CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

219019Orig1s000

OTHER REVIEW(S)

Division of Hepatology and Nutrition Consultation

Drug-Induced Liver Injury (DILI) Team

IND/NDA/BLA	NDA 219019 (IND 125632)
Consultation Issue	Drug-induced liver injury (DILI)
Drug Product	Fitusian
Indication	Hemophilia A & B with or without inhibitors
Applicant	Sanofi
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Signatory Authority	Paul H. Hayashi, MD, MPH Associate Director for DILI, OND/DHN
Assessment Date	Mar 21, 2025

Context: Fitusiran is a synthetic double stranded, small interfering RNA (siRNA) covalently linked to three N-acetyl galactosamine residues. The siRNA sequence is complimentary to antithrombin (AT) messenger RNA (mRNA) and delivered to the liver via binding the asialoglycoprotein receptor (ASGPR) on hepatocytes followed by endosomal uptake. Complimentary binding to the AT mRNA leads to its targeted destruction. Consequent decrease in AT production is expected to decrease bleeding in hemophilia A and B patients without or with factor inhibitors. Fitusiran is given subcutaneously with the dose dependent on anti-thrombin levels (ATDR dosing). The Division of Non-malignant Hematology (DNH) noted liver enzyme elevations associated with fitusiran use and consulted the Division of Hepatology and Nutrition (DHN) DILI Team to provide input on *"the type, severity and significance of any potential hepatotoxicity safety signals and whether this may be attributed to fitusiran"* and to *"review labeling" and "mitigation strategies ...when labeling this risk."*

Executive Summary: We conclude that there is a substantial risk of both idiosyncratic hepatocellular DILI and indirect DILI via gallbladder (GB) complications associated with fitusiran. The mechanism of both injuries is unclear; whether the pathophysiology of the two injuries is related or can be concurrent is also unknown. However, we can support approval with risk management and surveillance through labeling and pharmacovigilance, respectively, if the efficacy and need are high.

Besides having two different DILI signals, the assessment of liver injury risk was further complicated by the dose change from 80 mg monthly to the lower ATDR late in clinical trials, preventing clear definition of DILI risk for the labeled ATDR dosing based on contemporaneous, randomized controlled data. Nevertheless, the study level and subject level data provide strong evidence for both DILI types with the 80 mg dosing. There was one Hy's Law case who recovered and marked unfavorable imbalances in Temple's Corollary quadrants for the pivotal trials using 80 mg. The total exposed to fitusiran was only 335, and without solid evidence for substantially lower risk with ADTR, we are concerned that more severe idiosyncratic DILI could appear post-market. Similarly, there were clearly more GB adverse events including gallstone formation, cholecystectomy, and gallstone pancreatitis in subjects exposed to fitusiran at the 80 mg dose compared to controls. Like the idiosyncratic DILI risk, we are not convinced that the risk is substantively mitigated by ADTR dosing. Although the twelve subjects requiring cholecystectomy did well, bleeding risk and need for specialized care are increased with hemophilia. Thus, substantial GB related morbidity and mortality could arise in a larger post-market population.

If fitusiran is approved, we recommend a boxed Warning be considered for at least the gallbladder complication risk if not the idiosyncratic DILI risk as well. Otherwise, both liver injuries should appear in the Highlights Warnings and Precautions and be more fully described in Section 5.0 of the label. Enhanced pharmacovigilance should be implemented at a minimum. Our full assessment and recommendations are in Section 5 of this consult.

Consultation Sections:

Abbreviations:
ADA: antidrug antibody
ADME: absorption, distribution, metabolism, excretion
ALP or AP: alkaline phosphatase
ALT: alanine aminotransferase
AP: alkaline phosphatase

ASGPR: asialoglycoprotein receptor
AST: aspartate aminotransferase
AT: aminotransferase (ALT and/or AST)
AT-DR: antithrombin based dosing regimen
BMI: body mass index
BPA: bypass agent (agents that activate clotting independent of factor VIII or IX)
CYP: cytochrome P450
DB: direct bilirubin
DDI: drug-drug interaction
DILI: drug-induced liver injury
eDISH: evaluation for Drug Induced Severe Hepatotoxicity (Hepatocellular DILI screening plot)
eDISH-AP: eDISH-alkaline phosphatase (Cholestatic DILI screening plot)
FAERS: FDA Adverse Events Reporting System
GB: gallbladder
GGT: gamma-glutamyl transferase
HAV: hepatitis A virus
HBV: hepatitis B virus
HCV: hepatitis C virus
HDS: herbal and dietary supplements
HEV: hepatitis E virus
LTE: long term extension
mAb: monoclonal antibody
RISC: RNA induced silencing complex
R-value: ALT/ULN ÷ ALP/ULN
SC: subcutaneous
TB: total bilirubin
TFPI: tissue factor pathway inhibitors
US: ultrasound
ULN: upper limit of normal

1.0 Target Disease and Rationale

1.1 Target Disease: Though hemophilia is an uncommon disease overall, it is the most common severe hereditary hemorrhagic disorder. Hemophilia A and B result from factor VIII and factor IX deficiency or dysfunction, respectively. Hemophilia C is the rarest of the three types and results from clotting factor XI deficiency.[1] Characteristics of hemophilia include prolonged and excessive bleeding after minor trauma or spontaneously. Prevention and treatment of bleeding episodes consists of factor replacement [recombinant factor VIII or IX], and there are several forms of these factors in use.[2,3] Emicizumab (HEMLIBRA®) is approved for bleeding prophylaxis. It is a bispecific monoclonal antibody (mAb) that binds both factor IXa and factor X thereby taking over factor VIII's role as a scaffold for IXa and X. Marstacimab (ALPROLIX®),

[1] Iorio A, et al. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. *Ann Intern Med*. 2019;171(8):540-6.
[2] Butterfield J.S.S., Hege KM, Herzog RW, Kaczmarek R. A Molecular Revolution in the Treatment of Hemophilia, *Molecular Therapy*. 2020; 28: 997-1015. https://doi.org/10.1016/j.ymthe.2019.11.006.
[3] UpToDate®. https://www.uptodate.com/contents/hemophilia-a-and-b-routine-management-including-prophylaxis?search=hemophilia&source=search_result&selectedTitle=1%7E150&usage_type=default&display_rank=1#H63155290 (Accessed Feb 9, 2025).

approved in October 2024 for bleeding prophylaxis, blocks the anti-tissue factor pathway inhibitor (TFPI), a serine protease inhibitor that hinders thrombin formation. Therefore, inhibiting TFPI enhances thrombin formation and coagulation.[4]

The average lifespan of hemophilia patients prior to the 1970s was 11 years.[5] With early initiation of advanced therapies, life expectancy in developed countries approaches that of the general population.[6] Although transfusion associated disorders (e.g., viral hepatitis, HIV infection, iron overload disorders including cirrhosis and liver cancer) and cardiovascular disease are the most common causes of death,[7] patients still die from bleeding (e.g. intracerebral hemorrhage), and morbidity can be substantial. Patients who develop inhibitor antibodies to administered factor can be less responsive to factor replacement and have higher risk of bleeding. Thus, there is continued need for alternative therapies in hemophilia.

1.2 Rationale for Drug Use (Drug Mechanism of Action): Fitusiran is a synthetic double stranded, small interfering RNA (siRNA) that is covalently linked to three N-acetyl galactosamine residues. Fitusiran targets liver cells by uptake via the asialoglycoprotein receptor (ASGPR) expressed on hepatocytes.[8] siRNAs are short double stranded RNAs that dissociate to single strands to anneal preferentially to target messenger RNA (mRNA) sequences. The fitusiran siRNA specifically inhibits antithrombin (AT) production in hepatocytes by annealing to antithrombin mRNA from the SERPINC1 gene leading to mRNA destruction via the RNA induced silencing complex (RISC) (**Figure 1**). AT reduction leads to more thrombin and hemostasis regardless of hemophilia type (A or B) and factor inhibitor status. Fitusiran is being developed by Genzyme corporation as a first-in-class siRNA treatment for hemophilia A and B with or without factor VIII or IX inhibitors.

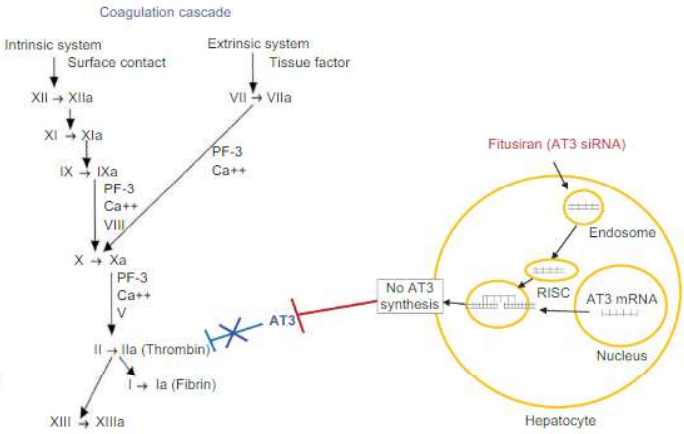


Figure 1. Mechanism of action of fitusiran in the coagulation cascade.[9]

[4] *Nature Reviews Drug Discovery*. https://www.nature.com/articles/d41573-024-00175-4#:~:text=Marstacimab%20is%20the%20first%20anti,antibody%20to%20secure%20FDA%20approval.
[5] Ishaikhli A, Killeen RB, Rokkam VR. Hemophilia B. [Updated 2023 Oct 29]. In: *StatPearls* [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2025 Jan-. Available from: HYPERLINK "https://www.ncbi.nlm.nih.gov/books/NBK560792/"https://www.ncbi.nlm.nih.gov/books/NBK560792/
[6] Kloosterman F, et al. Hemophilia management: Huge impact of a tiny difference. *Res Pract Thromb Haemost*. 2020 Feb 28;4(3):377-385. doi: 10.1002/rth2.12314. PMID: 32211572; PMCID: PMC7086468.
[7] Hassan S, et al. Mortality, life expectancy, and causes of death of persons with hemophilia in the Netherlands 2001-2018. J Thromb Haemost. 2021 Mar;19(3):645-653. doi: 10.1111/jth.15182. Epub 2020 Dec 18. PMID: 33217158; PMCID: PMC7986360.
[8] Cummings RD, et al. C-type Lectins. *Essentials of Glycobiology*. Cold Spring Harbor Laboratory Press; 2009;Chap31:439-57.
[9] Machin N, Ragni MV. An investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia A and B. *J Blood Med*. 2018; 22:135-140.

For clinical context, **Figure 2** shows fitusiran with other hemophilia drugs and their treatment pathways.

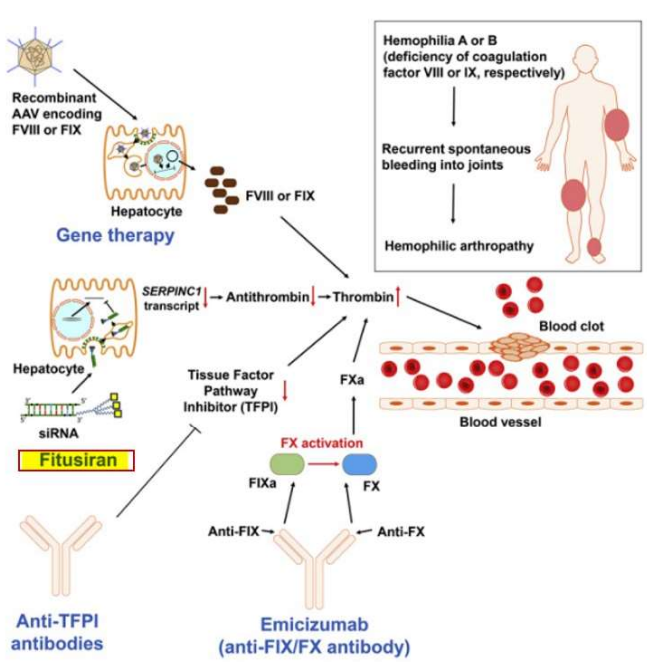


Figure 2. Fitusiran and three other treatments for hemophilia: gene therapy, anti-TFPI mAb, anti-IX/X mAb.[10]

2.0 Drug structure, pharmacokinetic and pharmacodynamic data pertinent to DILI

The DILI Team consulted the Division of Applied Regulatory Science (DARS), Office of Clinical Pharmacology (OCP), who provided cheminformatics assessment of fitusiran's hepatotoxicity potential in relation to other marketed siRNA products based on chemical structure and in silico modeling. Some portions of Section 2 are based on their findings.

2.1 Structural Information Pertinent to DILI:

The structural formula of fitusiran is in **Figure 3**. Like other siRNAs, fitusiran is stabilized by 2'O-methylated and deoxy-2'-Fluoro nucleotides which do not occur naturally. Despite early concerns that 2' fluorinated nucleotides could lead to mitochondrial toxicity,[11] DARS found no confirmed toxicity signals with these structures.

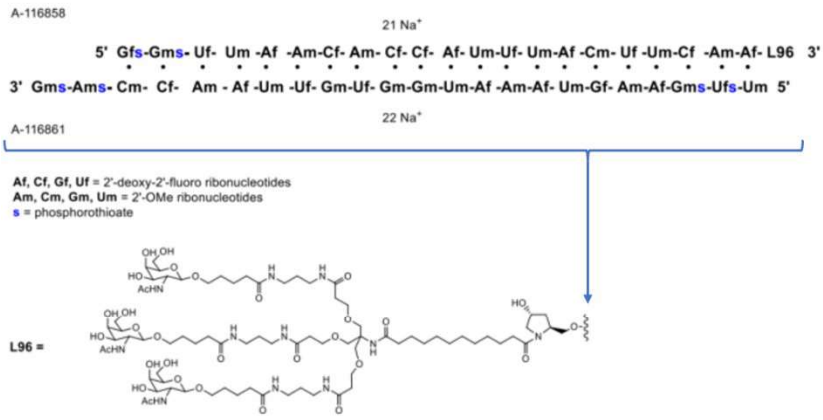


Figure 3: Chemical structure of fitusiran.[12]

2.2 Absorption: At the recommended therapeutic doses of 20 and 50 mg, fitusiran was rapidly absorbed after subcutaneous (SC) administration. Median Tmax was 3.8 hours and

[10] Butterfield JSS, et al. A Molecular Revolution in the Treatment of Hemophilia. *Molecular Therapy*. 2020; 28: 997-1015.

[11] (b) (4)

[12] NDA219019 (219019 - 0004 - (4) - 2024-04-29 - ORIG-1 /Clinical/Clinical Information) - Structure (#2)

accumulation ratio for 50 mg and 80 mg were both <1.5. Thus, the drug is not expected to accumulate in plasma, but intrahepatic levels and accumulation are unknown.

2.3 Distribution: There is active uptake of fitusiran into the liver via ASGPR with a long duration of action in terms of reduction of AT activity. The drug also distributes into the kidney and reticuloendothelial system.

2.3 Metabolism: Per DARS analyses, the in vitro metabolism of fitusiran evaluated in serum and liver S9 fractions[13] of mice, rats, dogs, monkeys, and humans produced several fitusiran metabolites. However, none were more than 10% of the total amount delivered upon repeated 80 mg fitusiran dosing. The Applicant did not do in vitro toxicity studies for the intermediate metabolites. siRNAs enter the target cell via endosome formation, but the intracellular trafficking and pharmacokinetics thereafter is often less clear, particularly the rate and mechanism of siRNA escape from the endosome to engage its mRNA target in the cytoplasm. For example, intracellular pH changes may alter the amount escaping and cellular accumulation (**Figure 4**).

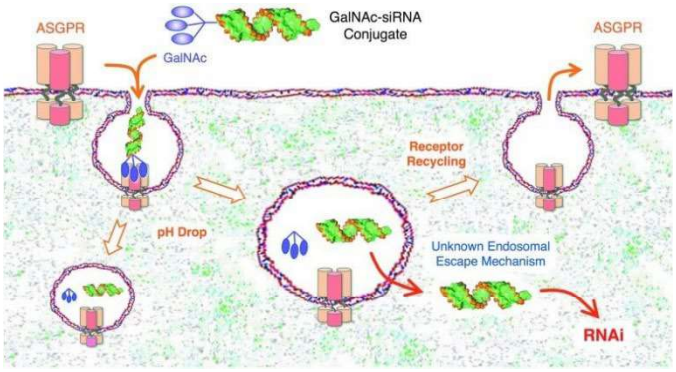


Figure 4. Intracellular localization and delivery of RNAI (aka siRNA) products into the hepatocellular endosome.[14]

2.4 Excretion: Only about 10% of the administered dose is accounted for in excretion studies and the rest of the product may be retained in the liver. How much is retained in smaller nucleotide forms (metabolites) that may be recycled within the hepatocyte is unclear. The mean plasma terminal $T_{1/2}$ of fitusiran was 8.0 hours. Mean fraction excreted in urine up to 24 hours after 50 mg dosing was 14.6%. Oligonucleotides are mainly cleared by catabolism via exo- and/or endonucleases into smaller oligonucleotides excreted in the urine. While the non-naturally occurring nucleotides are likely excreted, it is unclear to us how much of the other extra-cellular RNA nucleotides are excreted or recycled.

A summary ADME findings are in **Table 1**.

Table 1: ADME summary table[15]

Item	Finding
Absorption	Rapid, Tmax: 3.8 hours
Distribution	Active uptake into the liver, low to moderate plasma protein binding
Metabolism	Catabolism via exo- and/or endonuclease intrahepatically

[13] Supernatant fraction of homogenized organs.
[14] Springer, A, D.; et al. GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. *Nucleic Acid Therapeutics*. 2018, 28(3): 109–118.
[15] Made by DILI Team

Elimination	Small portion (<10%) eliminated in urine and $T_{1/2}$ estimate: 8 hours

2.5 Drug-Drug Interactions (DDI): Fitusiran is not an inhibitor or inducer of major CYPs likely because it is not a CYP substrate. At clinical doses, there is low potential for DDI between fitusiran and other co-administered drugs that are metabolized by the CYP450 system. Fitusiran had no effect on BCRP, BSEP, MDR1, MATE-1, MATE-2, OAT1, OAT3, OATP1B1, PATP1B3, OCT1, and OCT3 transporters. While the non-clinical summary data suggests no inhibition of "MDR", MDR2 was not cited specifically.

2.6 Immunogenicity: There was a low incidence of anti-drug antibody (ADA) in clinical studies and no relevant effect on the fitusiran exposure. There was no association between ADA and signs of liver injury.

2.7 Pharmacodynamic Effects and Hepatotoxicity: Per our DARS colleagues, there was no evidence that the knockdown of antithrombin production would cause hepatotoxicity. Microvascular thrombosis created by increased coagulation could be injurious to the liver but such intrahepatic microthrombi formation with fitusiran is speculative. DARS found limited data on the degree of degradation of non-thrombin mRNAs, and what if any affect such degradation would have on the liver.

3.0 Non-clinical data related to DILI:

3.1 In vitro data: In an in vitro human whole blood assay, there was no increase in cytokines and or acute phase markers. We found no other in vitro data specific to hepatotoxicity risk.

3.2 Animal data: Species differences in liver toxicity are reported in **Table 2**. Fitusiran's maximum clinical dose is 50 mg/month, or 0.5 and 0.6 mg/kg/month for an average adult US male (average weight, 91 kg) and female (average weight, 78 kg) respectively.

Table 2: Animal data regarding DILI risk.[16]

Duration	NOAEL*	Human NOAEL	Mortality	Liver Related Findings
Mouse				
26 weeks	10-30 mg/kg/wk.	0.8-2.4 mg/kg/wk.	Deaths mainly in the control group	Minimal hepatocellular necrosis at NOAEL dose in 1 of 22 Bile duct hyperplasia in 1 of 24
Rat				
7 weeks	1-3 mg/kg/wk.	0.08-0.2 mg/kg/wk.	61% mortality at 3mg/kg/wk with hemorrhage and/or thrombosis as important	Hepatic necrosis, portal mononuclear infiltrate; biliary hyperplasia, bile duct dilatation; increase in ATs, ALP, total bilirubin.

[16] Made by DILI Team

			contributing factors	
26 weeks	0.5 mg/kg/wk.	0.04 mg/kg/wk.	13 mortalities at 1 mg/kg/wk. due to hemorrhage and/or thrombosis in various organs	NA
Dog				
4 weeks with 12-week follow-up	NA		NA	At ≥ 3 mg/kg: Reversible transaminase elevations with liver cell degeneration, necrosis, and inflammation; reversible at 12 weeks
Monkey				
3 weeks	0.5-1.0 mg/kg/wk SC	0.16-0.3 mg/kg/wk	3/3 deaths at 30 mg/kg/week - pleural effusion, GI hemorrhage	hepatic mononuclear cell inflammation
7 weeks with 8-week recovery	0.3-1.0 mg/kg/wk SC	0.1-0.3 mg/kg/wk	2 deaths - hemorrhage	NA
39 weeks	0.5 mg/kg/wk	0.16 mg/kg/wk	NA	NA

NA = Not reported in the non-clinical summary.
*For reference the label dose in human is 50 mg/month, or 0.5 and 0.6 mg/kg/month in male and female, respectively.

Overall, animal studies suggest minimal to moderate hepatotoxicity with some necrosis, biliary hyperplasia, and bile duct dilation in rodents, but at doses generally higher than the NOAEL. Single cell hepatocellular necrosis indicative of apoptosis was also seen in high dose acute exposures. Hepatic vacuoles with lipid droplets are reported in Kupffer and in other reticuloendothelial cells with siRNAs in general.[17]

4.0 Clinical data:

4.1 In class or near class data: We found five marketed siRNA products (**Table 3**); only givosiran is labeled for liver toxicity in the Highlighted Warnings and Precautions.

Table 3: Approved siRNAs with DILI information by label and LiverTox®.[18] Agents listed in order of approval year.

