studies indicate that BEQVEZ treatment was generally well-tolerated. No adverse events associated with the use of the nAbCyte Anti-AAVRh74var HB-FE Assay occurred during the clinical studies. No participants discontinued from the study as a result of a treatment emergent adverse event (TEAE). Long-term safety data was not collected as part of the clinical study. The long-term safety of BEQVEZ therapy is unknown. Not all transaminase elevations were reported as adverse reactions. Please refer to BEQVEZ labeling for additional safety information on the treatment.

Table 23. Adverse Reactions (Incidence ≥5%) Following Treatment with BEQVEZ

Adverse Reactions	Clinical Study 1 Subjects (%) (N=45)	Clinical Study 2 All Subjects (%) (N=15)
Transaminases increased*	24 (53.3%)	2 (13.3%)

* Includes terms alanine transaminase (ALT) increased, aspartate transaminase (AST) increased, hepatic enzyme increased, hepatic function abnormal, liver function test abnormal, transaminases increased.

2. Probable Benefit Results

The probable benefits of the nAbCyte Anti-AAVRh74var HB-FE Assay as a companion diagnostic device for the detection of AAVRh74var neutralizing antibodies in human serum to aid in the selection of patients with moderate to

severe hemophilia B for treatment with BEQVEZ are based on data from 45 subjects in clinical study 1 (NCT03861273) who had a "Negative" result. The clinical study 1 is an ongoing, prospective, open-label, single-arm, multi-national Phase 3 study. The study enrolled 45 adult male patients with moderately severe to severe hemophilia B (factor IX activity ≤2 IU/dL). All patients completed a prospective lead-in study of at least six months for baseline data collection while they received routine factor IX prophylaxis in the usual care setting before entering clinical Study 1. Enrolled patients then received a single intravenous infusion of BEQVEZ at a dose of 5×10^{11} vg/kg of body weight and entered a follow-up (FU) period of 6-years. Of the 45 patients, 41 completed at least 15 months of FU. The median FU of the 45 treated patients was 2.0 years (range: 0.4 to 3.2 years) from the time of infusion.

Only patients who were negative for pre-existing neutralizing antibodies to AAVRh74var capsid were eligible. Other key exclusion criteria included active hepatitis B or C infection, ALT/AST/ALP >2 times ULN, bilirubin >1.5 times ULN, unstable liver or biliary disease, and significant liver fibrosis.

Enrolled patients were 73% White, 16% Asian and 2.2% Black. The median age was 29 years (range: 18 to 62 years). A total of 13 (29%) and 15 (33%) patients had a history of hepatitis B and C, respectively. One (2%) patient was HIV positive.

The main efficacy outcome was a non-inferiority (NI) test of annualized bleeding rate (ABR) during the efficacy evaluation period (EEP, Week 12 (Day 82) to data cutoff following BEQVEZ treatment) compared with baseline ABR during the lead-in period. The ABR included treated and untreated bleeds, excluding procedural bleeds. The NI margin on the difference between the mean EEP ABR and the mean baseline ABR was 3.0 bleeds/year.

Efficacy results for clinical study 1 using the patient population selected in part by the nAbCyte Anti-AAVRh74var HB-FE Assay are summarized in Table 24. The results showed that the mean ABR was 4.5 bleeds/year (95% CI: 1.9, 7.2) during the baseline period and 2.5 bleeds/year (95% CI: 1.0, 3.9) during post-BEQVEZ EEP, resulting in a difference between the mean post-BEQVEZ EEP ABR and the baseline ABR of -2.1 bleeds/year (95% CI: -4.8, 0.7). The upper bound of the 95% CI in the difference was less than 3.0 bleeds/year, meeting the NI study success criterion. Six out of 45 patients (13%) resumed routine factor IX prophylaxis after BEQVEZ treatment, starting from 0.4 years to 1.7 years after BEQVEZ infusion. An additional patient had intermittent exogenous factor IX use and had a higher ABR post BEQVEZ (5.0 bleeds/year) compared to baseline (1.2

bleeds/year) with a factor IX activity <5% (SynthASil assay) starting at 0.4 years. The results from this study support the clinical benefit of the nAbCyte Anti-AAVRh74var HB-FE Assay in the selection of patients with moderate to severe hemophilia B for treatment with BEQVEZ.

Table 24. Efficacy results in clinical study 1 (NCT03861273)

	Baseline Prospective Lead-in Period (N=45)	**Post-BEQVEZ Efficacy Evaluation Period**[#] (N=45)
Median (range) of follow-up time (years)	1.2 (0.6, 2.4)	1.8 (0.2, 3.0)
Total follow-up time (person-years)	59	83
Median (min, max) ABR (bleeds/year)[b]	1.3 (0.0, 53.9)[c]	0.0 (0.0, 19.0)
Model derived mean ABR [bleeds/year] (95% CI)[b,d]	4.5 (1.9, 7.2)	2.5 (1.0, 3.9)
n (%) of patients without any bleeds	13 (29%)	27 (60%)
Total number of observed bleeds	225	98
Number of observed spontaneous bleed count (proportion of total bleeds)	157 (70%)	60 (61%)
Number of observed joint bleed count (proportion of total bleeds)	184 (82%)	71 (72%)