Drug (Brand), Target Disease(s)	Mechanism of action; target	Approval year	Labeling regarding DILI in • Box Warning (BW)	LiverTox® category[18]

[17] Janas MM, Harbison CE, Perry VK, et al. The Nonclinical Safety Profile of GalNAc-conjugated RNAi Therapeutics in Subacute Studies. *Toxicologic Pathology*. 2018;46(7):735-745. doi:10.1177/0192623318792537
[18] LiverTox® https://www.ncbi.nlm.nih.gov/books/NBK547852/

			• Warnings/Precautions, Highlights (W/P, HL) • Adverse Reactions, Highlights (AR, HL) • Sections 5 & 6 (S5 & S6)	
Patisiran (ONPATTRO®) PNP/amyloidosis	siRNA; transthyretin	2018	• BW: No • W/P, HL: No • AR, HL: No • S5: No • S6: No	E: unlikely cause of clinically apparent liver injury
Givosiran (GIVLAARI®), AHP	siRNA; 5-aminolevulinic acid synthase 1	2019	• BW: No • W/P, HL: Yes, hepatic toxicity: stop for clinically significant transaminase increased. • AR, HL: No • S5: Yes. Transaminase elevations • S6: Yes, transaminase elevations	E*: unproven but suspected cause of clinically apparent liver injury
Lumasiran (OXLUMO®) PH1	siRNA; hydroxyacid oxidase 1 (HAO1)	2020	• BW: No • W/P, HL: N/A • AR, HL: No • S5: N/A • S6: No	E: unlikely cause of clinically apparent liver injury
Inclisiran (LEQVIO®) HeFH	siRNA; proprotein convertase subtilisin/kexin type 9	2021	• BW: No • W/P, HL: N/A • AR, HL: No • S5: N/A • S6: No	E: unlikely cause of clinically apparent liver injury
Vutrisiran (AMVUTTRA®) PNP/amyloidosis	siRNA transthyretin	2022	• BW: No • W/P, HL: No. • AR, HL: No • S5: No • S6: No	E: unlikely cause of clinically apparent liver injury

AR = Adverse Reactions; BW = box warning; HL = highlighted (page 1 of label); NA = not available; S5 = Label Section 5; S6 = Label Section 6; W/P = Warnings and Precautions
PNP: polyneuropathy, AHP: acute hepatic porphyria, PH1: primary hyperoxaluria type 1, HeFH: heterozygous familial hypercholesterolemia,

PubMed search using two different search syntaxes yielded one case report each for givosiran and inclisiran (**Table 4**).[19] We did not find cases of liver injury associated with patisiran, lumasiran, and vutrisiran. According to the FDA Adverse Events Reporting System (FAERS) public dashboard, there have been 18, 20, 2, 44, and 7 hepatobiliary disorder related reports for patisiran, givosiran, lumasiran, incisiran, and vutrisiran, respectively (**Table 4**).[20]

[19] PubMed https://pubmed.ncbi.nlm.nih.gov/?otool=mdufdrlib "(drug name) AND ((hepatotoxicity) or (liver injury) or (DILI))," and "(drug name) AND Human[MH] AND (drug induced liver injury OR jaundice/CI OR bile duct diseases/CI OR liver/DE OR liver diseases/CI) AND ("1900/1/1"[EDat]:"2999/12/31"[EDat])"
[20] https://fis.fda.gov/sense/app/95239e26-e0be-42d9-a960-9a5f7f1c25ee/sheet/33a0f68e-845c-48e2-bc81-8141c6aaf772/state/analysis

Table 4: Results of FAERS and PubMed Searches related to DILI for marketed siRNA.

Drug (Approval year)	FAERS DILI or hepatotoxicity cases since 2010	Metys®[21] based rate of DILI or hepatotoxicity cases per patients prescribed drug since 2010 (%) *	PubMed Search Search syntax: (Drug Name) AND ((hepatotoxicity) or (liver injury) or (DILI))
Patisiran (2018)	1	0.39	0 reports of DILI
Givosiran (2019)	2	14.3^	1 reports of DILI
Lumasiran (2020)	0	0	0 reports of DILI
Inclisiran (2021)	13	0.25	1 reports of DILI
Vutrisiran (2022)	3	1.02	0 reports of DILI

* Metys® only captures prescription data since 2010.
^ Metys data suggests just 14 patients prescribed Givosiran.

4.2 Clinical Drug Development Program

4.2.1 **Pivotal Studies**: Pivotal studies are described below in sections (a) and (b) with a summary **Table 5**. Study schemas for each study are in the **Appendix**. Section (c) describes a critical change in dosing. We did not do a detailed assessment of the long term extension (LTE), Study 14762 that enrolled 34 subjects from a phase 1 trial, but we did include these cases in the overall number of exposed subjects (safety population) including two subjects with gallbladder related adverse events (AE).

a). Efficacy assessment relied primarily on two studies:

- Study EFC147**68** (ALN-AT3SC-003) *A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients with Hemophilia A or B, with Inhibitory Antibodies to Factor VIII or IX (ATLAS INH)*: Hereafter we refer to this study as Study 68. It is a randomized controlled study originally designed to compare fitusiran 80 mg monthly to by-pass agent (BPA) on demand.[22] At the time of submission, the study had completed, with 60 patients enrolled, of whom 57 were randomized (38 fitusiran and 19 control) and three patients assigned directly to the treatment arm per request from the Chinese Health Authority.

- Study EFC147**69** (ALN-AT3SC-004) *A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients With Hemophilia A or B, without Inhibitory Antibodies to Factor VIII or IX (ATLAS-A/B)*: Hereafter we refer to this study as Study 69. It is a randomized controlled study originally designed to compare fitusiran 80 mg monthly to factor on demand in patients with hemophilia A or B, without inhibitory antibodies to factor VIII or IX, not receiving prophylaxis. This study has completed

[21] Metys® https://metys.symphonyhealth.com/metys/#/home/prescriptionQuery
[22] BPAs are agents that activate clotting independent of factor VIII or IX, thus bypassing the inhibitors of VIII and XI. (e.g., recombinant factor VIIa and activated prothrombin complex concentrate).

with 120 enrolled, 80 fitusiran treated subjects and 40 controls. One participant in the active arm withdrew before the first dose.

b). Safety assessment relied on the above two phase 3 studies and two others:

- EFC151**10** (ALN-AT3SC-009), *An open-label, multinational, switching study to describe the efficacy and safety of fitusiran prophylaxis in patients with hemophilia A and B previously receiving factor or bypassing agent (ATLAS-PPX)*: Hereafter we refer to this study as Study 10. This is a phase 3 study. The study is complete with 80 enrolled; 67 received 80 mg monthly, and two subjects received an antithrombin based dosing regimen (AT-DR), starting dose at 50 mg every two months.

- LTE151**74** (ALN-AT3SC-005) *An Open-label, Long-term Safety and Efficacy Study of Fitusiran in Patients with Hemophilia A or B, with or without Inhibitory Antibodies to Factor VIII or IX (ATLAS-OLE)*: Hereafter, we refer to this study as Study 74. This is a phase 3 study which also assessed pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity of fitusiran in patients who previously participated in Studies 68 (ATLAS-INH), 69 (ATLAS-A/B), and 10 (ATLAS-PPX). The study is ongoing, with 281 enrolled (180 received 80 mg QD; 166 switched to 50 mg every two months (ATDR).

Table 5: Summary of four pivotal studies for fitusiran development program.[23]

Study	Population/ # enrolled	Study Design	Primary Objective/Endpoint	Dose	Duration
EFC14768 (ATLAS-INH) **PARENT**	-Males aged ≥12 years with severe hemophilia A or B with inhibitors -57	Randomized, controlled, open-label	Efficacy of fitusiran prophylaxis compared to on-demand BPA/Annualized Bleeding Rate (ABR)	80 mg QM	9 months
EFC14769 (ATLAS-A/B) **PARENT**	-Males aged ≥12 years with severe hemophilia A or B without inhibitors -120	Randomized, controlled, open-label	Efficacy of fitusiran prophylaxis compared to on-demand factor/ABR	80 mg QM	9 months
EFC15110 (ATLAS-PPX) **PARENT**	-Males aged ≥12 years with severe hemophilia A or B with or without inhibitors receiving factor/BPA prophylaxis -80	Open-label switching study "before-after" study	Efficacy of fitusiran prophylaxis compared to factor/BPA prophylaxis/ABR	80 mg QM, 50 mg Q2M (to target an AT level of 15-35%)	7 months
LTE15174 (ATLAS-OLE) **AT-DR**	-Adult males who completed EFC14768, EFC14769, or EFC15110 -281	Open-label long-term safety and efficacy study	Safety and tolerability	50 mg Q2M, 50 mg QM, 80 mg QM, 20 mg Q2M, or 20 mg QM to target an AT level of 15-35%	Up to 76 months

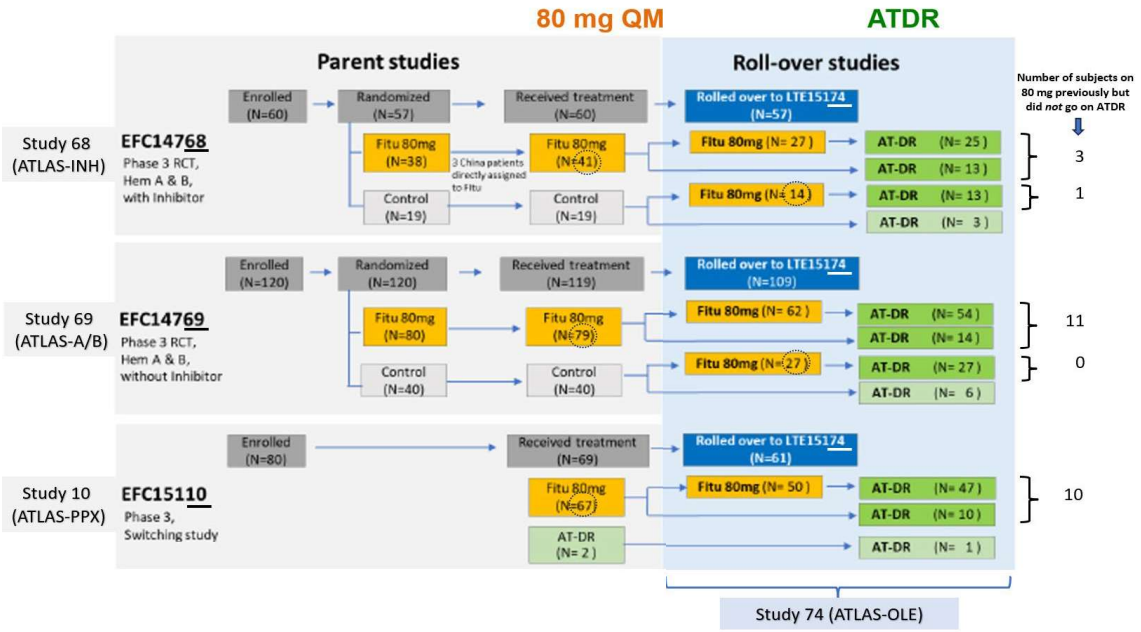
BPA = Bypassing Agent

c). Change in dosing: On Oct 30, 2020, the Applicant paused dosing due to clinically significant thromboses in subjects taking fitusiran (see **Appendix, Figure E** for regulatory history timeline.) Upon restart, the fixed dose of 80 mg monthly was struck

[23] DNH mid-cycle meeting, Aug 28, 2024 and NDA219019 (219019 - 0001 - (1) - 2024-03-28 - ORIG-1 /Multiple Categories/Subcategories) - Summary of Clinical Safety (#29)

from the development program in favor of an antithrombin based dosing regimen (ATDR), which stipulates dose adjustment based on periodically checked antithrombin levels and starts dosing at 50 mg monthly. This dosing change substantially hampers comparison of the now labeled ADTR dose to any comparator groups because Studies 68 and 69, had already completed and Study 10 had only two of the 69 on fitusiran switch to or initiate ATDR. Therefore, the two pivotal, randomized controlled trials (Studies 68 and 69), compared only the 80 mg monthly dosing to control therapy, not the labeled ADTR dosing (**Figure 5, a**). Overall, only 65 of the 335 pooled safety population received exclusively ADTR (**Figure 5, b**), and none of these 65 could be compared to a contemporaneous control group in a randomized controlled fashion. Subjects who rolled over from studies 10, 68, and 69 into study 74, the long-term safety study, were to receive ATDR after Oct 30, 2020, (**Figure 5, a**), but many initially received the 80 mg monthly dosing before switching to ATDR. Also, 25 (11%) of 228 subjects on 80 mg monthly in Studies 68, 69, or early 74, did not roll over to ADTR.

(a)



(b)

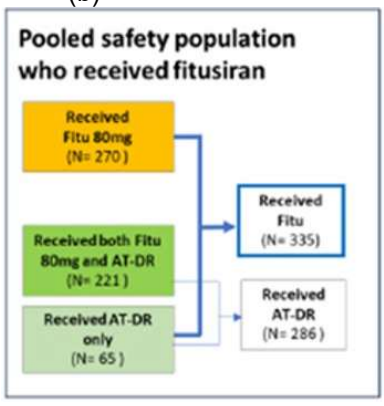


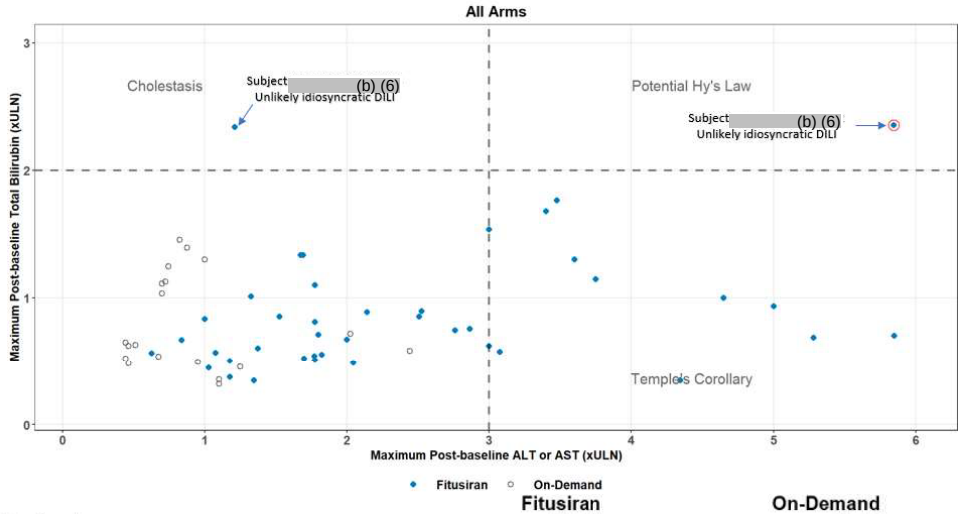
Figure 5: (a) Subject flow for the four pivotal studies (Studies 68, 69, 10, and 74) showing transitions from 80 mg monthly (QM) to antithrombin based dosing regimen (ATDR) after Oct 30, 2020.[24] The dotted circles identify the 228 subjects who received 80 mg QM *and* could potentially have moved on to ADTR in Study 74. Numbers on the far right, outside the blue shaded area, show the number of subjects who took at least one dose of 80 mg QM previously but did *not* go on to ADTR in Study 74. Thus, 25 (11%) of 228 subjects previously given 80mg QM did not transition to ADTR. (b) Pooled safety population of subjects receiving at least one dose of fitusiran including the four pivotal studies and Study 14726. Only 65 received ATDR without having first taken the 80 mg monthly dosing.

[24] Adapted from mid-cycle presentation, Aug 8, 2024

4.3 Study level data: Study level data required several analyses due to the dosing change from 80 mg QM to ADTR and the identification of substantial gallbladder adverse events across the studies. As a guide, we have the following notes for this Section 4.3: (i) We present Study 68 data first and most fully, including eDISH and eDISH-AP plots. (ii) Next, we discuss Study 69 data but more briefly because the results were similar to Study 68. (iii) We then discuss pooled gallbladder/biliary adverse event (AE) data from Studies 68 *and* 69. (iv) Fourthly, we cover Study 10 in more detail because it had the only Hy's law case. (v) Finally, we present Study 74 data in detail because it is the only study that used the labeled ATDR dosing substantially. We finish Section 4.3 with a summary table.

4.3.1. **Study 68** [*A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients with Hemophilia A or B, with Inhibitory Antibodies to Factor VIII or IX (ATLAS INH)]*

a). Hepatocellular DILI screening scatterplot (eDISH): eDISH plotting for Study 68 shows a clear overall shift toward higher aminotransferases (AT) values with fitusiran compared to the On-Demand therapy with 11 (29.3%) fitusiran subjects in Temple's Corollary quadrant versus none for On-Demand (**Figure 6**). One subject (ID (b) (6) plotted to the Potential Hy's law quadrant, but we assessed this case as unlikely idiosyncratic DILI due to fitusiran.



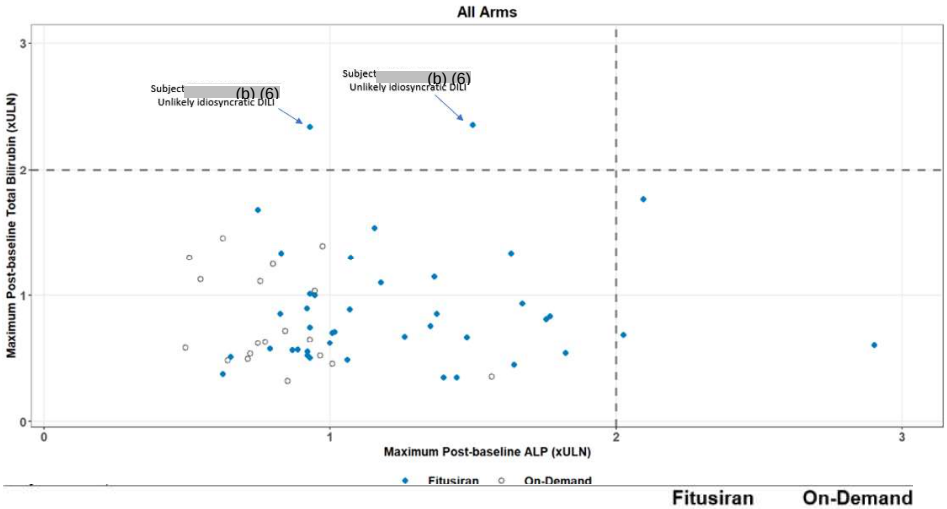
Quadrant	Fitusiran N=41	On-Demand N=19
Potential Hy's Law (right upper)	1/41 (2.4)	0/19 (0)
Cholestasis (left upper)	1/41 (2.4)	0/19 (0)
Temple's corollary (right lower)	12/41 (29.3)	0/19 (0)
Total	14/41 (34.1)	0/19 (0)

Source: adlb.xpt; Software: R
Abbreviations: DILI, drug-induced liver injury
Source:Clinical Data Scientist; adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALT or AST versus their maximum total bilirubin values in the post-baseline period. A potential Hy's Law case (red circle) was defined as having any post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after a post-baseline ALT or AST equal to or exceeding 3X ULN. All patients with at least one post-baseline ALT or AST and bilirubin are plotted. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; TB, total bilirubin; ULN, upper limit of normal

Figure 6: eDISH with quadrant counts for Study 68.[25]

[25] Made by CDS

b). Cholestatic DILI screening scatterplot (eDISH-AP): eDISH using alkaline phosphatase (AP) instead of ATs did not have any subjects in the right upper quadrant at cut-offs of TB ≥2x ULN and AP ≥2x ULN (**Figure 7**). However, there was an overall shift toward higher peak AP levels with fitusiran compared to the On-Demand therapy with three (7.3%) fitusiran subjects having AP levels over 2x ULN versus none for On-Demand. Subject (b) (6), who plots to the potential Hy's law quadrant on a standard eDISH was now in the left upper quadrant on eDISH-AP, suggesting this subject did not have substantial AP elevations. Subject (b) (6) was the only other jaundiced subject (TB >2x ULN) and remained in the left upper quadrant on both eDISH and eDISH-AP, meaning this subject did not have substantial elevations in either ATs or AP; this subject was not evaluated further.



Quadrant	Fitusiran N=41	On-Demand N=19
>=2X ULN Bilirubin and >=2X ULN ALP (right upper)	0/41 (0)	0/19 (0)
>=2X ULN Bilirubin and <2X ULN ALP (left upper)	2/41 (4.9)	0/19 (0)
<2X ULN Bilirubin and >=2X ULN ALP (right lower)	3/41 (7.3)	0/19 (0)
Total	5/41 (12.2)	0/19 (0)

Source: adlb.xpt; Software: R
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALP versus their maximum total bilirubin values in the post-baseline period.
A potential cholestatic DILI case (red circled) was defined as having a maximum post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after post-baseline ALP became equal to or exceeding 2X ULN.
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Figure 7: eDISH-AP with quadrant counts for Study 68.[26]

c). Shifts in liver blood tests by cut-off levels: **Table 6** for Study 68 reflected the eDISH and eDISH-AP findings with fitusiran being associated with a 31.7% rate of ALT elevations ≥3x ULN compared to 0% for On-Demand therapy.

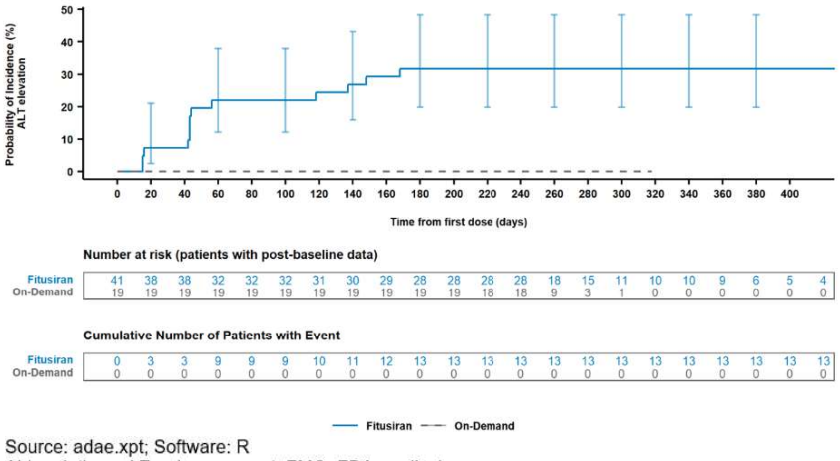
Table 6: Number and frequency of liver blood test elevations by set cut-offs, Fitusiran versus On-Demand, Study 68.[27]

[26] Made by CDS.
[27] Made by CDS.

Laboratory Abnormality	Fitusiran N=41 n (%)	On-Demand N=19 n (%)	Risk Difference (%) (95% CI)
ALT			
>=1X ULN	37/41 (90.2)	5/19 (26.3)	63.9 (38.8, 80.8) *
>=3X ULN	13/41 (31.7)	0/19 (0)	31.7 (13.1, 47.1) *
>=5X ULN	3/41 (7.3)	0/19 (0)	7.3 (-10.2, 19.6)
>=10X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
>=20X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
AST			
>=1X ULN	35/41 (85.4)	1/19 (5.3)	80.1 (57.9, 90.3) *
>=3X ULN	5/41 (12.2)	0/19 (0)	12.2 (-5.5, 25.7)
>=5X ULN	2/41 (4.9)	0/19 (0)	4.9 (-12.5, 16.3)
>=10X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
>=20X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
ALP			
>=2X ULN	3/41 (7.3)	0/19 (0)	7.3 (-10.2, 19.6)
>=3X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
Total Bilirubin			
>=2X ULN	2/41 (4.9)	0/19 (0)	4.9 (-12.5, 16.3)
>=5X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
>=8X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
Direct Bilirubin			
>=2X ULN	1/41 (2.4)	0/19 (0)	2.4 (-14.8, 12.7)
>=5X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
GGT			
>=2X ULN	10/41 (24.4)	1/19 (5.3)	19.1 (-2.7, 35.6)
INR			
>=1.5X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
>=3X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)
>=5X ULN	0/41 (0)	0/19 (0)	0.0 (-17.1, 8.7)

Source: adlb.xpt; Software: R
The frequency represented here are based on peak levels. Appropriate cutoff for liver biochemistries should be adjusted based on the study population (e.g., pediatric population, those with underlying liver disease etc.). For patients with chronic liver disease, cutoff should be established using multiples of baseline (e.g. 2X, 3X, 5X).
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; GGT, gamma-glutamyl transferase; INR, prothrombin international normalized ratio; N, number of patients in group; n, number of patients meeting criteria; ULN, upper level of normal

d*). Liver injury time to onset*: By ALT ≥ 3x ULN, the onset of liver injury tended to be early with approximately two-thirds occurring within 60 days and all within 175 days (**Figure 8**).



Source: adae.xpt; Software: R
Abbreviations: AE, adverse event; FMQ, FDA medical query

Figure 8: Time to onset of ALT ≥ 3x ULN, Safety Population, Study 68.[28]

[28] Made by CDS

4.3.2 **Study 69** *[A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients with Hemophilia A or B, without Inhibitory Antibodies to Factor VIII or IX (ATLAS-A/B)]***:** Study 69's eDISH, eDISH-AP, and liver blood test shift table data were similar to Study 68 with more AT and AP elevations associated with fitusiran compared to On-Demand therapy (**Appendix Figures F and G, Table A**). Like Study 68, there were no Hy's law cases. While the differences in proportions with liver blood test elevations between fitusiran and On-Demand arms were substantial in both studies, they were less pronounced in study 69 compared to Study 68. For example, the proportions of patients with ALT ≥ 3x ULN on fitusiran was 17.7% in Study 69 (without inhibitors) compared to 31.7% in Study 68 (with inhibitors). Still, the proportions were 0% in the On-Demand arms for both studies. There was also a lower portion of subjects with AP ≥ 2x ULN in Study 69 compared to 68: 2.5% versus 7.3%, respectively (0% in the On-Demand subjects for both studies). The onset of liver injury by ALT ≥ 3x ULN was longer with approximately two-thirds of the injuries occurring within 120 days in study 69 compared to 60 days for Study 68 (**Appendix Figure H**).

4.3.3 **Hepatobiliary AE and SAE analysis for Studies 68 and 69 (pooled)**: Hepatobiliary related AEs and SAEs were more common in subjects on fitusiran (**Tables 7**). By comparison, gallbladder and biliary issues were conspicuously absent (0%) in the On-Demand subjects in both Study 68 and 69. These gallbladder and gallstone events are discussed in more detail in the case level analyses (**Section 4.4.3**).

Table 7: Number and proportions of (i) any SAE and hepatobiliary SOC and (ii) any AE, cholecystitis (+/- chronic) and cholelithiasis in Studies 68 and 69.[29]

(i)

	Inhibitor				Non-Inhibitor			
	Hemophilia A		Hemophilia B		Hemophilia A		Hemophilia B	
System Organ Class Preferred Term	Fitusiran N=32 n (%)	On-Demand N=16 n (%)	Fitusiran N=9 n (%)	On-Demand N=3 n (%)	Fitusiran N=61 n (%)	On-Demand N=31 n (%)	Fitusiran N=18 n (%)	On-Demand N=9 n (%)
Any SAE	5 (15.6)	4 (25.0)	2 (22.2)	1 (33.3)	3 (4.9)	2 (6.5)	2 (11.1)	3 (33.3)
Hepatobiliary disorders (SOC)	2 (6.2)	0	0	0	1 (1.6)	0	2 (11.1)	0
Biliary colic	1 (3.1)	0	0	0	0	0	0	0
Cholecystitis acute	1 (3.1)	0	0	0	0	0	0	0
Cholecystitis chronic	1 (3.1)	0	0	0	0	0	0	0
Cholecystitis	0	0	0	0	1 (1.6)	0	0	0
Cholelithiasis	0	0	0	0	0	0	2 (11.1)	0

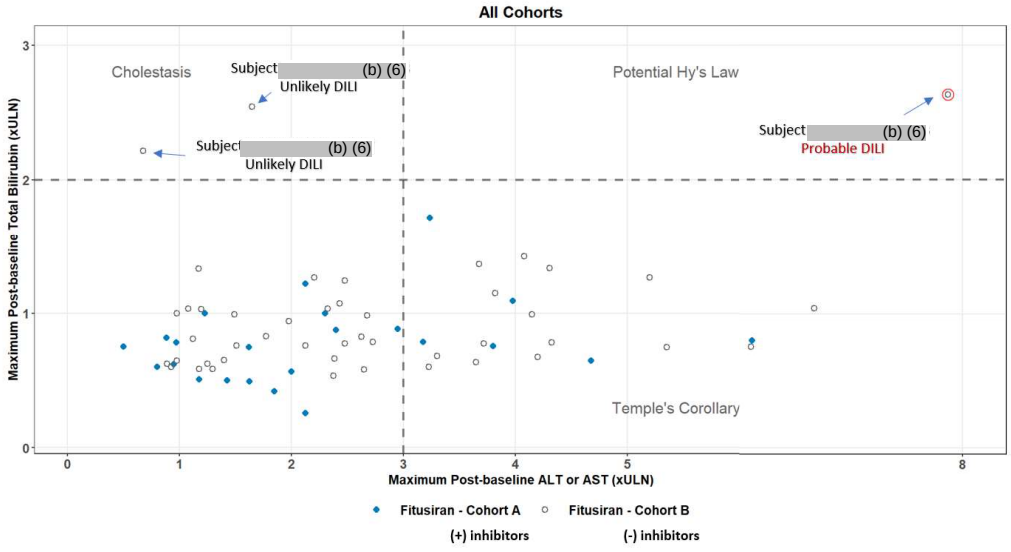
(ii)

[29] Made by CDS

Preferred Term	EFC14768 (Inhibitor) Fitusiran N=41 n (%)	On-Demand N=19 n (%)	Risk Difference % (95% CI)	EFC14769 (Non-Inhibitor) Fitusiran N=79 n (%)	On-Demand N=40 n (%)	Risk Difference % (95% CI)
Any AE	38 (92.7)	11 (57.9)	34.8 (12.8, 57.7) *	62 (78.5)	18 (45.0)	33.5 (15.3, 50.3) *
Cholecystitis	2 (4.9)	0	4.9 (-12.5, 16.3)	2 (2.5)	0	2.5 (-6.4, 8.8)
Cholecystitis chronic	2 (4.9)	0	4.9 (-12.5, 16.3)	0	0	0.0 (-8.8, 4.7)
Cholelithiasis	2 (4.9)	0	4.9 (-12.5, 16.3)	3 (3.8)	0	3.8 (-5.1, 10.6)

4.3.4 **Study 10** *[An open-label, multinational, switching study to describe the efficacy and safety of fitusiran prophylaxis in patients with hemophilia A and B previously receiving factor or bypassing agent (ATLAS-PPX)]:* This study had no non-fitusiran comparator arm, so we analyzed the data comparing Cohort A, patients with inhibitors versus Cohort B, patients without inhibitors; all but two patients received 80 mg dosing.

a). Hepatocellular DILI screening scatterplot (eDISH): The proportions of subjects plotting to Temple's Corollary were similar to Studies 68 and 69 (**Figure 9**); but unlike Studies 68 and 69, the proportions were lower for those with inhibitors (Cohort A) compared to those without (Cohort B), 26.1% and 32.6%, respectively. Also, Study 10 had one subject (Subject (b) (6)) plotting to the Potential Hy's Law quadrant that we assessed as probable DILI and thus meeting Hy's law. The two other jaundiced subjects remained in the left upper quadrant on eDISH-AP (**Appendix, Figure I**), and thus had no substantial elevations in ATs or AP. The mild jaundice was transient and unlikely to be acute DILI. These cases were not further analyzed.



Quadrant	Cohort A: Inhibitor Fitusiran N=23	Cohort B: Non-inhibitor Fitusiran N=46
Potential Hy's Law (right upper)	0/23 (0)	1/46 (2.2)
Cholestasis (left upper)	0/23 (0)	2/46 (4.3)
Temple's corollary (right lower)	6/23 (26.1)	15/46 (32.6)
Total	6/23 (26.1)	18/46 (39.1)

Source: adlb.xpt; Software: R
Abbreviations: DILI, drug-induced liver injury

Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALP versus their maximum total bilirubin values in the post-baseline period.
A potential cholestatic DILI case (red circled) was defined as having a maximum post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after post-baseline ALP became equal to or exceeding 2X ULN.
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Figure 9: eDISH with quadrant counts for Study 10.[30]

b). *Cholestatic DILI screening scatterplot (eDISH-AP)* **Appendix, Figure I**): There were no subjects plotting to the right upper quadrant, and the three in the left upper quadrant (jaundiced with AP <2x ULN) are discussed above (. There were two subjects with transient and modest AP elevations (<3.5 xULN) without substantial hyperbilirubinemia. These subjects were not analyzed further.

c). Shifts in liver blood tests by cut-off levels: **Appendix Table B** for Study 10 reflected the eDISH and eDISH-AP plots with fitusiran being associated with ALT or AST >3x ULN rates of 21.7% to 34.8%, and three jaundiced subjects in Cohort B (non-inhibitor).

d). *Liver injury time to onset*: For ALT ≥ 3x ULN, the time to onset of liver injury was more evenly spread out to a maximum of about 165 days (**Appendix**, **Figure J**) when compared to Studies 68 and 69.

e). *Hepatobiliary events by System Organ Class (SOC) preferred terms (PT)*: There was only one gallbladder event (cholelithiasis) yielding a rate of 4.8% which is similar to Study 68 (with inhibitors), whereas there were no such events in Cohort B (no inhibitors) compared to 3.8% for Study 69 (no inhibitors) (**Table 8**).

[30] Made by CDS.

Table 8. Patients with Serious Adverse Events by System Organ Class and Preferred Term, Safety Population, Study 10, Fitusiran Treatment Period.[31]

	Inhibitor			Non-Inhibitor		
System Organ Class Preferred Term	Hemophilia A N=14 n (%)	Hemophilia B N=7 n (%)	Overall N=21 n (%)	Hemophilia A N=36 n (%)	Hemophilia B N=10 n (%)	Overall N=46 n (%)
Gastrointestinal disorders (SOC)	1 (7.1)	0	1 (4.8)	0	0	0
Pancreatitis acute	1 (7.1)	0	1 (4.8)	0	0	0
Hepatobiliary disorders (SOC)	1 (7.1)	0	1 (4.8)	0	0	0
Cholelithiasis	1 (7.1)	0	1 (4.8)	0	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps) (SOC)	0	0	0	1 (2.8)	0	1 (2.2)
Biliary neoplasm	0	0	0	1 (2.8)	0	1 (2.2)

4.3.5 **Study 74** *An Open-label, Long-term Safety and Efficacy Study of Fitusiran in Patients with Hemophilia A or B, with or without Inhibitory Antibodies to Factor VIII or IX (ATLAS-OLE)*: This study had no non-fitusiran comparator arm. The dose changed from 80 mg monthly (QM) to ADTR after Oct 30, 2020. Just ten patients went on ADTR only, while 193 (95%) of 203 subjects had received the 80 mg QM dosing in either Study 74 itself, or prior Studies 10, 68, or 69. The ten who only received ATDR had been in the On-Demand groups from Studies 68 and 69 or received ATDR in Study 10 (**Section 4.2.1, Figure 5**, above). On the other hand, 25 (11%) of subjects who had been on the 80 mg monthly dosing in the feeder studies did not enter Study 74 and therefore, did not make the transition to ADTR. The DILI Team did not have details on these 25 subjects in terms of liver injury signals or reason for not moving on to ADTR.

a). Hepatocellular DILI screening scatterplot (eDISH): Elevations in ATs occurred but at a substantially lower rate compared to the fitusiran treated subjects in the other three pivotal studies. The proportions of subjects in Temple's with and without inhibitors were 8.7% and 8% (**Appendix**, **Figure K**), respectively, compared to 29.3% for Study 68 (with inhibitors) and 19% for Study 69 (without inhibitors). The corresponding Temple's rates for Study 10 were 26.1% and 32.6%. Two subjects plotted to the Potential Hy's Law quadrant, but both were considered unlikely idiosyncratic DILI due to fitusiran. One subject ((b) (6)) had alcohol related injury and the jaundice was not coincident with the AST elevations. The other subject ((b) (6)) had gallstone related liver injury and is discussed later.

b). *Cholestatic DILI screening plot (eDISH-AP), liver blood test shift table and liver injury time to event*: eDISH-AP, shift table and time to event graph are in the **Appendix** as **Figure L**, **Table C,** and **Figure M,** respectively. One subject ((b) (6)) plotted to the right upper quadrant (ALP>2x ULN and TB >2x ULN) on eDISH-AP but was considered unlikely DILI. The ALP peak was highest at baseline and TB elevation transient during a hospitalization for colitis. Time to ALT >3x ULN had a wider range

[31] Made by CDS

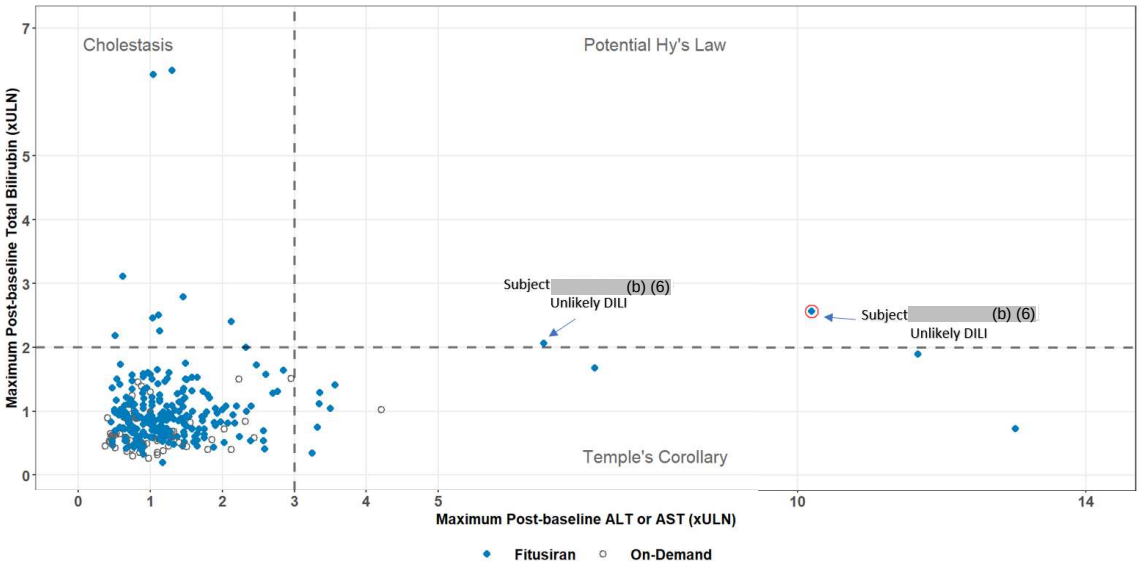
compared to the other three pivotal studies (approximately 15 to 170 days). Otherwise, the findings from these figures and tables were similar to the other three pivotal studies.

c). *Gallbladder related SAE and AEs*: Seventeen (6%) of subjects experienced a gallbladder related adverse event and these are discussed further in **Section 4.4.3**.

4.3.5.1: *Study 74 ATDR only vs. On-Demand (Studies 68 & 69):* The study-level results discussed thus far include the original 80 mg dosing as well as the ADTR without discrimination between the two because many subjects transitioned at different points in the trials. Only about 20% of the safety population received the ATDR dosing only. Indeed, the only randomized controlled periods amongst all four pivotal trials were in Studies 68 and 69 where all subjects received the 80 mg QM dosing exclusively; there are no randomized controlled data for the labeled ATDR dosing.

Therefore, the Sponsor, DNH, and the DILI Team did post-hoc analyses comparing subjects on the labeled ATDR dosing in Study 74 to the On-Demand control subjects from the feeder Studies 68 and 69. The DILI Team's ATDR cohort data is limited to the ATDR dosing period for each patient.

a). *Hepatocellular DILI screening scatterplot (eDISH)*: Despite the lower ATDR dosing, there was still on overall shift toward higher ATs and TB for fitusiran subjects compared to the On-Demand controls (**Figure 10**). Proportionately more fitusiran subjects fell in Temple's compared to On-Demand (3.4% versus 1.7%), though the proportion was substantially lower than in Studies 68 and 69 that used 80 mg QM dosing, 29.3% and 19%, respectively. The two subjects plotting to the Potential Hy's Law quadrant are discussed Section 4.3.4, (a) above. Both were unlikely DILI. There was a remarkable imbalance in the cholestasis quadrant with ten (3.8%) fitusiran ATDR subjects versus none for the On-Demand groups.

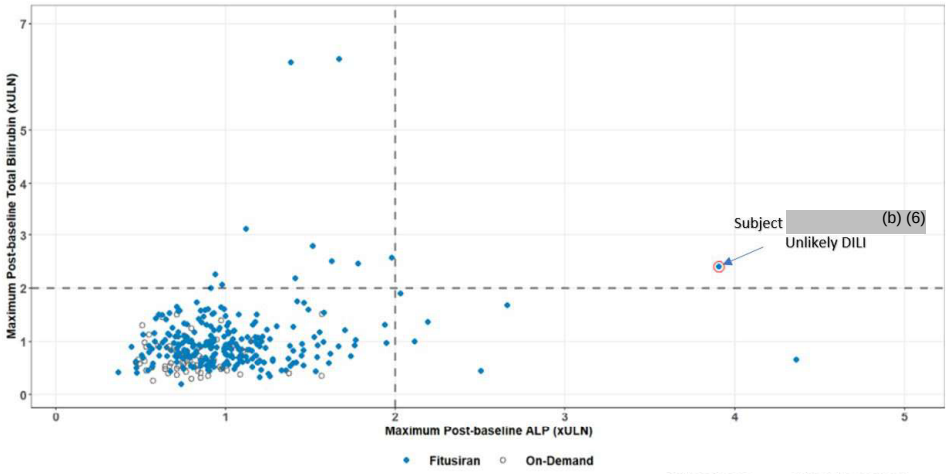


Quadrant	Fitusiran N=267	On-Demand N=59
Potential Hy's Law (right upper)	2/266 (0.8)	0/58 (0)
Cholestasis (left upper)	10/266 (3.8)	0/58 (0)
Temple's corollary (right lower)	9/266 (3.4)	1/58 (1.7)
Total	21/266 (7.9)	1/58 (1.7)

Source: adlb.xpt; Software: R
Abbreviations: DILI, drug-induced liver injury
Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALP versus their maximum total bilirubin values in the post-baseline period.
A potential cholestatic DILI case (red circled) was defined as having a maximum post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after post-baseline ALP became equal to or exceeding 2X ULN.
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Figure 10: eDISH with quadrant counts for ATDR fitusiran from Study 74 and On-Demand subjects from Studies 68 and 69.[32]

b). *Cholestatic DILI screening plot (eDISH-AP*): There was a persistent shift toward higher AP and TB levels in the ATDR fitusiran subjects compared to the On-Demand controls (**Figure 11**). Unlike the shifts in ATs on eDISH which were more than On-Demand but lower than in Studies 68 and 69, the shifts in AP were similar to Studies 68 and 69. The one subject ([redacted] (b) (6)) in the right upper quadrant on eDISH-AP was considered unlikely DILI as previously discussed. Otherwise, no other subject in the eDISH cholestasis quadrant plotted to the right upper quadrant suggesting only mild to no AP elevations for these jaundiced patients.



Quadrant	Fitusiran N=267	On-Demand N=59
>=2X ULN Bilirubin and >=2X ULN ALP (right upper)	1/266 (0.4)	0/58 (0)
>=2X ULN Bilirubin and <2X ULN ALP (left upper)	11/266 (4.1)	0/58 (0)
<2X ULN Bilirubin and >=2X ULN ALP (right lower)	6/266 (2.3)	0/58 (0)
Total	18/266 (6.8)	0/58 (0)

Source: adlb.xpt; Software: R
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal
Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALP versus their maximum total bilirubin values in the post-baseline period.
A potential cholestatic DILI case (red circled) was defined as having a maximum post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after post-baseline ALP became equal to or exceeding 2X ULN.
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

Figure 11: eDISH-AP plot and quadrant counts for ATDR fitusiran from Study 74 and On-Demand subjects from Studies 68 and 69.[33]

[32] Made by CDS.
[33] Made by CDS.

c). *Shifts in liver blood tests by cut-off levels:* The table for numbers and rates of liver blood tests elevations by cut-off levels is in the **Appendix, Table D**. This tabular data largely reflects the eDISH and eDISH-AP plots with higher proportions having ALT, AST, AP, and TB elevations in the fitusiran ATDR group compared to the On-Demand group. Indeed, the On-Demand group had only one of 59 with an AST between 3x and 5x ULN; there were no other On-Demand subjects with abnormal liver enzymes or TB.

d). *Gallbladder AEs by preferred terms*: As with the other pivotal studies, there were more gallbladder related AEs with fitusiran, even at the lower ATDR dosing, compared to the On-Demand controls from Studies 68 and 69 (**Table 9**). The higher rate of hepatotoxicity AEs is in line with the higher rates of liver blood tests abnormalities discussed above.

Table 9: Grouped query, preferred term gallbladder disorders in the safety population, ATDR subjects in Study 74 compared to On-Demand controls from Studies 68 and 69.[34]

Grouped Query Preferred Term	Fitusiran N=267 n (%)	On-Demand N=59 n (%)	Risk Difference (%) (95% CI)
Gallbladder disorders (GQ)	11 (4.1)	0	4.1 (-2.1, 7.2)
Cholecystitis	4 (1.5)	0	1.5 (-4.6, 3.8)
Cholelithiasis	4 (1.5)	0	1.5 (-4.6, 3.8)
Gallbladder polyp	3 (1.1)	0	1.1 (-5.0, 3.3)
Cholecystitis acute	2 (0.7)	0	0.7 (-5.4, 2.7)
Pancreatitis	1 (0.4)	0	0.4 (-5.8, 2.1)
Cholangitis	1 (0.4)	0	0.4 (-5.8, 2.1)
Cholecystitis chronic	1 (0.4)	0	0.4 (-5.8, 2.1)

4.3.6**: Summary of study level, liver injury data across the four pivotal trials**: eDISH and eDISH-AP suggest the potential for hepatocellular and cholestatic liver injury, respectively. The numbers and proportions of subjects for the two major quadrants of concern (right upper and lower) for both plots and for all four studies including the post-hoc ADTR analysis are in **Table 10**.

For hepatocellular injury, there were consistently higher rates of subjects in Potential Hy's Law and Temple's quadrants compared to On-Demand subjects. There were three fitusiran subjects in the Hy's law quadrants versus no On-Demand subjects, and the proportions of fitusiran subjects in Temple's quadrants ranged from 3.4% to 29.3% versus 0% to 2.6% for the On-Demand groups. There was only one subject in Study 10 that met Hy's Law. Studies 10 and 74 by themselves had no comparator arm so Temple's Corollary could not be tested, but their proportions in quadrants of interest were similar to the fitusiran arms in Studies 68 and 69.

[34] Made by CDS.

Table 10: Counts and percentages in eDISH and eDISH-AP quadrants of concern across the four pivotal studies. FTS = fitusiran, and Q = quadrant. Only the first three rows have non-fitusiran comparator arms. Trials in the last two rows (italicized) had only subjects on fitusiran.