ABR = Annualized Bleeding Rate for all bleeds (treated and untreated with factor IX, excluding procedural bleeds).
CI = confidence interval; SD = standard deviation

a. Post-BEQVEZ efficacy evaluation period is from Week 12 (Day 82) to data cutoff.
b. A total of 7 participants (16%) had used factor IX replacement products during the efficacy evaluation period for extended prophylaxis that confounded the treatment effect of BEQVEZ, with a median start time at 0.8 (range: 0.4 to 1.1) years. An ABR of 20 bleeds/year was imputed for the confounded periods.
c. The results presented in this table included data on a participant with a baseline ABR of 53.9 bleeds/year, which disproportionately influenced the baseline ABR estimate. A post-hoc sensitivity analysis, excluding this participant, still met the non-inferiority study success criterion.
d. Model-based ABR estimates from a repeated measures generalized linear model with negative binomial distribution and identity link function.

E. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

XI. FINANCIAL DISCLOSURE

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator at the single test site Labcorp-Monogram Biosciences. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SAFETY AND PROBABLE BENEFIT ANALYSIS

A. Probable Benefit Conclusions

The probable benefits of the nAbCyte Anti-AAVRh74var HB-FE Assay are based on data collected in clinical study 1 (NCT03861273), an ongoing, prospective, open-label, single-arm, multi-national Phase 3 study consisting of 45 moderately severe to severe adult hemophilia B patients. A single intravenous dose of 5×10^{11} vg/kg of body weight of BEQVEZ met the pre-specified non-inferiority margin. The results from this study support the clinical benefit of the nAbCyte Anti-AAVRh74var HB-FE Assay in the selection of moderate to severe hemophilia B patients for the treatment with BEQVEZ.

B. Safety Conclusions

Failure of the device to perform as expected or failure to correctly interpret test results may lead to inaccurate or unreliable test results, and subsequently, inappropriate patient management decisions for hemophilia B patients for which BEQVEZ is being considered. Patients with false positive results would continue with the current standard of care. The risk associated with a false positive result is minimal. Patients with positive results were not enrolled in clinical study 1 (NCT03861273). Patients with false negative results would be inappropriately determined to be eligible for treatment with BEQVEZ. The benefit from gene therapy in the presence of pre-existing anti-AAVRh74var antibodies is unclear. Patients with false negative results may undergo treatment with BEQVEZ and may be exposed to short-term and long-term risks associated with BEQVEZ treatment.

Safety data collected in clinical study 1 (NCT03861273) have shown that BEQVEZ treatment was generally well-tolerated. The long-term safety of BEQVEZ therapy is unknown.

C. Probable Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in clinical study 1 (NCT03861273) as described above and the data from the study support the efficacy of BEQVEZ.

The probable risks of the device are also based on data collected in clinical study 1 (NCT03861273). The data for patients selected to receive treatment in part by the nAbCyte Anti-AAVRh74var HB-FE Assay demonstrated a favorable safety and tolerability profile for Fidanacogene elaparvovec. No subjects have withdrawn from the study as a result of an AE. No adverse events associated with the use of the nAbCyte Anti-AAVRh74var HB-FE Assay occurred during the clinical studies. No thromboembolic events have been reported, and no subjects have developed clinically meaningful anti-FIX inhibitors.

Additional factors, including the rarity of severe hemophilia B, the ability to manage false positive patients with standard of care and the lack of alternative testing methods are considered in the assessment of benefit-risk. Additionally, the current device supports filling an unmet medical need for more effective gene therapy treatment of severe hemophilia B, an irreversibly debilitating disease.

When considering the above factors and additional risk mitigations provided by appropriate labeling, the probable benefit of this device outweighs the probable risk, and the data provide a reasonable assurance of probable benefits and safety for the proposed indications for use.

1. Patient Perspective

This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the HDE for this device.

In conclusion, given the available information described above, the data support that probable benefit of use of this device to identify adult patients with moderate to severe hemophilia B without pre-existing anti-AAVRh74var neutralizing antibodies to determine eligibility to receive BEQVEZ outweighs the probable risks associated with the device, when considering the mitigations provided by appropriate labeling.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and probable benefit of this device when used in accordance with the indications for use.

Therefore, it is reasonable to conclude that the probable benefits to health from using the device for the target population outweighs the risk of illness or injury, taking into account the probable risks and benefits of currently available devices or alternative forms of treatment when used as indicated in accordance with the directions for use.

XIII. PANEL RECOMMENDATION

In accordance with the provisions of section 520(m) of the act as amended by the Safe Medical Devices Act of 1990, this HDE was not referred to the Hematology Panel, an FDA

advisory committee, for review and recommendation because the information in this HDE did not raise any unanticipated safety concerns.

XIV. CDRH DECISION

CDRH has determined that, based on the data submitted in the HDE, the nAbCyte Anti-AAVRh74var HB-FE Assay will not expose patients to an unreasonable or significant risk of illness or injury and the probable benefit to health from using the device outweighs the risks of illness or injury. CDRH issued an approval order on April 25, 2024.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See the device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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