Studies	N	Hepatocellular DILI Assessment		Cholestatic DILI Assessment		Gallbladder AEs n (%)
		eDISH Potential Hy's Law Q n (%)	eDISH Temple's Corollary Q n (%)	eDISH-AP Right upper Q (AP >2x ULN + TB >2x ULN) n (%)	eDISH-AP Right lower Q (AP >2x ULN + TB <2x ULN) n (%)	
68	41 fitusiran versus 19 On-Demand	1 (2.4) vs 0 (0)	12 (29.3) vs 0 (0)	0 (0) vs 0 (0)	3 (7.3) vs 0 (0)	6 (14.6) vs 0 (0)
69	79 FTS versus 40 On-Demand	0 (0) vs 0 (0)	15 (19) vs 1 (2.6)	0 (0) vs 0 (0)	2 (2.5) vs 0 (0)	5 (6.3) vs 0 (0)
74 vs. 68/69~	267 ATDR fitusiran versus 59 On-Demand	2 (0.8) vs 0 (0)	9 (3.4) vs 1 (1.7)	1 (0.4) vs 0 (0)	6 (2.3) vs 0 (0)	11 (4.1) vs 0 (0)
10	*69 fitusiran (23 Inhibitor; 46 Non-inhibitor)*	***1 (1.4) ^***	*21 (30.4) **	*0 (0)*	*2 (2.9)*	*9 (13.0)*
74	*281 fitusiran (105 Inhibitor; 176 Non-inhibitor)*	*2 (0.7)*	*23 (8.2) ***	*1 (0.4)*	*9 (3.2)*	*17 (6)*

~ Fitusiran ADTR data from Study 74 versus On-Demand controls from Studies 68 and 69.
^ met Hy's Law
* 6/23 (26.1 %) inhibitor and 15/46 (32.6 %) noninhibitor
** 9/104 (8.7%) inhibitor and 14/175 (8.0%) non-inhibitor
FTS = fitusiran; Q = quadrant.

4.4 Case level analyses

4.4.1 *DILI case* identification

a). Case criteria: On January 2024, the DNMH requested the Applicant provide case level data for subjects in the following categories:

1. *Post-Day 0 ALT or AST ≥5x ULN*
2. *Post-Day 0 ALT or AST ≥3x ULN and any of the following:*
   a. TB ≥2x ULN
   b. INR ≥1.5
   c. Signs of liver failure (e.g., ascites, encephalopathy)
3. *Post-Day 0 ALP ≥2x ULN*

   Any co-occurring hepatobiliary AE leading to drug discontinuation (but not TEAE, AESI, SAE) were listed.

The data fulfilling the information request for the 66 (19.7%) subjects so identified comes from the following sources in the NDA:

1. Narratives in the original NDA application[35]
2. Narratives in the cholecystitis module[36]
3. Additional detail on the case of Hy's law[37]
4. Graphical profiles (line graphs of liver blood tests over time)[38]

4.4.2 *Case assessment for DILI*: Of the 66, we assessed five as at least probable DILI due to fitusiran, six as possible, and 55 as unlikely or indeterminate. Several of the six possibles were borderline probable, but we relegated them to possible because they had no jaundice nor AT elevations >5x ULN, thus not meeting minimum criteria for DILI case definition.[39] We summarize the five at least probable cases with ATs >5x ULN in **Table 11**. All but one of the five were on the 80 mg QM dosing. Injuries tended to be hepatocellular with median R-value (ALT) of 8.0; using either ALT or AST, all cases had hepatocellular injury (R-values ≥5). Latencies from drug start were short with a median of 42 days (range 22 to 56 days). One subject met Hy's law.

Table 11: Summary of five subjects with at least probable DILI and ATs >5x ULN.

#	ID	Causality Score*	Dose	Study	Age (y)	Sex	Race	Hy's Law	Latency from start drug (d)	Latency from stop drug (d)~	ALT peak (U/L)	AST peak (U/L)	ALP peak (U/L)	Bilirubin peak (mg/dL)	R value peak (ALT)	R value peak (AST)
1	(b) (6)	2	80 mg	10	20	M	Asian	Yes	28	[148]	307	157	118	3.16	7.96	4.07
2		2	80 mg	69	28	M	Unknown	No	22	[203]	346	217	119	1.4	8.89	5.58
3		3	80 mg	10	14	M	Unknown	No	45	[123]	331	247	156	1.17	6.49	4.84
4		3	80 mg	10	12	M	Unknown	No	42	19	165	251	382	0.88	4.85	7.38
5		3	50 mg	74	NA	M	Unknown	No	56	55	624	164	121	0.7	15.77	4.15
				Average	*19*				*38.6*	*[80]*	*355*	*207*	*179*	*1.46*	*8.8*	*5.2*
				Std.	*6.2*				*12.2*	*99.6*	*149*	*40*	*102*	*0.88*	*3.7*	*1.2*
				Median	*17*				*42*	*[123]*	*331*	*217*	*121*	*1.17*	*8.0*	*4.8*
				Max.	*28*				*56*	*55*	*624*	*251*	*382*	*3.16*	*15.8*	*7.4*
				Min.	*12*				*22*	*[203]*	*165*	*157*	*118*	*0.7*	*4.9*	*4.1*

*1=definite, 2=highly likely, 3=probable, 4=possible, 5=unlikely, 6=indeterminate
^R-value adjusted for pediatric ULN
^^ Age not provided but subject is an adult (>18 years)
~Bracketed [ ]day values mean the drug continued for that many days after injury onset.
R-value = (ALT/ULN) ÷ (AP/ULN) or (AST/ULN) ÷ (AP/ULN); hepatocellular: R-values ≥ 5; mixed: 2-5; cholestatic: R-value < 5
ULNs used for R-values: ALT 34 U/L, AST 34 U/L, AP 104 U/L, TB 1.2 mg/dL unless otherwise stated/footnoted
NA = not available or not applicable

DILI cases of interest:

i. *Subject* (b) (6) *(Study 10); Highly likely DILI; met Hy's law criteria.*

[35] NDA219019 (219019 - 0001 - (1) - 2024-03-28 - ORIG-1 /Multiple Categories/Subcategories) - EFC14768 8.3.3 Participant narratives - SAEs
[36] NDA219019 (219019 - 0001 - (1) - 2024-03-28 - ORIG-1 /Multiple Categories/Subcategories) - ISS Appendix 11 - Narratives of cholelithiasis/cholecystitis events by HGLT
[37] NDA219019 (219019 - 0004 - (4) - 2024-04-29 - ORIG-1 /Clinical/Clinical Information) - Tabular Outputs and Listings by Liver Function (#5)
[38] NDA219019 (219019 - 0004 - (4) - 2024-04-29 - ORIG-1 /Clinical/Clinical Information) - Tabular Outputs and Listings by Liver Function (#8)
[39] Aithal GP, et al. Case Definition and Phenotype Standardization in Drug-Induced Liver Injury. *Clin Pharm Therap*. 2011; 89:806-815

Summary: This subject was a 20-year-old male with hemophilia A, who developed elevation in aminotransferase approximately 28 days after starting fitusiran, 80 mg QM. He had repeat AT elevation and jaundice on rechallenge, meeting Hy's law.

At baseline, he had no other medical problems. He denied alcohol. There was no mention of other concomitant medications or herbal/dietary supplements (HDS). His ALT, AST, AP, and TB were 11 U/L, 12 U/L, 77 U/L and <ULN, respectively.

On (b) (6) (Day 28) his ALT was 71 and AST 35. AP and TB remained normal. He had no symptoms and fitusiran continued. Liver enzymes continued to rise after the second dose. They fell slightly, only to rise to and ALT of 171 U/L and AST to 85 U/L with the third dose (**Figure 12**). The next dose was delayed and both enzymes fell to nearly normal. However, on two more rechallenges, ATs rose again, the last rechallenge giving the highest AT levels (ALT 307 U/L; AST 157 U/L) with jaundice (TB of 3.16; DB 1.21 mg/dL). Fitusiran was stopped, and liver blood tests fell to baseline within a few weeks. Serologies for hepatitis A, B, C, E, EBV, and CMV were negative. ANA was 1:160; ASMA and IgG were not provided. Ultrasound was unrevealing.

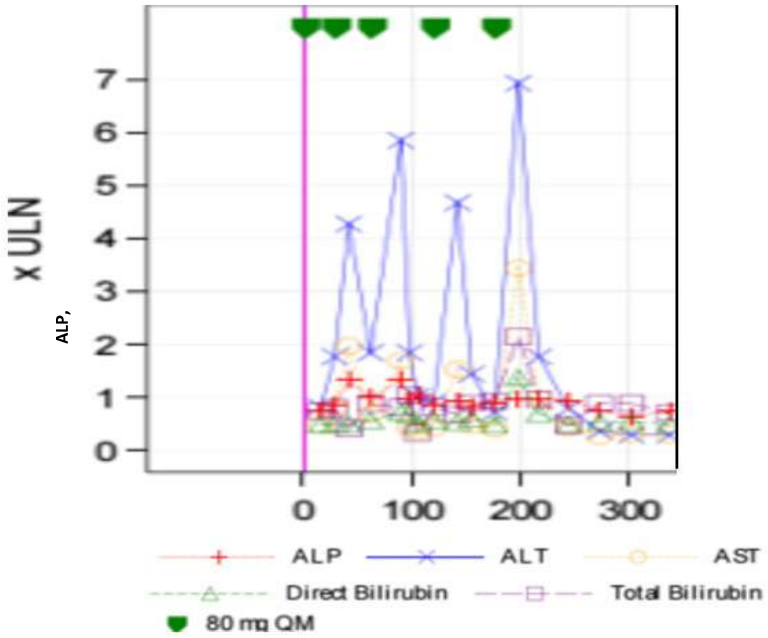


Figure 12: Liver blood tests by study day for Subject (b) (6) [40]

Assessment: We assessed this case as highly likely to definite fitusiran hepatotoxicity due to the multiple positive rechallenges and dechallenge washout of liver enzymes. The last rechallenge resulted in jaundice and met Hy's law criteria.

ii. Subject (b) (6) (Study 69): Highly likely DILI and adaptation.

[40] Adapted from NDA219019 (219019 - 0004 - (4) - 2024-04-29 - ORIG-1 /Clinical/Clinical Information) - Tabular Outputs and Listings by Liver Function (#293)

Summary: This is a 28-year-old male of unknown or unstated race, with hemophilia, who developed elevated aminotransferases approximately 22 days after starting fitusiran. Initial dose was 80 mg.

At baseline, the subject's BMI was 19 kg/m^2. Relevant medical history included just the target disease. There was no mention of metabolic syndrome diagnoses or liver disease. Alcohol history was not provided. Concurrent medications relevant to DILI risk included use of “energy drinks” but no other medications or HDS products of concern for DILI risk based on timing or type of agent. The subject's ALT, AST, AP, and TB were 16 U/L, 20 U/L, 94 U/L, and 0.6 mg/dL, respectively.

The subject started fitusiran, 80 mg dose on Day 1. On Day 15, ALT, AST, AP, and TB were 66 U/L, 36 U/L, 91 U/L and normal, respectively. The subject had no symptoms. There was no mention of study drug change. By Day 22, ALT, AST, AP, and TB were now 346 U/L, 217 U/L, 119 U/L and 1.4 mg/dL, respectively. Still no symptoms were mentioned. The study drug was held, and liver enzymes fell to baseline by Day 57. On Day 57, fitusiran 80 mg dose was restarted. This was followed by a repeat rise in ATs (**Figure 13** and **Table 12**) and the fitusiran was held for the second time to allow ATs to fall again. Fitusiran was restarted on Day 162. There was another rise in ATs but smaller than the first two. He had no liver related symptoms. Serologic tests for CMV and EBV are reported but mainly IgG antibodies. The narrative makes no mention of hepatitis virus testing or imaging. He continued with monthly doses of 80 mg fitusiran with minor and diminishing rises in liver enzymes. His last dose was on Day 225 at study completion, and he enrolled in Study 74 receiving ATDR without substantial increase in liver enzymes. His TB never rose higher than 1.4 mg/dL.

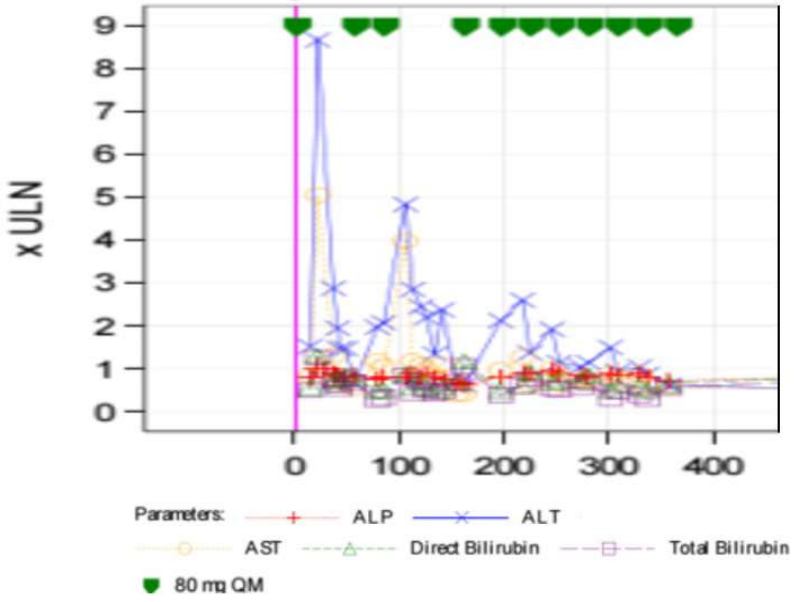


Figure 13: Line graph of liver blood tests by study day for Subject (b) (6).[41]

[41]Adapted from NDA219019 (219019 - 0004 - (4) - 2024-04-29 - ORIG-1 /Clinical/Clinical Information) - Tabular Outputs and Listings by Liver Function (#270)

Table 12: ALT values by study day for Subject [redacted] (b) (6) Red marks indicate dosing of fitusiran 80 mg. Peak ALT increases are indicated by orange lines and highlighting.[42]

Laboratory Test		Study day	Visit number (name)	Study period	Finding (normal range)
ALT		Day -43	SCREENING	SCREENING	13 (10-40) IU/L
ALT	Fitusiran 80 mg	Day -2	UNSCHEDULED VISIT 1.03	SCREENING	16 (.-49) IU/L
ALT		Day 1	DAY1	SCREENING	16 (10-40) IU/L
ALT		Day 15	DAY15	TREATMENT	61 (10-40) IU/L
ALT		Day 22	UNSCHEDULED VISIT 5.01	TREATMENT	346 (10-40) IU/L
ALT		Day 37	UNSCHEDULED VISIT 6.02	TREATMENT	115 (10-40) IU/L
ALT		Day 41	DAY43	TREATMENT	78 (10-40) IU/L
ALT		Day 43	UNSCHEDULED VISIT 7.01	TREATMENT	59 (10-40) IU/L
ALT		Day 49	UNSCHEDULED VISIT 7.02	TREATMENT	59 (10-40) IU/L
ALT	Fitusiran 80 mg	Day 52	UNSCHEDULED VISIT 7.03	TREATMENT	48 (0-49) IU/L
ALT		Day 57	MONTH2	TREATMENT	27 (10-40) IU/L
ALT		Day 65	UNSCHEDULED VISIT 8.01	TREATMENT	45 (0-49) IU/L
ALT		Day 73	UNSCHEDULED VISIT 8.02	TREATMENT	76 (0-49) IU/L
ALT		Day 79	UNSCHEDULED VISIT 8.03	TREATMENT	79 (10-40) IU/L
ALT		Day 85	MONTH3	TREATMENT	83 (10-40) IU/L
ALT		Day 106	UNSCHEDULED VISIT 9.01	TREATMENT	193 (10-40) IU/L
ALT		Day 113	MONTH4	TREATMENT	114 (10-40) IU/L
ALT		Day 121	UNSCHEDULED VISIT 10.01	TREATMENT	98 (10-40) IU/L
ALT		Day 126	UNSCHEDULED VISIT 10.03	TREATMENT	88 (10-40) IU/L
ALT		Day 134	UNSCHEDULED VISIT 10.08	TREATMENT	54 (10-40) IU/L
ALT		Day 141	MONTH5	TREATMENT	95 (10-40) IU/L
ALT	Fitusiran 80 mg	Day 155	UNSCHEDULED VISIT 11.01	TREATMENT	26 (10-40) IU/L
ALT		Day 162	MONTH6	TREATMENT	25 (10-40) IU/L
ALT		Day 196	UNSCHEDULED VISIT 12.02	TREATMENT	85 (.-49) IU/L
ALT	Fitusiran 80 mg	Day 197	MONTH7	TREATMENT	85 (10-40) IU/L
ALT		Day 197	UNSCHEDULED VISIT 13.01	TREATMENT	92 (0-50) IU/L
ALT	Fitusiran 80 mg	Day 218	UNSCHEDULED VISIT 13.02	TREATMENT	104 (10-40) IU/L
ALT		Day 225	MONTH8_END OF TREATMENT	TREATMENT	55 (10-40) IU/L
ALT		Day 246	MONTH9_END OF STUDY/EARLY TERMINATION	TREATMENT	77 (10-40) IU/L

Assessment: We assessed this case as highly likely to definite DILI due to fitusiran. The latency is appropriate and may have been even shorter at Day 15 when ALT was up to 61 U/L from the subject's baseline of 16 U/L. Line graph and Table of ALTs clearly shows positive rechallenges. Evaluation testing was limited, but the main competing cause would be repeated gallstone passage, which is less likely than the positive rechallenges. The patient was asymptomatic. Unlike Subject [redacted] (b) (6) (Case i. above), this patient showed adaptation with less severe ALT increases on rechallenge and ability to finish the study.

iii. *Subject* [redacted] (b) (6) *(Study 74): Probable DILI.*

Summary: This subject was a 21-year-old male with a history of hemophilia A who developed elevated ATs 27 to 56 days after taking one 50 mg dose of fitusiran.

At baseline, he had no other medical problems relevant to the liver injury except a "gallbladder polyp," size not provided. His BMI was 21 kg/m^2. He did not drink alcohol or take HDS products. He took ibuprofen, 200 mg/d on Study Days 1 and 2; he took

[42] Adapted from NDA219019 (219019 - 0001 - (1) - 2024-03-28 - ORIG-1 /Multiple Categories/Subcategories) - EFC14769 8.3.3 Participant narratives - AESIs (#15)

levofloxacin 200 mg BID on Days 2 and 3. Otherwise there were no other medications pertinent to this liver injury event. His ALT, AST, AP, and TB were 35 U/L, 24 U/L, 111 U/L and 0.58 mg/dL.

He enrolled in Study 74 without prior exposure to fitusiran; therefore, he only received the ATDR dose of 50 mg on Day 1. On Day 26, his ALT and AST were still low, at 38 U/L and 30 U/L, respectively. However, on Day 56, his ALT, AST, AP, and TB were 624 U/L, 164 U/L, AP 121 U/L, and TB 0.7 mg/dL. There is no mention of symptoms. Fitusiran was discontinued. Thereafter, his ATs fell to normal baseline within 12 days (**Figure 14**). There is no mention of evaluation testing.

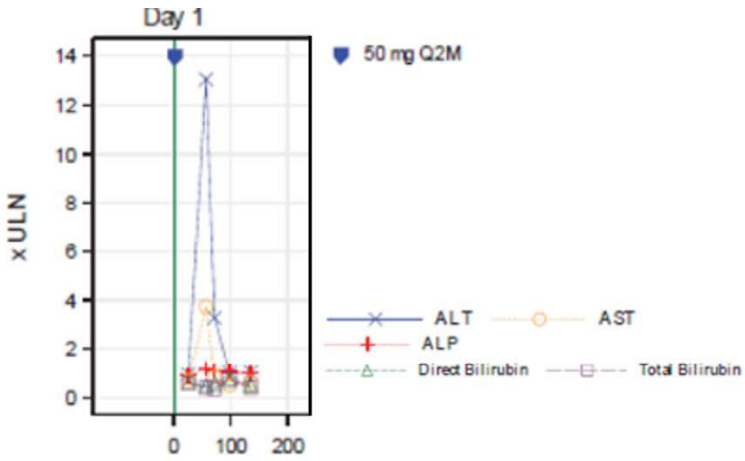


Figure 14: Liver blood tests by study day for Subject [redacted] (b) (6)
[redacted] Source: Adapted from applicant response to Information Request

Assessment: We considered this case as probable DILI due to fitusiran based on appropriate latency and dechallenge washout. This case is noteworthy for use of ATDR dosing only. Fitusiran's long tissue half-life, particularly in the liver makes a latency of 26 to 56 days plausible, and the injury onset and peak were probably earlier than 56 days because the enzymes fell immediately after the initial elevations were noted. Evaluation testing was not provided, but we are not inclined to give the benefit of such doubt to the study drug. Ibuprofen can cause high ATs but it is quite rare, often accompanied by immunoallergic features and at high dosages.[43] The dose was 200 mg/d for two days, and the patient was asymptomatic. Levofloxacin hepatotoxicity is well-described, but onset is most commonly within one to two weeks,[44] and ATs were normal at Day 26.

4.4.3 Case assessments for gallbladder (GB) AE: **Tables 13, 14, and 15** describe the gallbladder related SAEs and AEs. We reviewed narratives for each case and agree with assignments of GB AEs. Brief notes and Study Day onset are in the tables.

Overall, there were 36 discrete subjects with gallbladder (GB) AEs across the four pivotal studies and Study 14762, yielding a crude rate of 11% (36 of approximately 335 subjects exposed). Across just the four pivotal studies, rates ranged from 4.1% to 14.6% (see **Section 4.3.6, Table 10**). Four (11%) of 36 subjects experienced gallstone pancreatitis. Twelve (28%) required a cholecystectomy. All twelve did well with eleven able to continue fitusiran therapy after surgery. Average time to onset was 137 days (range 14 to 661 days). However, estimates of time to onset is hindered by the indolent nature of gallstone formation and cholecystitis with delay in recognition by provider and

[43] LiverTox® https://www.ncbi.nlm.nih.gov/books/NBK547845/
[44] Orman ES, et al. Clinical and histopathologic features of fluoroquinolone-induced liver injury. *Clin Gastro Hep*. 2011; 9:517-523.

patient. Similarly, we could not define a dose response for these GB AEs because recognition during the ATDR may merely reflect formation of gallstones during the prior 80 mg dosing.

Table 13: Gallbladder related AEs for Study 68

Subject ID	SAE	AE
(b) (6)	Cholecystitis Chronic (139) Cholangitis (1030)	Cholecystitis (85); AT increase (115) Pancreatitis
	Cholecystitis Acute (108), Biliary Colic (173 and 210)	no additional AEs
	None	ALT increase (43), Cholecystitis chronic (142), Cholelithiasis (142), Acute cholecystitis (15174-Day 569)
	None	Cholestasis (42)
	None	Cholecystitis (253)
	None	Cholelithiasis (140)

Source: DILI Clinical Reviewer. Abbreviations: SAE, serious adverse event; AE, adverse event; SD, study day (parent study unless otherwise noted).
Notes: *indicates the subject experienced gallbladder events in the parent study and in LTE 15174

Table 14: Gallbladder related AEs for Study 69

Subject ID	SAE (study day)	AE (study day)
(b) (6)	Cholelithiasis (89)	None
	Cholecystitis (72)	ALT increased (56)
	Cholelithiasis (223 Study 69; Day 108 Study 74)	ALT increase (110); AT increase (109 of Study 74)
	None	ALT increase (85); Cholelithiasis (173, 639, 680)
	None	Cholecystitis (39)

Source: DILI Clinical Reviewer. Abbreviations: SAE, serious adverse event; AE, adverse event; SD, study day (parent study unless otherwise noted).
Notes: *indicates the subject experienced gallbladder events in the parent study and in LTE 15174

Table 15: Gallbladder related AEs for Study 10

Subject ID	SAE (study day)	AE (study day)
(b) (6)	Biliary neoplasm (42)	Cholelithiasis (74), Cholecystitis Chronic (41), ALT increase (48)
	None	Cholecystitis (76)
	Cholelithiasis (146)	ALT increase (55), Cholelithiasis (106),Cholecystitis (146)
	Pancreatitis due to stone (39)	Cholelithiasis (39)
	None	Cholelithiasis (160), ALT increase (165)
	None	Cholecystitis (140)
	None	ALT increase (88), Cholelithiasis (94), AST increase (29 of Study 74), AT increase (395)
	None	ALT increase (112); gallbladder enlargement
	All events occurred in Study 74: ALT increased (151) Cholecystitis (151) Pancreatitis (15 and 217)	cholecystitis (132)

Source: DILI Clinical Reviewer. Abbreviations: SAE, serious adverse event; AE, adverse event; SD, study day (parent study unless otherwise noted).
Notes: *indicates the subject experienced gallbladder events in the parent study and in LTE 15174

Table y. Gallbladder related AEs for Study 74

Subject ID	SAE (study day)	AEs (study day)
(b) (6)	Biliary colic (52)	Biliary colic (57)
	Cholelithiasis (226)	Cholecystitis (84 and 133)
	Cholecystitis (207)	Cholelithiasis (83), ALT increase (209)
	Cholecystitis, acute (49)	pancreatitis acute (52)
	Cholecystitis (162)	Hyperplastic "cholecystopathy", (162), AST increased (1127)
	Cholecystitis, chronic (258)	ALT increased (113)
	Cholecystitis (479)	None
	None	GB enlargement (254 and 994)
	None	ALT increase (29), Cholelithiasis (113)
	None	Cholelithiasis (84), ALT increase (91) Cholelithiasis (929)
	None	Cholelithiasis (14)
	None	Cholecystitis (661), GB polyp (661)
	None	Cholelithiasis (77)
	None	Cholelithiasis (170)
	None	Cholecystitis (169)
	None	Acute cholecystitis (30, GB polyp (30), chronic cholecystitis (53)
	None	ALT increase (30), cholelithiasis (54)

Source: DILI Clinical Reviewer. Abbreviations: SAE, serious adverse event; AE, adverse event; SD, study day (parent study unless otherwise noted).

Two exemplary cases are described below. Common symptoms experienced by subjects included epigastric pain, indigestion, nausea, and vomiting.

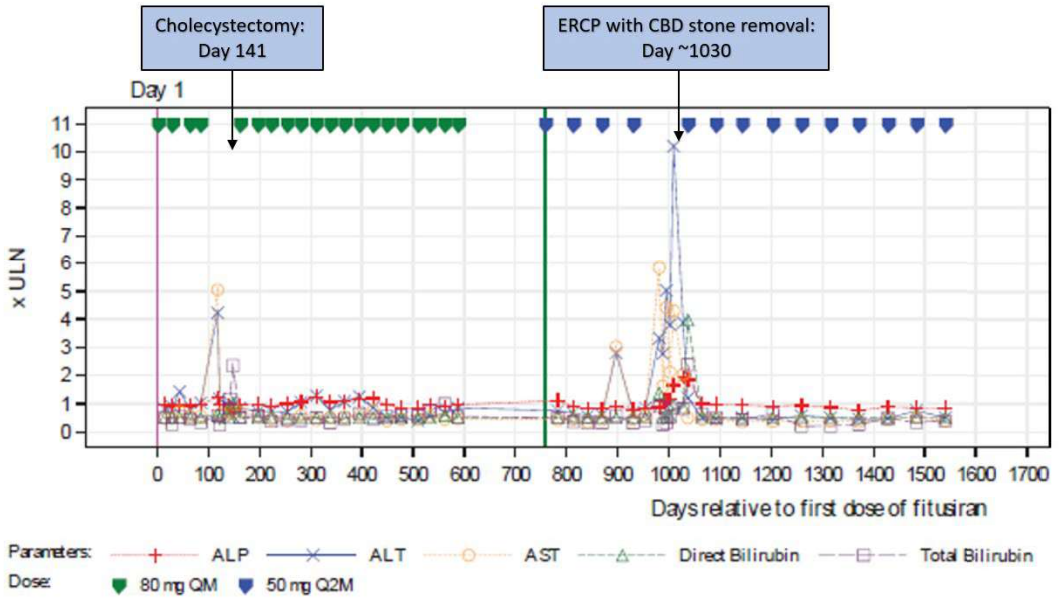
Gallbladder AE cases of interest:

i. Subject (b) (6) *(Probable cholecystitis due to fitusiran)*

Summary: This patient was a 50-year-old man with hemophilia A and inhibitors enrolled in Study 68 and started on treatment with fitusiran 80 mg QM on Day 1. He had diabetes mellitus, hypertension and history of hepatitis C. Concomitant medications included acarbose, glimepiride, metformin, and perindopril. On Day 85 the subject experienced abdominal pain. CT scan and ultrasound showed a gallstone, and his providers diagnosed cholecystitis; treatment was unclear at this time, but fitusiran was held. The patient went to emergency department on Days 105 and 129 for abdominal pain. ALT fluctuated between 1x to 5x ULN during this time. On Day 141, the patient had an elective laparoscopic cholecystectomy for cholecystitis. He received recombinant factor peri-operatively. On removal, his gallbladder was 8cm x 3cm with erosive and fibrotic mucosal surface of 0.7 cm thickness. Diagnosis was chronic cholecystitis with diffuse papillary hyperplasia, superimposed on acute erosive cholecystitis. The patient recovered postoperatively and restarted fitusiran 80 mg on Day 162. There was no mention of cholangiograms or MRCPs during this event.

On Day 930, liver enzyme rose prompting fitusiran to be held (**Figure 15**). On Day 1030, he presented with gallstone pancreatitis. ALT was >10x ULN and bilirubin >2x ULN. ERCP removed the common bile duct stone and the patient improved with decline in liver tests. Fitusiran (50 mg Q2M) was resumed on Day 1037. The patient remains on fitusiran treatment at a dose of 20 mg Q2M.

Figure 15: Liver enzymes and total bilirubin levels by Study Day, Subject (b) (6)



Source: Applicant response to Information Request

Assessment: We assessed this gallbladder AE as probably related to fitusiran. While this patient had risk factors for cholelithiasis (obesity and diabetes), the onset of within 120 days of starting fitusiran treatment and the overall incidence of gallbladder disorders in the clinical trials raises concern for fitusiran having a role in the development of cholecystitis in this patient. This case also highlights the risk of gallstone pancreatitis years after a cholecystectomy.

ii. Subject ALN-AT3SC-001- (b) (6)

Summary: This subject was a 40-year-old male with a history of hemophilia A without inhibitors who enrolled in study ALN-AT3SC-001 receiving 3 doses of fitusiran at (0.075 mg/kg) and later enrolled into the long-term extension (LTE) Study 14762 and go fitusiran 50 mg monthly.

On Day 678 (Day 90 of LTE) the patient reported burning epigastric pain radiating to the chest at night. Pain improved with antacids, so his providers diagnosed gastroesophageal reflux disease and prescribed pantoprazole. Yet, he still had intermittent upper abdominal and chest pain. ALT rose to 75 U/L from a normal baseline. Over the next 3 months, the patient made multiple trips to the emergency department and clinic for his symptoms. Providers gave him factor VIII thinking his

symptoms represented bleeding events, but factor did not provide relief. Upper endoscopy with normal.

On Day 737, abdominal ultrasound suggested mild acalculous cholecystitis. On Day 849 the patient was hospitalized due to cholecystitis. On Day 1025 (Day 438 of the LTE) the subject underwent a laparoscopic cholecystectomy. Fitusiran treatment had been paused months prior to the procedure due to the clinical hold on Oct 30, 2020. The patient received factor VIII perioperatively and recovered well post-operatively. He elected not to continue fitusiran treatment.

Assessment: We assessed this gallbladder AE as probably related to fitusiran. The patient had abdominal symptoms within 90 days of fitusiran, 50 mg QM start in the LTE study. The patient did not have any risk factors for gallstone disease and experienced a delay in diagnosis, likely due to low suspicion of acute GB disease in patients with hemophilia. He had unnecessary factor therapy before the cholecystitis was discovered, and ultimately needed a cholecystectomy six months after discontinuing fitusiran suggesting drug discontinuation may not necessarily reverse gallbladder disease.

5.0 Assessment & Recommendations

5.1 Assessment

Fitusiran is a synthetic double stranded, small interfering RNA (siRNA) covalently linked to three N-acetyl galactosamine residues. The siRNA sequence is complimentary to antithrombin (AT) messenger RNA (mRNA) and delivered to the liver via binding the asialoglycoprotein receptor (ASGPR) on hepatocytes followed by endosomal uptake. Complimentary binding to the AT mRNA leads to its targeted destruction. Consequent decrease in AT production is expected to decrease bleeding in hemophilia A and B patients without or with factor inhibitors. The drug is given subcutaneously every month with the dose dependent on antithrombin levels (labeled ATDR dosing). The Division of Non-malignant Hematology (DNH) noted liver enzyme elevations associated with fitusiran use and consulted the Division of Hepatology and Nutrition (DHN) DILI Team to provide input on attribution of the enzyme elevations to fitusiran, opinion on hepatotoxicity severity and risk, as well as labeling recommendations.

Non-clinical data suggest DILI risk with hepatocellular necrosis, biliary hyperplasia, and inflammation in rodents and/or dogs. While the plasma half-life is eight hours, intra-hepatocyte accumulation with longer half-life in the liver is likely. Indeed, persistence in hepatocytes probably allows monthly dosing with steady efficacy. In vitro data on liver risk were limited. Fitusiran does not inhibit most hepatocyte transporters, but we did not find data specifically related to MDR2, reactive metabolite formation, or evidence of off-target mRNA destruction leading to liver injury. The five marketed siRNAs for other indications do not suggest an obvious class effect. Only givosiran is labeled for hepatotoxicity (Highlighted Warnings and Precautions); it uses the ASGR delivery route, but another using this deliver means are not labeled for hepatotoxicity. Thus, the mechanism of siRNA liver injury is unclear.

Clinical assessment of liver risk was challenging because (a) we observed two liver injury signals and (b) the change from 80 mg monthly to the lower ATDR prevented comparisons to contemporaneous randomized control groups. We discuss the two DILI risks, idiosyncratic and indirect via gallbladder (GB) adverse events, sequentially. We discuss the effect of the late change in dosing within each DILI risk.

Idiosyncratic DILI: The evidence for idiosyncratic DILI was substantial.

Study level data (e.g., eDISH plotting) for the two randomized controlled, pivotal studies (Studies 68 and 69) show a clear increase in liver enzymes and bilirubin associated with fitusiran compared to by-pass agent on demand (On-Demand) therapy, with 19% and 29% of fitusiran treated patients plotting to Temple's Corollary quadrant (AT >3x ULN + TB <2x ULN) compared to 0% and 2.6% for On-Demand in Studies 68 and 69, respectively. However, Studies 68 and 69 only used the 80 mg QM dosing having completed before implementation of the lower ATDR dosing across clinical trials. In a post-hoc analysis comparing ATDR dosing in Study 74 to the On-Demand controls in 68 and 69, this risk difference persisted but was less prominent with the 3.4% of ATDR patients in Temple's versus 1.7% for the On-Demand patients from 68 and 69, suggesting a dose response for this DILI. However, selection bias could also be partly explanatory because those who tolerated fitusiran in 68 and 69 were more likely to roll-over into study 74 using ATDR. Indeed, 11% of subjects receiving 80 mg dosing in the feeder studies did not continue into study 74, and we did not have specifics on why they did not continue. Furthermore, some subjects on the ATDR dosing still had significant rises in ALT that led to drug discontinuation. Three fitusiran subjects plotted to the Potential Hy's Law quadrant compared to no comparator arm patients, across the pivotal studies. One of the three met Hy's law (see next paragraph).

We assessed 66 subjects (66 of 335 or 19.7%) that met criteria for DILI concern across the four pivotal and LTE studies. We assess two as highly likely DILI due to fitusiran, one meeting Hy's law. We considered three others as probable DILI and six as possible. Overall, the injuries were hepatocellular and of relatively short latency (median 42 days from drug start for at least probable DILI cases). The two highly likely cases had positive rechallenges and are instructive because the Hy's law case did not adapt, having progressively higher ALT levels on rechallenges with eventual jaundice. The other highly likely case also had two positive rechallenges but adapted with progressively lower ALT rises without jaundice and finished the 80 mg dosing. One probable DILI case only took the ATDR dose of 50 mg. So, this DILI likely has an idiosyncratic component in terms of adaptability and dose, even if the overall risk were lower with the labeled ATDR dosing. On the other hand, the recovery after repeated rechallenges suggests the injuries may be amenable to monitoring. Overall, one Hy's law case and unfavorable imbalances in Temple's Corollary appearing in under 400 subjects exposed raises concerns for fatal idiosyncratic DILI in a larger post-market population.[45]

[45] FDA Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation. 2009; https://www.fda.gov/media/116737/download

Indirect DILI: The evidence for indirect DILI by GB adverse events (AE) was substantial.

Gallstones and GB problems are rare in hemophilia.[46] Therefore, 36 GB AEs out of approximately 335 exposed is remarkably high (11%) compared to 0% observed for On-Demand patients. The crude annual rate of acute cholecystitis in US adults overall is only 0.08%[47,48] while there were ten fitusiran subjects with cholecystitis AEs (3%). Thus, there was a strong association between fitusiran and GB AEs.

GB events were less common on the ATDR dosing, but selection bias discussed for idiosyncratic DILI also applies here. Due the indolent nature of gallstone formation, potential delay in GB disease recognition, and most subjects receiving both 80 mg and ATDR, we could not establish a dose response relationship. However, at least one subject had cholecystitis on the ADTR dosing alone, and another had gallstone pancreatitis while on ADTR over 880 days after cholecystectomy, suggesting a persistent risk. The GB AE severity was substantial with twelve (33%) of 36 needing cholecystectomy. While all did well post-operatively, such surgery in hemophilia patients carries increased bleeding risk and requires careful management perioperatively.[49] In a larger post-market population, complications and increase morbidity may arise.

In summary, we see substantial dual liver injury risks should fitusiran be approved. The mechanisms of injury for both the idiosyncratic and indirect DILIs are unclear. Nevertheless, we can support approval with risk management and surveillance through labeling and pharmacovigilance, respectively, if the efficacy and need are high. Labeling will be especially important for the GB risk because providers do not expect GB problems in their hemophilia patients. So, we recommend a box Warning be considered for at least the GB risk if not the idiosyncratic DILI as well. Otherwise, both liver injury risks should appear in the Highlights Warnings and Precautions and be more fully described in Section 5.0 of the label. We recommend monitoring for liver injury for the first six months on a set schedule and as clinically indicated thereafter. Detailed recommendations follow.

5.2 **Recommendations**

1) Labeling recommendations for GB risk should fitusiran be approved.
   a. Discuss GB adverse event (AE) risk in a boxed Warning with detailed information in Section 5 (Warnings and Precautions).

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[46] Boddu, Siva Rama Krishna, Monish Ram SD, and Suresh Hanagavadi. "Surgical management in a classical haemophilia patient–A rare case report." IP Archives of Cytology and Histopathology Research 2022;7(3):186–188

[47] Gallaher JR, et al. Acute cholecystitis: A Review. JAMA. 2022; 327:965-975.

[48] https://www.census.gov/library/stories/2021/08/united-states-adult-population-grew-faster-than-nations-total-population-from-2010-to-2020.html#:~:text=In%202020%2C%20the%20U.S.%20Census,from%20234.6%20million%20in%202010.

[49] Sugiura, R., Kuwatani, M., Kawakubo, K. *et al.* Successful endoscopic sphincterotomy for choledocholithiasis in a patient with severe hemophilia A and inhibitors. *Clin J Gastroenterol* 11, 188–192 (2018). https://doi.org/10.1007/s12328-018-0826-8

b. Describe of GB AE incidence across the development program. May discuss lower incidence with ATDR dosing compared to 80 mg dose, but would mention that lower rate was not observed in a randomized controlled fashion.
c. Describe high cholecystectomy incidence in subjects with GB AEs
d. Consider describing the patient with gallstone pancreatitis more than a year after cholecystectomy.
e. Describe common symptoms cholangitis and cholecystitis in label and/or patient information material and need for prompt GB imaging.
f. Interrupt therapy for GB adverse events.
g. Avoid use in patients with hepatic impairment (Child-Pugh Class A, B and C).

2) Labeling recommendations for idiosyncratic DILI.
   a. Hepatotoxicity in Highlights, Section 5 (Warnings and Precautions) and Section 6, at DNH's discretion.
   b. Recommend baseline liver tests and then monthly for at least six months, and as clinically indicated thereafter.
   c. Describe liver injury as hepatocellular with incidence of ALT elevations for subjects on original dosing and ATDR.
   d. Describe latency (time from drug start to liver injury onset) as relatively short with ALT over 3x ULN occurring at 42.5 days (range 14-365) overall and a median of 42 days for at least probable cases of DILI with ATs over 5x ULN.
   e. Describe the Hy's Law case.
   f. Recommend liver imaging in initial evaluation for elevated liver blood tests and/or symptoms of liver injury.
   g. Interrupt fitusiran, AST or ALT rise > 5x ULN or >5x baseline. Allow liver tests to return to baseline before considering rechallenge.
   h. If AST or ALT elevations occur greater than 5x ULN reoccur, or jaundice occurs (total bilirubin ≥ 2.5 mg/dL) discontinue fitusiran permanently unless an obvious non-DILI cause is evident.
3) Enhanced pharmacovigilance to detect DILI and GB AEs events. Consider gallstone composition analysis in patients getting a cholecystectomy on fitusiran treatment to better understand the mechanism gallstone formation.
4) Encourage post-market clinical and non-clinical studies that may define intrahepatocyte accumulation and trafficking of fitusiran.

Paul H. Hayashi -S

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Date: 2025.03.21 18:14:39 -04'00'

(PH Hayashi signed on behalf of Dr. Hemmat who was on leave.)
Shirin Hemmat, MD MPH
Medical Officer, DILI Team, DHN
CDER/OND

Paul H. Hayashi -S

Digitally signed by Paul H. Hayashi -S
Date: 2025.03.21 18:15:09 -04'00'

(PH Hayashi signed on behalf of Dr. Navarro who was on leave.)
Eileen Navarro, MD
DILI Team Lead, OND/DHN

Paul H. Hayashi -S

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Date: 2025.03.21 18:15:40 -04'00'

Paul H. Hayashi, MD, MPH
DILI Team Lead, DHN
CDER/ON

Appendix:

Table A: Number and frequency of liver blood test elevations by set cut-offs, Fitusiran versus On-Demand, Study 69.[50]

Laboratory Abnormality	Fitusiran N=79 n (%)	On-Demand N=40 n (%)	Risk Difference (%) (95% CI)
ALT			
>=1X ULN	68/79 (86.1)	15/39 (38.5)	47.6 (29.7, 63.1) *
>=3X ULN	14/79 (17.7)	0/39 (0)	17.7 (8.2, 27.6) *
>=5X ULN	2/79 (2.5)	0/39 (0)	2.5 (-6.6, 8.8)
>=10X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
>=20X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
AST			
>=1X ULN	54/79 (68.4)	10/39 (25.6)	42.7 (24.0, 57.9) *
>=3X ULN	5/79 (6.3)	1/39 (2.6)	3.8 (-7.4, 12.0)
>=5X ULN	3/79 (3.8)	0/39 (0)	3.8 (-5.4, 10.6)
>=10X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
>=20X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
ALP			
>=2X ULN	2/79 (2.5)	0/39 (0)	2.5 (-6.6, 8.8)
>=3X ULN	1/79 (1.3)	0/39 (0)	1.3 (-7.8, 6.9)
Total Bilirubin			
>=2X ULN	2/79 (2.5)	0/39 (0)	2.5 (-6.6, 8.8)
>=5X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
>=8X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
Direct Bilirubin			
>=2X ULN	4/79 (5.1)	0/39 (0)	5.1 (-4.1, 12.3)
>=5X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)
GGT			
>=2X ULN	12/79 (15.2)	4/39 (10.3)	4.9 (-9.9, 16.7)
INR			
>=1.5X ULN	2/79 (2.5)	1/39 (2.6)	-0.0 (-10.9, 6.7)
>=3X ULN	0/79 (0)	1/39 (2.6)	-2.6 (-13.2, 2.2)
>=5X ULN	0/79 (0)	0/39 (0)	0.0 (-9.0, 4.7)

Table B: Number and frequency of liver blood test elevations by set cut-offs by Cohorts A and B in Study 10.[51]

Laboratory Abnormality	Cohort A: Inhibitor Fitusiran N=23 n (%)	Cohort B: Non-inhibitor Fitusiran N=46 n (%)
ALT		
>=1X ULN	18/23 (78.3)	41/46 (89.1)
>=3X ULN	5/23 (21.7)	16/46 (34.8)
>=5X ULN	0/23 (0)	5/46 (10.9)
>=10X ULN	0/23 (0)	0/46 (0)
>=20X ULN	0/23 (0)	0/46 (0)
AST		
>=1X ULN	12/23 (52.2)	33/46 (71.7)
>=3X ULN	2/23 (8.7)	4/46 (8.7)
>=5X ULN	1/23 (4.3)	1/46 (2.2)
>=10X ULN	0/23 (0)	0/46 (0)
>=20X ULN	0/23 (0)	0/46 (0)
ALP		
>=2X ULN	0/23 (0)	2/46 (4.3)
>=3X ULN	0/23 (0)	1/46 (2.2)
Total Bilirubin		
>=2X ULN	0/23 (0)	3/46 (6.5)
>=5X ULN	0/23 (0)	0/46 (0)
>=8X ULN	0/23 (0)	0/46 (0)
Direct Bilirubin		
>=2X ULN	1/23 (4.3)	1/46 (2.2)
>=5X ULN	0/23 (0)	0/46 (0)
GGT		
>=2X ULN	5/23 (21.7)	4/46 (8.7)
INR		
>=1.5X ULN	0/23 (0)	0/46 (0)
>=3X ULN	0/23 (0)	0/46 (0)
>=5X ULN	0/23 (0)	0/46 (0)

[50] Made by CDS.
[51] Made by CDS.

Table C: Number and frequency of liver blood test elevations by set cut-offs by Cohorts A and B in Study 74.[52]

Laboratory Abnormality	Inhibitor Fitusiran N=105 n (%)	Non-inhibitor Fitusiran N=176 n (%)
ALT		
>=1X ULN	74/104 (71.2)	130/175 (74.3)
>=3X ULN	9/104 (8.7)	13/175 (7.4)
>=5X ULN	4/104 (3.8)	2/175 (1.1)
>=10X ULN	2/104 (1.9)	1/175 (0.6)
>=20X ULN	0/104 (0)	0/175 (0)
AST		
>=1X ULN	56/104 (53.8)	97/175 (55.4)
>=3X ULN	4/104 (3.8)	6/175 (3.4)
>=5X ULN	1/104 (1.0)	2/175 (1.1)
>=10X ULN	0/104 (0)	1/175 (0.6)
>=20X ULN	0/104 (0)	0/175 (0)
ALP		
>=2X ULN	4/104 (3.8)	6/175 (3.4)
>=3X ULN	0/104 (0)	2/175 (1.1)
Total Bilirubin		
>=2X ULN	5/104 (4.8)	8/175 (4.6)
>=5X ULN	1/104 (1.0)	1/175 (0.6)
>=8X ULN	0/104 (0)	0/175 (0)
Direct Bilirubin		
>=2X ULN	12/104 (11.5)	12/175 (6.9)
>=5X ULN	1/104 (1.0)	1/175 (0.6)
GGT		
>=2X ULN	14/104 (13.5)	26/175 (14.9)
INR		
>=1.5X ULN	2/104 (1.9)	3/175 (1.7)
>=3X ULN	1/104 (1.0)	0/175 (0)
>=5X ULN	0/104 (0)	0/175 (0)

Source: adlb.xpt; Software: R

Table D: Number and frequency of liver blood test elevations for ATDR fitusiran from Study 74 and On-Demand subjects from Studies 68 and 69.[53]

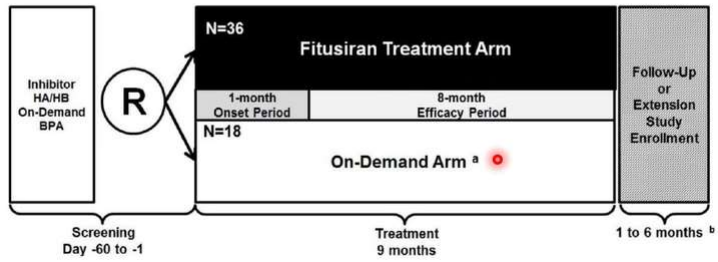
Laboratory Abnormality	Fitusiran N=267 n (%)	On-Demand N=59 n (%)	Risk Difference (%) (95% CI)
ALT			
>=1X ULN	143/266 (53.8)	20/58 (34.5)	19.3 (5.1, 31.9) *
>=3X ULN	9/266 (3.4)	0/58 (0)	3.4 (-2.9, 6.3)
>=5X ULN	4/266 (1.5)	0/58 (0)	1.5 (-4.7, 3.8)
>=10X ULN	3/266 (1.1)	0/58 (0)	1.1 (-5.1, 3.3)
>=20X ULN	0/266 (0)	0/58 (0)	0.0 (-6.2, 1.4)
AST			
>=1X ULN	87/266 (32.7)	11/58 (19.0)	13.7 (0.7, 23.9) *
>=3X ULN	7/266 (2.6)	1/58 (1.7)	0.9 (-6.6, 4.1)
>=5X ULN	3/266 (1.1)	0/58 (0)	1.1 (-5.1, 3.3)
>=10X ULN	1/266 (0.4)	0/58 (0)	0.4 (-5.9, 2.1)
>=20X ULN	0/266 (0)	0/58 (0)	0.0 (-6.2, 1.4)
ALP			
>=2X ULN	7/266 (2.6)	0/58 (0)	2.6 (-3.6, 5.3)
>=3X ULN	2/266 (0.8)	0/58 (0)	0.8 (-5.5, 2.7)
Total Bilirubin			
>=2X ULN	12/266 (4.5)	0/58 (0)	4.5 (-1.8, 7.7)
>=5X ULN	2/266 (0.8)	0/58 (0)	0.8 (-5.5, 2.7)
>=8X ULN	0/266 (0)	0/58 (0)	0.0 (-6.2, 1.4)
Direct Bilirubin			
>=2X ULN	19/266 (7.1)	0/58 (0)	7.1 (0.8, 10.9) *
>=5X ULN	2/266 (0.8)	0/58 (0)	0.8 (-5.5, 2.7)
GGT			
>=2X ULN	28/266 (10.5)	5/58 (8.6)	1.9 (-8.6, 8.6)
INR			
>=1.5X ULN	5/266 (1.9)	1/58 (1.7)	0.2 (-7.3, 3.1)
>=3X ULN	1/266 (0.4)	1/58 (1.7)	-1.3 (-8.8, 0.8)
>=5X ULN	0/266 (0)	0/58 (0)	0.0 (-6.2, 1.4)

Source: adlb.xpt; Software: R

[52] Made by CDS.
[53] Made by CDS.

Safety Design for Trial EFC14768

Figure 1 - Study design



Abbreviations: AT = antithrombin; BPA = bypassing agent; HA/HB = hemophilia A/hemophilia B; R = randomization

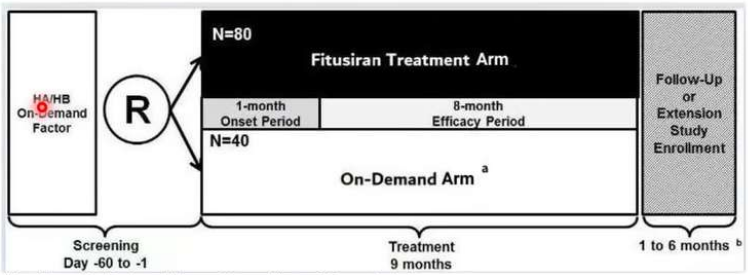
a On-demand local BPA as routinely prescribed by physician per local standard practice.

b For participants in the fitusiran arm who do not enroll in the extension study, the AT activity level was monitored at monthly intervals following the final fitusiran dose until activity levels returned to approximately 60% (per the central laboratory) or per Investigator discretion in consultation with the study Medical Monitor.

Figure A: Schema for Study 68.[54]

Safety Design for Trial EFC14769

Figure 1 - Study design



Abbreviations: HA/HB = hemophilia type A / hemophilia type B; R = randomized

a On-demand factor concentrates as routinely prescribed by physician per local standard practice.

b For participants in the fitusiran prophylaxis arm who do not enroll in the extension study: AT activity level will be monitored at monthly intervals following the final fitusiran dose until activity levels return to approximately 60% (per the central laboratory) or per Investigator discretion in consultation with the study Medical Monitor.

Figure B: Schema for Study 69[55]

Safety Design for Trial EFC15110

Figure 1 - Study design

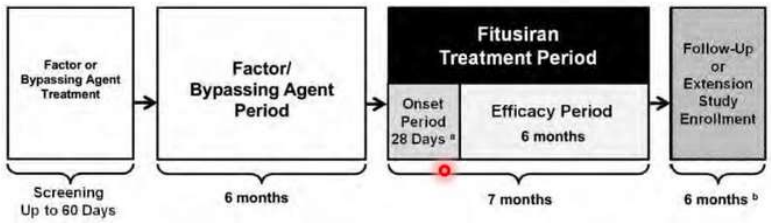


Figure C: Schema for Study 10.[56]

[54] DNH mid-cycle meeting, Aug 28, 2024
[55] DNH mid-cycle meeting, Aug 28, 2024
[56] DNH mid-cycle meeting, Aug 28, 2024

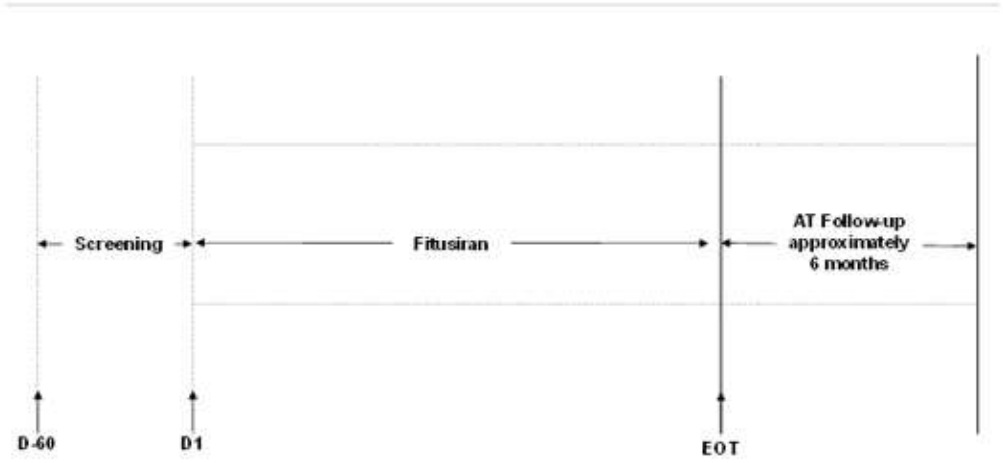


Figure D: Schema for Study 74. As of Jun 14, 2023, there were 281 subjects enrolled, including 227 rollover subjects who completed a parent phase 3 study (57 from Study 68; 61 from Study 69; 54 de novo subjects from China).[57]

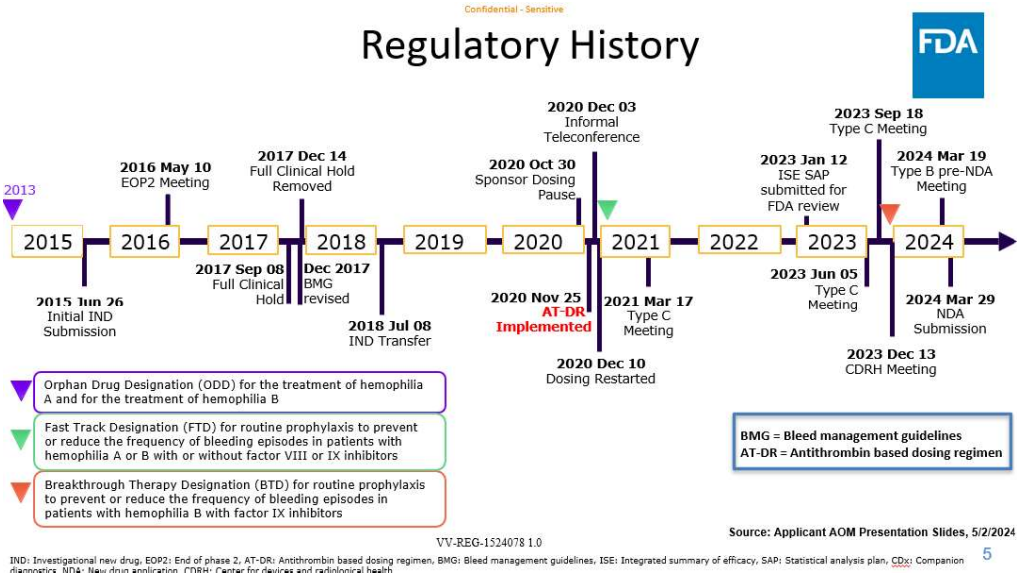


Figure E: Regulatory History by year.

[57] DNH mid-cycle meeting, Aug 28, 2024

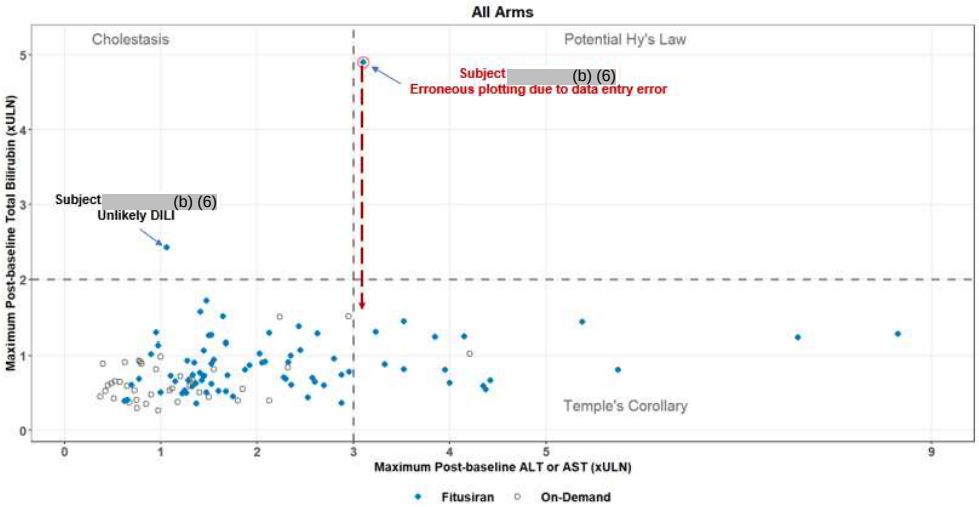


Figure F: eDISH for Study 69.[58]

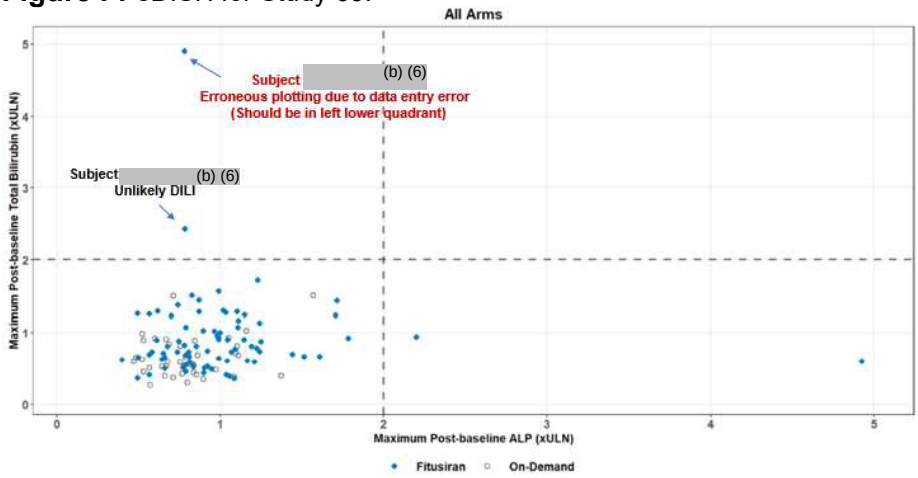


Figure G: eDISH-AP for Study 69 [59]

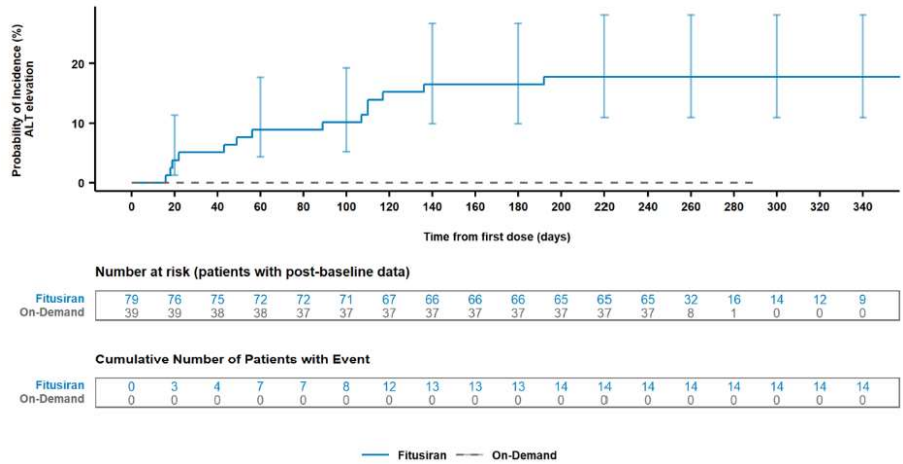


Figure H: Time to onset of ALT ≥ 3x ULN for Safety Population, Study 69.[60]

[58] Made by CDS.
[59] Made by CDS.
[60] Made by CDS

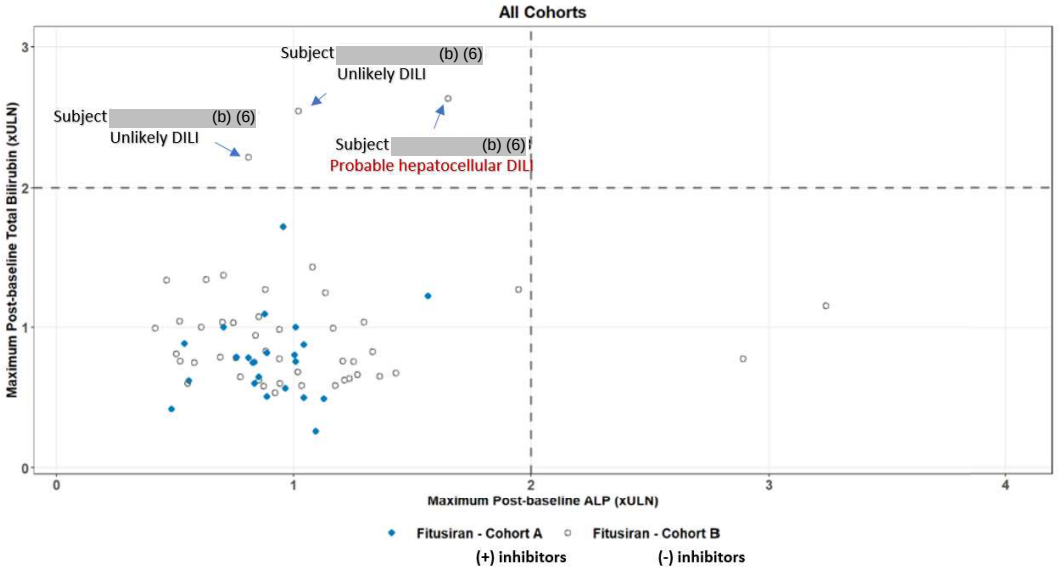


Figure I: eDISH-AP plot for study 10.[61]

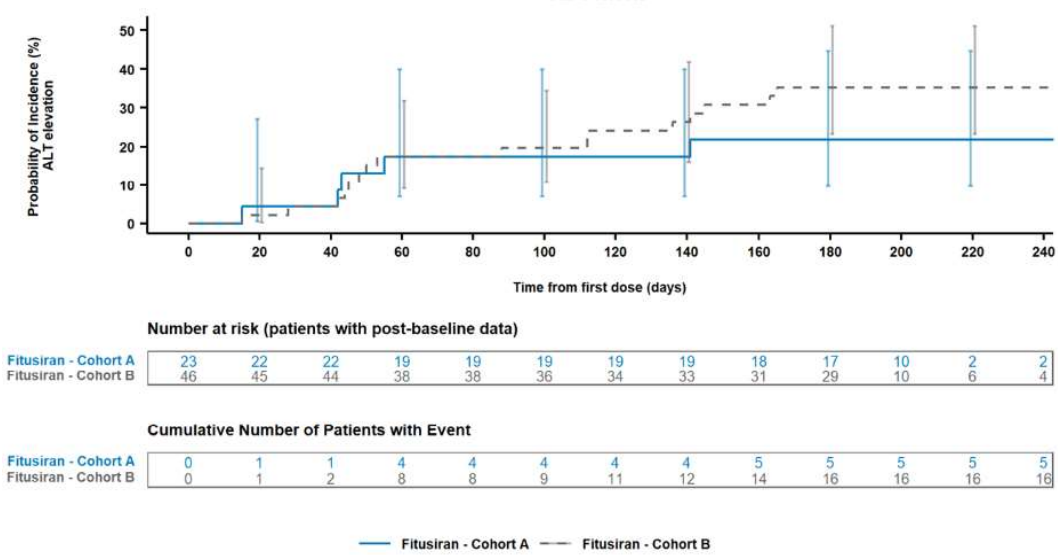
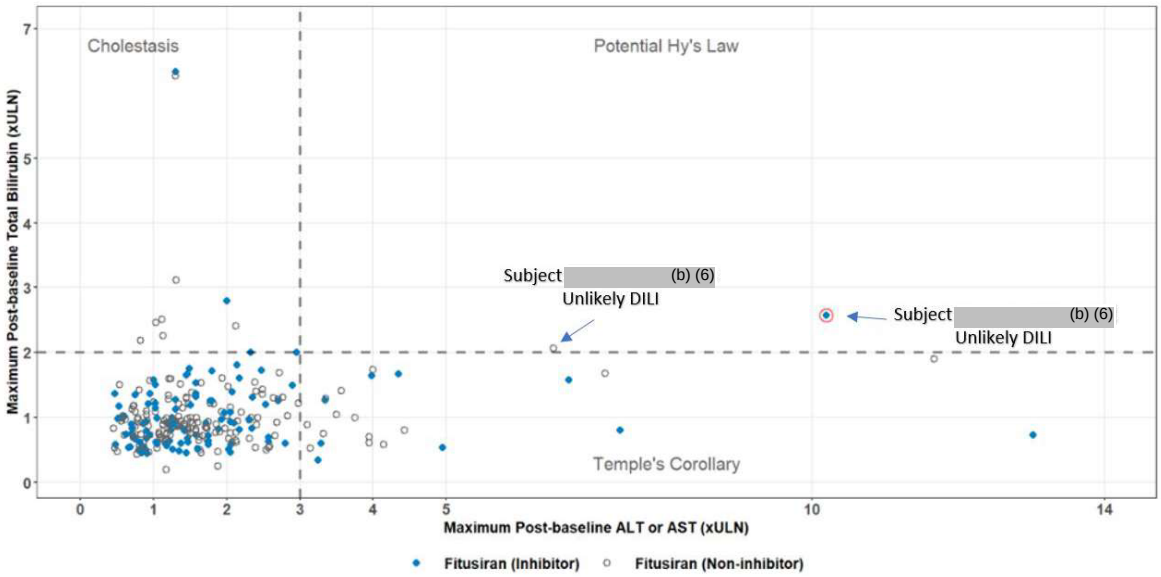


Figure J: Time to onset of ALT ≥ 3x ULN for Safety Population, Study 10.[62]

[61] Made by CDS.
[62] Made by CDS



Quadrant	Inhibitor Fitusiran N=105	Non-inhibitor Fitusiran N=176
Potential Hy's Law (right upper)	1/104 (1)	1/175 (0.6)
Cholestasis (left upper)	4/104 (3.8)	7/175 (4)
Temple's corollary (right lower)	9/104 (8.7)	14/175 (8)
Total	14/104 (13.5)	22/175 (12.6)

Source: adlb.xpt; Software: R
Abbreviations: DILI, drug-induced liver injury

Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALT or AST versus their maximum total bilirubin values in the post-baseline period.
A potential Hy's Law case (red circle) was defined as having any post-baseline total bilirubin equal to or exceeding 2X ULN within 30 days after a post-baseline ALT or AST equal to or exceeding 3X ULN. All patients with at least one post-baseline ALT or AST and bilirubin are plotted.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; TB, total bilirubin; ULN, upper limit of normal

Figure K: eDISH with quadrant counts for Study 74.[63]

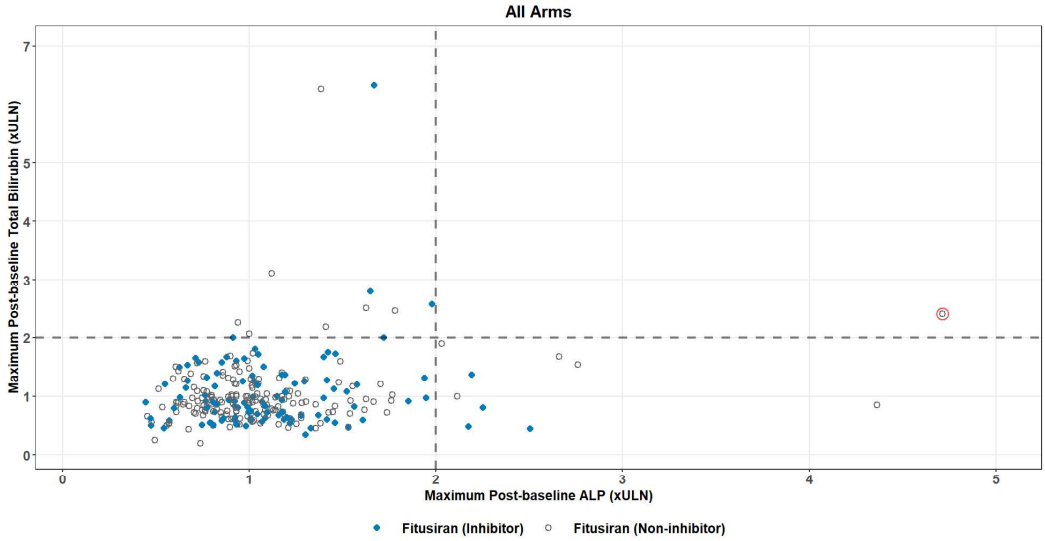


Figure L: eDISH-AP plot for study 74.[64]

[63] Made by CDS.
[64] Made by CDS.

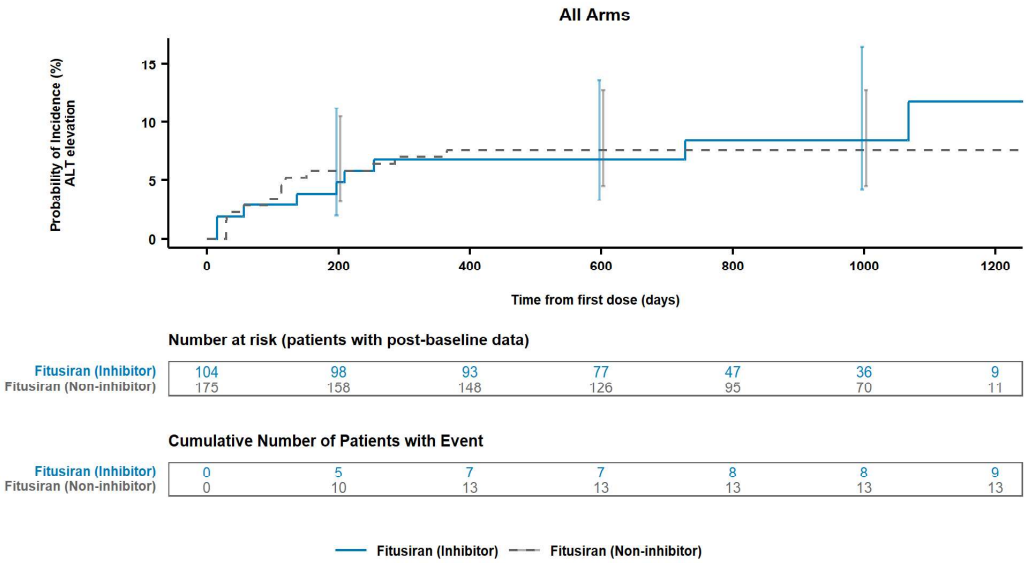


Figure M: Time to onset of ALT ≥ 3x ULN for Safety Population, Study 74.[65]

[65] Made by CDS

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/s/

PAUL H HAYASHI
03/21/2025 06:22:26 PM

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING

Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

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Date of This Review:	March 18, 2025
Requesting Office or Division:	Division of Non-Malignant Hematology (DNH)
Application Type and Number:	NDA 219019
Product Name, Dosage Form, and Strength:	Qfitlia (fitusiran) injection, 50 mg/0.5 mL prefilled pen and 20 mg/0.2 mL single-dose vial
Applicant Name:	Genzyme Corporation, a Sanofi company (Genzyme)
FDA Received Date:	February 14, 2025
TTT ID #:	2024-8888- 2
DMEPA 2 Safety Evaluator:	Shabana Rauf, PharmD
DMEPA 2 Team Leader:	Millie Shah, PharmD, BCPS

1 PURPOSE OF MEMORANDUM

Genzyme Corporation, a Sanofi company (Genzyme) submitted revised container labels and carton labeling received on February 14, 2025 for Qfitlia. The Division of Non-Malignant Hematology (DNH) requested that we review the revised container labels and carton labeling for Qfitlia (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.[a]

2 CONCLUSION

Genzyme Corporation, a Sanofi company (Genzyme) implemented all of our recommendations and updated the expiration date format in the instructions for use (IFU) to be consistent with the expiration date format on carton and container. Therefore, we have no additional recommendations at this time.

[a] Rauf, S. Memorandum Review of Revised Label and Labeling for Qfitlia (NDA 219019). Silver Spring (MD): FDA, CDER, OSE, DMEPA 2 (US); 2025 Jan 22. TTT ID: 2024-8888-1.

APPENDIX A. IMAGES OF LABELS AND LABELING RECEIVED ON FEBRUARY 14, 2025

Instruction for Use can be accessed in EDR via:
\\CDSESUB1\EVSPROD\nda219019\0045\m1\us\proposedpi-20mg.doc

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/s/

SHABANA RAUF
03/18/2025 08:37:55 AM

MILLIE B SHAH
03/18/2025 09:36:34 AM

Consult MEMORANDUM

Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Devices and Radiological Health
Office of Health Technology VII (OHT7)

Date: March 3, 2025

To: Bijal Patel, Regulatory Project Manager
Division of Nonmalignant Hematology (DNH)
Division of Regulatory Operations - Cardiology, Hematology, Endocrinology and Nephrology (DROCHEN)
Office of Regulatory Operations (ORO)
Office of New Drugs (OND)
Center for Drug Evaluation and Research (CDER)

From: Megan Bannister, Reviewer
Hematology Branch (HEMB)
Division of Immunology and Hematology Devices (DIHD)
Office of Health Technology VII (OHT7)
Office of Product Evaluation and Quality (OPEQ)
Center for Devices and Radiological Health (CDRH)

Through: Min Wu, Branch Chief
HEMB/DIHD/OHT7/OPEQ/CDRH

Takeesha Taylor-Bell, Deputy Director
DIHD/OHT7/OPEQ/CDRH

Lea Carrington, Director
DIHD/OHT7/OPEQ/CDRH

Subject: Antithrombin (AT) assay for monitoring NDA 219019

Tracking Number: ICCR # 00988771

ICCR Received: 5/4/2024
ICCR Due: 3/7/2025

Background:
Genzyme Corporation (Genzyme) has developed a first-in-class synthetic small interfering RNA (siRNA) which reduces blood antithrombin (AT) levels and leads to a corresponding increase in thrombin generation thus rebalancing coagulation and reducing the risk of bleeding episodes in patients with hemophilia A or B with or without VIII or IX inhibitors, called QFITLIA (fitusiran).

QFITLIA is filed under NDA 219019. QFITLIA was granted orphan drug designation (ODD) in the U.S. for the treatment of hemophilia A (ODD # 13-4028) and of hemophilia B (ODD # 13-4029) in 2013 and granted Fast Track Designation on December 23, 2020, for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in patients with hemophilia A or B with or without factor VIII or IX inhibitors.

Early in the clinical development program AT levels were measured to understand the pharmacodynamic effect of fitusiran, and subsequently to guide the dosing of fitusiran on a revised AT-based dose regimen. In October 2020, concerns regarding thrombotic events potentially related to fitusiran led Genzyme to voluntarily pause the ongoing clinical studies to evaluate risk factors. As a risk mitigation strategy, an on-therapy AT activity target range between 15% and 35% was implemented with implications that fitusiran dosing would be modified to maintain AT levels within the target range. Genzyme and CDER concluded that QFITLIA requires monitoring of the patient's AT levels to maintain the AT activity between 15–35% AT. Dose adjustments are made based on most recent AT levels.

During the fitusiran clinical trials, AT levels were tested using the INNOVANCE Antithrombin assay (K081769) on the BCS-XP system and (b) (4) . However, based on the field studies conducted with several AT assays, Genzyme determined that a human FXa-based chromogenic antithrombin activity assay (i.e., Siemens INNOVANCE Antithrombin) is recommended to monitor fitusiran. The Intended Use/Indications for Use statement for the Siemens INNOVANCE Antithrombin assay indicates that it is used for the "*quantification of functionally active antithrombin in human citrated plasma and can be used as an aid in the diagnosis of antithrombin deficiency."* INNOVANCE Antithrombin is not intended to be used for the monitoring of QFITLIA. On September 25, 2024, Siemens Healthcare Diagnostic Products GmbH submitted a 510(k) to expand the intended use to include the monitoring of AT activity to support fitusiran dosing for the INNOVANCE Antithrombin assay (K242952).

Indications for Use for QFITLIA (fitusiran) in NDA 219019:

QFITLIA is a an antithrombin-directed small interfering ribonucleic acid indicated for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and pediatric patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors.

Device Description:
AT is a main inhibitor of enzymatic activity in the coagulation processes and is synthesized in liver cells. This serine protease inhibitor irreversibly inhibits different coagulation factors, with thrombin

and factor Xa being the primary target enzymes. The inhibition of thrombin and factor Xa by antithrombin is accelerated approximately 1,000-fold in the presence of heparin.

The INNOVANCE Antithrombin assay is suitable for the determination of physiologically active antithrombin on automatic analyzers and enables the diagnosis of inherited or acquired antithrombin deficiencies. A genetically caused antithrombin deficiency leads to reduced antithrombin activity with reduced protein concentration (type I deficiency) or normal protein concentration (type II deficiency). Acquired antithrombin deficiency occurs as a result of reduced synthesis, increased protein consumption, or as a result of protein loss in conditions such as DIC (disseminated intravascular coagulation), sepsis, acute hemolytic transfusion reaction, increased protein loss in nephrotic syndrome, thrombotic microangiopathies, malignant diseases, acute thrombotic episodes and asparaginase therapy. The INNOVANCE Antithrombin assay enables the detection of patients with antithrombin deficiency.

The INNOVANCE Antithrombin assay utilizes a chromogenic measuring principle. An excess of human factor Xa is added to citrated plasma. In the presence of heparin, a portion of the enzyme is complexed and inactivated by the antithrombin present in the sample. Excess, uninhibited factor Xa then cleaves a specific chromogenic substrate, causing the release of a dye. The rate of the substrate cleavage is determined by the increase in the absorbance value at 405 nm.

Scope:
CDER has requested CDRH to:

- Provide the regulatory status of the CDx antithrombin (AT) assay [t]o be used with fitusiran;
- Confirm the adequacy of the Applicant's proposal to (b) (4)
- Confirm the adequacy of the Applicant's proposal to (b) (4)
- Concurrence on (b) (4).

Regulatory Status:
As of February 25, 2025, the premarket submission for the Siemens INNOVANCE Antithrombin assay (K242952) is currently on hold. A deficiency letter requesting additional information was issued on November 22, 2024. In the deficiency letter, CDRH requested that the sponsor perform additional precision studies using multiple reagent lots to determine the true within-laboratory precision and total reproducibility of the assay because reagent lots are a common source of variation encountered in clinical laboratories. Without the results of a reproducibility study that includes multiple reagent lots, we are unable to determine that the INNOVANCE Antithrombin is a reliable test to monitor fitusiran. CDRH also requested that the sponsor perform a limit of quantitation (LoQ) study to determine the lowest amount of antithrombin activity that can be quantitatively determined with acceptable accuracy. A LoQ study will adequately characterize performance in the low-end region of the measuring interval (10–35% AT activity) to ensure patient safety and prevent patient harm as patients on fitusiran are considered to be at risk of a vascular thrombotic event with prolonged exposure to AT activity levels less than 10%. Notably, both analytical studies mentioned above (i.e., precision and LoQ) are common/routine studies

performed by manufacturers in order to validate and characterize the performance of quantitative assays.

On February 28, 2025, CDRH received a supplement to the Traditional 510(k) and it is currently under review. As of the date of this memorandum, CDRH has 29 days remaining in the review cycle and the due date is April 1, 2025, which can be modified to an earlier date to align with the PDUFA action date for QFITLIA.

(b) (4)

(b) (4) This comment was discussed with Siemens, Genzyme, and CDER during pre-submission (Q232462) teleconference held on December 13, 2023. CDRH expects a contemporaneous authorization of the INNOVANCE Antithrombin assay with the approval of QFITLIA.

QFITLIA Labeling:

(b) (4)

CDRH agrees with CDER that INNOVANCE Antithrombin assay is considered a companion diagnostic device for QFITLIA and therefore the use of the FDA-authorized CDx device should be included in QFITLIA labeling. CDRH has provided CDER with the most current thinking regarding wording to describe the companion diagnostic device in the QFITLIA package insert. This language includes the use of 'FDA-authorized' to describe the INNOVANCE Antithrombin assay as it is meant to support and cover all regulatory pathways that future, potential companion diagnostic tests to Fitusiran may utilize.

If you have any questions or comments regarding this review, please email me at Megan.Bannister@fda.hhs.gov.

Sincerely,

MEGAN BANNISTER -S

Digitally signed by MEGAN BANNISTER -S
Date: 2025.03.03 11:41:15 -05'00'

Megan Bannister
Reviewer
Division of Immunology and Hematology Devices
OHT7: Office of In Vitro Diagnostics Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Through

Takeesha Taylor-bell -S

2025.03.03 12:05:48 -05'00'

for Lea Carrington
Director
Division of Immunology and Hematology Devices
OHT7: Office of In Vitro Diagnostics Office of Product Evaluation and Quality
Center for Devices and Radiological Health

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/s/

BIJAL R PATEL
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MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING

Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

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Date of This Review:	02/12/2025
Requesting Office or Division:	Division of Non-Malignant Hematology (DNH)
Application Type and Number:	NDA 219019
Product Name, Dosage Form, and Strength:	Qfitlia (fitusiran) injection, 50 mg/0.5 mL prefilled pen and 20 mg/0.2 mL single-dose vial
Applicant Name:	Genzyme Corporation, a Sanofi company (Genzyme)
FDA Received Date:	January 29, 2025
TTT ID #:	2024-8888- 1
DMEPA 2 Safety Evaluator:	Shabana Rauf, PharmD
DMEPA 2 Team Leader:	Millie Shah, PharmD, BCPS

1 PURPOSE OF MEMORANDUM

Genzyme Corporation, a Sanofi company (Genzyme) submitted revised container labels and carton labeling received on January 29, 2025 for Qfitlia. The Division of Non-Malignant Hematology (DNH) requested that we review the revised container labels and carton labeling for Qfitlia (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.[a]

2 CONCLUSION

The container labels and carton labeling are unacceptable from a medication error perspective. Upon our review of the revised IFU, we identified an inconsistency between the expiration date format in the images of the container labels and carton labeling and actual container labels and carton labeling.

3 RECOMMENDATIONS FOR GENZYME CORPORATION, A SANOFI COMPANY (GENZYME)

A. Container Labels and Carton Labeling for Single-Dose Vial (SDV) and Prefilled Pen (PFP)

The expiration date format in the IFU in Steps A3 (for the PFP) and Step A2 (SVD) is (b) (4), which is inconsistent with the actual expiration date format (i.e., YYYY-MM) on the container labels and carton labeling. Inconsistent formats of the expiration date may result in confusion and risk for deteriorated drug medication errors. We acknowledge your proposal to keep the expiration date format in the IFU as (b) (4) per FDA Guidance Product Identifiers Under the Drug Supply Chain Security Act Questions and Answers Guidance for Industry. Your response states, "The applicant will revise the 50 mg prefilled pen (PFP) and 20 mg single-dose vial carton labeling expiration date format during the next submission." Please submit the revised container labels and carton labeling for the SDV and PFP with the revised expiration date format.

3 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

[a] Rauf, S. Human Factors Study Report and Label and Labeling Review for Qfitlia (NDA 219019). Silver Spring (MD): FDA, CDER, OSE, DMEPA 2 (US); 2025 Jan 22. TTT ID: 2024-8888; 2024-9027.

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MILLIE B SHAH
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COA Tracking ID: C2024184
NDA Number: 219019/Referenced IND for NDA: 125632

CLINICAL OUTCOME ASSESSMENT (COA) CONSULT REVIEW

COA Tracking ID:	C2024184
NDA#/Referenced IND for NDA:	NDA 219019/ IND 125632
Applicant:	Genzyme Corporation- A Sanofi Company
Established Name/Trade Name:	Qfitlia / Fitusiran Solution for Injection
Indication:	Routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors. ☒Rare Disease/Orphan Designation ☒Pediatrics
PDUFA Goal Date:	03/28/2025
Review Division:	Nonmalignant Hematology
Clinical Reviewer(s):	Donna Whyte-Stewart (Efficacy) Alison Moliterno (Safety)
Clinical Team Leader (TL)	Carrie Diamond
Regulatory Project Manager:	Bijal Patel
COA Reviewer:	Daphney Jean, PhD
COA TL/ Deputy Director:	Selena Daniels, PharmD, PhD
COA Division Director:	David Reasner, Ph.D.
Instruments reviewed:	1. Hemophilia Quality of Life Questionnaire for Adults (Haem-A-QoL)- Physical Health domain score ☒ Patient-reported outcome (PRO)

Table of Contents

1. EXECUTIVE SUMMARY

In this submission, the Applicant is seeking approval of fitusiran for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients (≥12 years old) patients with hemophilia A or B with or without factor VIII or IX inhibitors. (b) (4)

(b) (4)

The secondary efficacy patient-reported outcome (PRO) endpoint (b) (4):

- Change from baseline in Hemophilia Quality of Life Questionnaire for Adults (Haem-A-QoL) Physical Health domain score to Month 9 in the treatment period (Studies EFC14768 and EFC14769; referred to as the parent trials)
- Change from baseline in Haem-A-QoL Physical Health domain score to Month 9 in the treatment period (Study LTE15174)

The data from Studies EFC14768 and EFC14769 demonstrated that fitusiran had statistically significant improvement in the selected secondary efficacy endpoints compared to the on-demand arm[1].

From a COA perspective, the Haem-A-QoL Physical Health domain score and its corresponding endpoint does not adequately support (b) (4) the comparison between antithrombin dose regimen (AT-DR)[2] data (change from baseline to the end of AT-DR treatment) with control data (change from baseline to the parent study) due to issues related to the study design and data interpretability (refer to the Key Issues Identified section).

2 REVIEW CONCLUSIONS

The Haem-A-QoL Physical Health domain[3] has previously been determined to appear fit-for-purpose to assess patient-reported hemophilia symptoms (swellings and joint pain) and physical functioning in the target population. As such, this instrument was not reviewed for content validity and the other measurement properties as those have already been established; therefore, these sections are not included in this review. The focus of this review was on the score interpretability of the Haem-A-QoL Physical Health domain score. While the Haem-A-QoL Physical Health domain score may be fit-for-purpose for this target population, it is inadequate (b) (4) (b) (4) due to several limitations related to study design and data interpretability (refer to the Key Issues Identified section).

[1] On-demand bypassing agent (BPA) for treatment of breakthrough bleeding episodes.
[2] Due to safety concerns with excess events of thrombosis, the original dosing regimen (fitusiran 80mg) was modified to an antithrombin dose regimen (AT-DR).
[3] Data from the Haem-A-QoL Physical Health domain has been previously labeled with HEMLIBRA® for HAVEN 1 study. The Physical Health domain was a multiplicity-adjusted secondary endpoint that reached statistical significance.

Review Summary

- The Haem-A-QoL Physical Health appears fit-for-purpose to assess patient-reported hemophilia symptoms (swellings and joint pain) and physical functioning in the target patient population.
- Review of the distribution of responses for the Haem-A-QoL Physical Health domain at the item-level by study visit showed that all items were generally moving in a positive direction (higher proportion of patients were responding to a least severe category from where they started at baseline). The distribution of responses also showed that improved patients were moving from one category to another despite the use of transformed scores.
- The threshold for meaningful change is unknown; however, the change from baseline in the Haem-A-QoL Physical Health domain score to Month 9 showed a clear separation between the treatment (fitusiran 80mg) and on-demand arm across a range that likely includes a clinically meaningful change threshold for Studies EFC14768 and EFC14769.
- It is difficult to compare fitusiran 80mg to the fitusiran AT-based dose regimen due to the study design (refer to the Key Issues Identified section).

Key Issues Identified

Issue #1: Data Interpretability

- The open-label trial design limits interpretability of COA data because patient's knowledge of treatment assignment may lead to systematic overestimation or underestimation of treatment effect, the magnitude of which is currently unknown. In settings where blinding is not feasible, or there is high likelihood of inadvertent unblinding due to toxicity, lack of blinding will need to be overcome by demonstrating a large and durable magnitude of effect in the setting of strict adherence to a carefully conducted clinical trial.
- (b) (4) not based on the pre-specified alpha-controlled secondary efficacy endpoint, but a post-hoc analysis that compared the fitusiran AT-based dose regimen with on-demand standard of care (SOC).
- The fitusiran AT-based dosing regimen does not have any true baseline values (i.e., the transition from fitusiran 80mg to the AT-based dosing regimen consisted of a variable period that is individualized.), as such it is difficult to compare the data between fitusiran 80mg and the fitusiran AT-based dose regimen from an efficacy standpoint. Further, there is no matching control for the fitusiran AT-based dosed regimen in the primary efficacy period.
- The analysis result is not robust against different assumptions for missing data per discussion with Biostatistics.

3 RECOMMENDATIONS FOR FUTURE STUDIES

For future clinical trials in this indication, sponsors should consider the following:

- Sponsors should engage with FDA early (e.g., Pre-IND) and throughout drug development to discuss their COA measurement strategy to ensure the selected instruments are fit-for-purpose and the studies are designed appropriately for the context of use prior to initiation of pivotal studies.

- [redacted] (b) (4) (b) (4) we recommend a clear endpoint definition and formal statistical testing with adjustment for multiplicity.

4 BACKGROUND AND CORRESPONDENCE ON CLINICAL OUTCOME ASSESSMENT(S)

Regulatory Background:

- The Applicant received Orphan Drug Designation for fitusiran on August 6, 2013 (treatment of hemophilia A), and August 12, 2013 (treatment of hemophilia B).

- Fitusiran was granted Breakthrough Therapy Designation from FDA for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in patients with hemophilia B with factor IX inhibitors on December 6, 2023.

- There has been previous communication related to the Applicant's COA measurement strategy, which includes the following:

    - Type C Meeting Minutes (IND 125632) dated October 12, 2023:
        - Identified the limitations of open-label study design as it relates to the interpretability patient reported outcome (PRO) data.
        - Noted concerns that the time lag between the parent studies (i.e., study EFC14768, EFC14769 and EFC15110) and the extension study LTE15174 could limit the interpretation of the PRO data.

Reviewer comment (s): *The comments and recommendations provided in this correspondence were from the review division.*

Previous COA Reviews:

- C2023248_IND125632_Chung, dated August 10, 2023 (DARRTS Reference ID:5224227)

Disease Background:

Hemophilia A and hemophilia B are X-linked recessive inherited bleeding disorders, characterized by deficiency of coagulation Factor VIII (FVIII) or Factor IX (FIX), leading to a profound defect of thrombin generation with impaired hemostasis and increased risk of bleeding. Patients with mild hemophilia typically experience bleeding after a serious injury or surgery; patients with moderate hemophilia experience bleeding episodes associated with injuries, and may have spontaneous bleeding episodes; severe hemophilia patients experience substantial bleeding with injury and may have frequent spontaneous bleeding episodes resulting in debilitating musculoskeletal damage that can markedly impair a patient's mobility and adversely affect various aspects of life.

Investigational Product:

Fitusiran is a triantennary N-acetyl galactosamine- small interfering ribonucleic acid (GalNAc-siRNA) conjugate that reduces production of AT, leading to lower plasma AT levels. By reducing plasma AT, fitusiran is designed to improve thrombin generation and hemostasis in individuals

with hemophilia, regardless of hemophilia type or presence of inhibitory antibodies to factor VIII or IX.

Materials reviewed:

(b) (4) the Applicant submitted a COA evidence dossier ("Use of Haem-A-QoL Physical Health Domain for the Evaluation of Physical Health in People With Hemophilia A or B, with or without inhibitors: A Clinical Outcome Assessment (COA) Evidence Dossier (b) (4) dated 12 December 2023; Final version)

5 CLINICAL OUTCOME ASSESSMENT REVIEW

5.1 Clinical Trial Population

The target population for Studies EFC14768 and EFC14769 were males aged >12 years with severe Hemophilia A or B[4] with and without inhibitors, respectively.

The target population for Study LTE15174 was participants with severe hemophilia A or B who had completed a Phase 3 fitusiran clinical trial.

A complete list of the inclusion and exclusion criteria is summarized in sections 5.1 and 5.2 of the clinical study protocol for Studies EFC14768, EFC14769, and LTE15174**.**

5.2 Clinical Trial Design

The Applicant conducted three phase 3 studies, but this review focused on the following two phase 3 studies:

- Study EFC14768 is a multicenter, multinational, randomized, open-label phase 3 study designed to evaluate the efficacy and safety of fitusiran in male patients aged ≥12 years with hemophilia A or B, with inhibitory antibodies to FVIII or FIX, who are not receiving prophylactic therapy.

  Eligible participants were randomized in a 2:1 ratio to:
  - Fitusiran treatment arm: Fitusiran 80 mg administered subcutaneously (SC) as prophylaxis once monthly, with use of on-demand bypassing agent for treatment of breakthrough bleeding episodes
  - On-demand arm: On-demand bypassing agent for treatment of breakthrough bleeding episodes

- Study EFC14769 is a multicenter, multinational, randomized, open-label phase 3 study designed to evaluate the efficacy and safety of fitusiran in male patients aged ≥12 years with hemophilia A or B, without inhibitory antibodies to FVIII or FIX, who are not receiving prophylactic therapy.

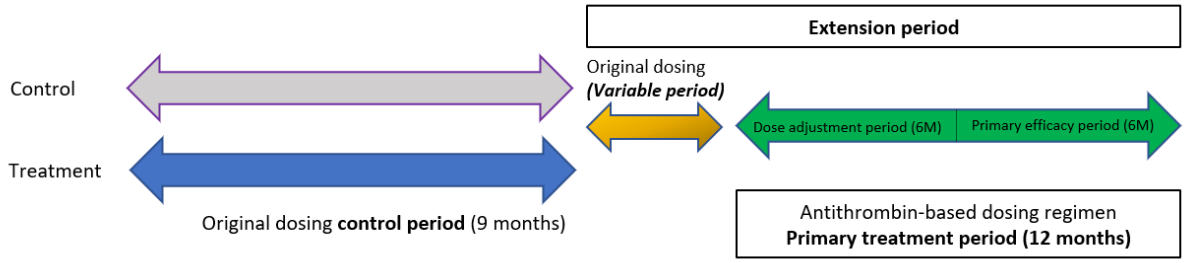
[4] Evidenced by (1) A central laboratory measurement or documented medical record evidence of FVIII <1% or FIX level ≤2% at Screening and (2) On-demand use of bypassing agents to manage bleeding episodes for at least the last 6 months prior to Screening, and meet one of the Nijmegen-modified Bethesda assay results criteria (listed in Section 5.1 in Clinical Study Protocol ALN-AT3SC-003 and Clinical Study Protocol ALN-AT3SC-004.

Eligible participants were randomized in a 2:1 ratio to:

- Fitusiran treatment arm: Fitusiran 80 mg administered SC as prophylaxis once monthly with use of on-demand factor concentrates for treatment of breakthrough bleeding episodes
- On-demand arm: On-demand factor concentrates for treatment of breakthrough bleeding episodes

For both Studies EFC14768 and EFC14769, all participants were treated for a total of 9 months[5]; patients randomized to the fitusiran treatment arm received a total of 9 SC injections of fitusiran. The schema for the study design for Studies EFC14768 and EFC14769 is shown in Figure 1.

Figure 1. Schema for Studies EFC14768 and EFC14769



The Applicant also conducted the following long-term extension study:

- Study LTE151746 is a multicenter, multinational, open-label extension study of the long-term safety and efficacy of fitusiran in males ≥12 years of age with hemophilia A or B with or without inhibitory antibodies to FVIII or FIX.

The study consists of a screening period of up to 30 days, a 48-month open-label treatment period, and an up to 6-month follow-up period after the last dose of fitusiran. The duration of treatment is up to 48 months or until fitusiran becomes commercially available, whichever comes first.

5.3 Endpoint Position, Definition, and Assessment Schedule

The placement of the COAs in the endpoint hierarchy, including the endpoint definition for Studies EFC14768, EFC14769, and LTE15174 is summarized below.

<u>Primary efficacy endpoint</u>

- Annualized Bleeding Rate (ABR) in the treatment period (EFC14768, EFC14769)
- Incidence, severity, relatedness, and seriousness of AEs, and laboratory assessments (LTE15174)

[5] Because the full pharmacodynamic (PD) effect of fitusiran is not achieved until approximately 28 days after receiving the first dose, efficacy will be assessed during the final 8 months on study (Day 29 to Month 9).
[6] Participants from all three phase 3 studies may be considered for enrollment.

Secondary efficacy COA endpoint(s) (multiplicity-adjusted[7])

- Change from baseline in Haem-A-QoL Physical Health domain score in the treatment period (EFC14768, EFC14769, LTE15174)
- Change from baseline in Haem-A-QoL total score in the treatment period (EFC14768, EFC14769, LTE15174)

(b) (4)

[redacted]

[7] The secondary efficacy COA endpoints were adjusted for multiplicity for Studies EFC14768 and EFC14769 only.

(b) (4)

5.5 Clinical Outcome Assessment(s)

5.5.1 Clinical Outcome Assessment Description(s)

5.5.1.1 Hemophilia Quality of Life Questionnaire for Adults (Haem-A-QoL) Physical Health domain

The Haem-A-QoL Physical Health domain is a 5-item domain in the 46-item Haem-A-Qol, a PRO instrument. The Physical Health domain is designed to assess patient-reported hemophilia-related symptoms (painful swellings and presence of joint pain) and physical functioning (pain with movement and difficulty walking far). Each item is rated on a 5-point verbal rating scale ranging from "Never" to "All the time." The Haem-A-QoL was administered by paper at baseline, and month 9[8]. The recall period for the instrument is the previous 4 weeks. For copy of the instrument see Appendix A Hemophilia Quality of Life Questionnaire for Adults Physical Health Domain.

5.5.2 Conceptual Framework(s)

The conceptual framework for the Haem-A-QoL Physical Health domain is shown in Table 1.

Table 1. Conceptual Framework of Haem-A-QoL Physical Health domain

Items ("In the past 4 weeks…")	Subdomain
1. …my swellings hurt 2. …I had pain in my joints 3. …it was painful for me to move 4. …I had difficulty walking as far as I wanted to 5. …I needed more time to get ready because of my condition	Physical Health

5.5.3 Scoring Algorithm

5.5.3.1 Haem-A-QoL Physical Health domain

The Haem-A-QoL Physical Health domain score is derived by summing the five items into a raw score, which ranges from 1-25. The raw scores are transformed to a normalized score using the formula below, which ranges from 0-100 where higher scores indicate greater impairment or poorer health-related quality of life.

$$\text{Transformed Score} = \frac{[(\text{Actual raw score} - \text{lowest possible raw score})}{(\text{Possible raw score range})]} * 100$$

[8] The administration schedule in LTE15174 was at baseline, month 12, month 24, month 36, and month 48.

The transformed score for a domain is calculated based on the number of items answered. The score is considered to be missing if >50% of item scores are missing.

Reviewer's comment(s): *The use of transformed scores often can be misleading. A 1-category change in a single item equates to about 6.25 points on a 0-100 scale.*

5.5.4 Item-level descriptives

While this review did not focus on the other measurement properties of the Haem-A-QoL Physical Health domain, the distribution of responses for the Haem-A-QoL Physical Health domain at the item-level were reviewed by study visit. An information request (IR) was submitted to the Applicant on October 30, 2024 requesting item-level descriptives. The results are presented in Tables 2a and 2b.

Table 2a- Summary of median Haem-A-QoL Physical Health domain scores (raw) at item-level and domain-level at baseline and Month 9 in Study EFC14768.

	BPA on-demand (N=18)			Fitusiran (N=32)		
	Baseline (N=18)	Month 9 (N=17)	**Change from Baseline to Day 253**	Baseline (N=32)	Month 9 (N=32)	**Change from Baseline to Day 253**
My swellings hurt	4.0	3.0	**0**	3.0	1.0	**-1**
I had pain in my joints	3.0	3.0	**0**	3.0	2.0	**-1**
It was painful for me to move	4.0	3.0	**-1**	3.0	1.0	**-1**
I had difficulty walking as far as I wanted to	4.0	3.0	**0**	3.0	2.0	**-1**
I needed more time to get ready because of my condition	3.0	3.0	**0**	3.0	1.0	**-1**
Physical Health domain score	3.6	3.2	**-.4**	2.9	1.6	**-1.4**

Source: FDA Reviewer's Table

Table 2b. Summary of median Haem-A-QoL Physical Health domain scores (raw) at item-level and domain-level at baseline and Month 9 in Study EFC14769.

	BPA on-demand (N=38)			Fitusiran (N=72)		
	Baseline (N=37)	Month 9 (N=34)	Change from Baseline to Day 253	Baseline (N=71)	Month 9 (N=69)	Change from Baseline to Day 253
My swellings hurt	3.0	3.0	**0**	3.0	1.0	**-1**
I had pain in my joints	4.0	3.0	**0**	3.0	2.0	**-1**
It was painful for me to move	3.0	3.0	**0**	3.0	2.0	**-1**
I had difficulty walking as far as I wanted to	3.0	3.0	**0**	3.0	2.0	**-1**
I needed more time to get ready because of my condition	2.0	2.0	**0**	3.0	1.0	**-1**
Physical Health domain score	3.0	3.0	**0**	3.0	1.8	**-1**

Source: FDA Reviewer's Table

Reviewer's comment(s):
Within the IR dated October 30, 2024, the following was requested:

1. *Baseline Haem-A-QoL item and Physical Health domain scores on raw score scale along with item distributions by response categories for both EFC14768, and EFC14769*
    - *Takeaway from the Applicant's response: Most patients were symptomatic at baseline.*
2. *Item-level descriptive statistics for the Haem-A-QoL Physical Health domain scores and change from baseline in the Haem-A-QoL Physical Health domain scores by treatment arm and by each study visit.*
    - *The median change from baseline to month 9 was -.4 in the BPA arm compared to -1.4 in the fitusiran arm of Study EFC14768.*
    - *The median change from baseline to month 9 was 0 in the BPA arm compared to -1.0 in the fitusiran arm of Study EFC14769.*
    - *Takeaway from the Applicant's response: most items contributed equally to observed change in the domain score in both studies.*
3. *Completion rates for the Haem-A-QoL Physical Health domain in EFC14768, and EFC14769*
    - *Key takeaway from the Applicant's response: Data quality appears reasonable.*
4. *Line graph of the mean Haem-A-QOL Physical Health domain score and mean change from baseline by treatment arm.*
    - *Key take away from the Applicant's response: Change is observed from baseline to Month 9. However, the number of data collection points was limited.*

5.5.5 Interpretation of Meaningful Within-Patient Score Changes

5.5.5.1 Haem-A-QoL Physical Health domain

The Applicant proposed that a 10 to 15 point reduction (improvement) on a transformed 0-100 scale) represents a clinically meaningful within-patient score change in the Haem-A-QoL Physical Health domain score**.**

To support this threshold of clinically meaningful within patient change, the Applicant performed the following analyses using data from LTE15174:

- Anchor-based analyses
  - Anchor-based empirical cumulative distribution function (eCDF)
- Distribution-based analysis

The Applicant utilized the following anchors:

- EuroQol 5 dimensions 5 level questionnaire (EQ-5D-5L) Pain/Discomfort dimension
  The Pain/Discomfort dimension of the 6-item EQ-5D-5L, a generic health status measure is designed to assess the severity of pain/discomfort using a 5-point VRS ranging from "I have no pain or discomfort" to "I have extreme pain or discomfort." The recall period is momentary ("today"). The EQ-5D-5L was administered at baseline, month 9, month 12, month 24, month 36, and month 48. However, data collected at month 12 were used for the anchor based analysis.

- EQ-5D-5L Usual Activities dimension
  The Usual Activities dimension of the 6-item EQ-5D-5L is designed to assess problems with doing usual activities using a 5-point VRS ranging from "I have no problems doing my usual activities" to "I am unable to do my usual activities." The recall period is momentary ("today"). The EQ-5D-5L was administered at baseline, month 9, month 12, month 24, month 36, and month 48. However, data collected at month 12 were used for the anchor based analysis.

- EQ-5D-5L Mobility dimension
  The Mobility dimension of the 6-item EQ-5D-5L is designed to assess problems with walking about using a 5-point VRS ranging from "I have no problems in walking about" to "I am unable to walk about." The recall period is momentary ("today"). The EQ-5D-5L was administered at baseline, month 9, month 12, month 24, month 36, and month 48.

- Treatment Satisfaction Questionnaire for Medication version 9 (TSQM-9) item 2
  Item 2 of the TSQM-9 is a single item within the TSQM-9, a 9-item PRO measure. Item 2 is designed to assess satisfaction with symptom relief from the medication using a 7 point VRS ranging from 1 ("Extremely dissatisfied") to 7 ("Extremely satisfied"). The recall period varies from last two to three weeks or since the last time the medication was used. The instrument was administered at baseline, month 9, month 12, month 24, month 36, and month 48. However, data collected at month 12 were used for the anchor based analysis.

Copies of the anchor scales are in Appendix B: EuroQoL-5 Dimensions-5 Level Questionnaire (EQ-5D-5L) and Appendix C: Treatment Satisfaction Questionnaire for Medication (TSQM-9) Item 2**.**

Reviewer's comment (s):

- *This reviewer noted the following limitations with the anchors*
    - *The concepts measured in some of the anchor scales do not completely align with the concepts measured in the Haem-A-QoL Physical Health domain. Selected anchor scales should be associated with the target COA endpoint in a way that addresses the question of clinical meaningfulness of the target COA endpoint.*
    - *The recall period of the anchors is not consistent with the assessment time period of the Haem-A-QoL Physical Health endpoint.*
    - *The time point used for the anchor based analysis (Month 12) does not align with the time point of the pre-specified endpoint (Month 9).*
    - *The correlation for EQ-5D-5L mobility with the Haem-A-QoL Physical Health score was low (r= 0.218). The sponsor relied only on the EQ-5D-5L Pain/Discomfort and Usual activities dimensions to derive meaningful change thresholds for the Haem-A-QoL Physical Health domain.*
    - *The response categories of the EQ-5D-5L may be potentially indistinguishable to patients. The anchor scale's response categories should be distinct and non-overlapping and should represent meaningful differences among adjacent response categories.*

Due to these limitations, an IR was submitted on October 30, 2024 requesting eCDF curves by treatment arm for the COA-based endpoint change scores. Initially the Applicant provided line plots instead of the step function plot. As such, a new IR was sent on November 22, 2024 requesting updated eCDF figures.

Key takeaway from the Applicant's response: there is clear separation of the mean change Haem-A-QoL Physical Health domain score from baseline to Month 9 between treatment arms indicative of treatment benefit in both Studies EFC14768 and EFC14769 (see Figures 2a-2d). However, the range of clinically meaningful change score(s) is unclear.

Figure 2a. Empirical Cumulative Distribution Function: Mean Change Haem-A-QoL Physical Health Domain Score From Baseline to Month 9 by Treatment Arm for Study EFC14768.

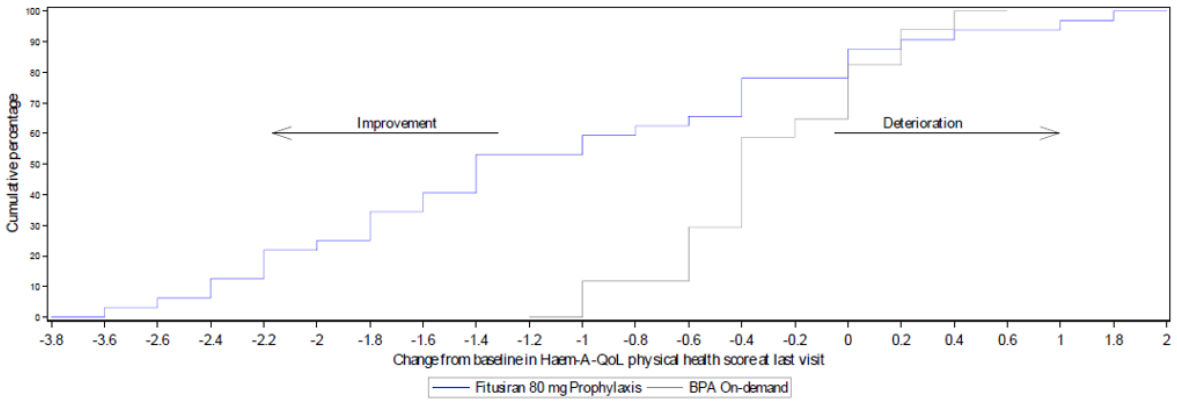


Figure 2b. Empirical Cumulative Distribution Function: Mean Change Haem-A-QoL Physical Health Domain Score From Baseline to Month 9 by Treatment Arm for Study EFC14768 (Transformed score).

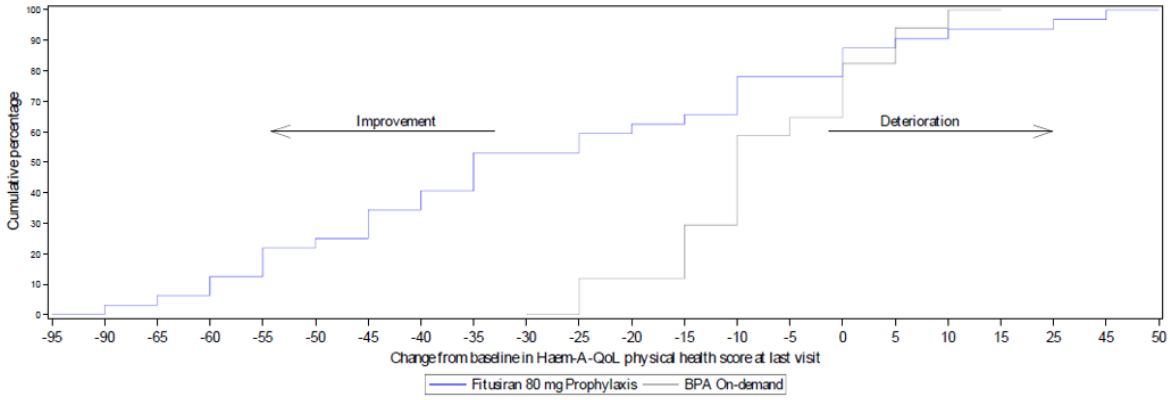


Figure 2c. Empirical Cumulative Distribution Function: Mean Change Haem-A-QoL Physical Health Domain Score From Baseline to Month 9 by Treatment Arm for Study EFC14769.

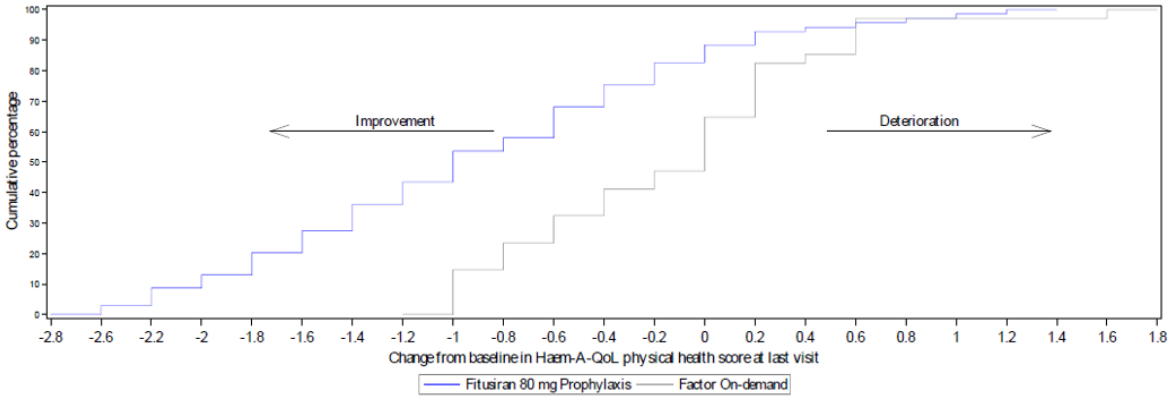
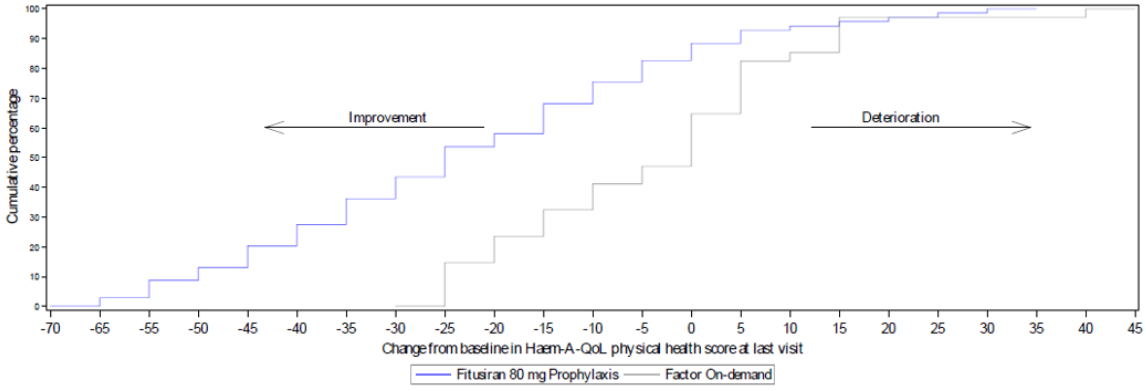


Figure 2d. Empirical Cumulative Distribution Function: Mean Change Haem-A-QoL Physical Health Domain Score From Baseline to Month 9 by Treatment Arm for Study EFC14769 (Transformed Score).



Based on discussion with our PFSS colleague, a review of anchor-based eCDF curves would be needed to gain a better insight into what may constitute a a threshold or range of clinically meaningful change in the Haem-A-QoL Physical Health domain. While there are limitations with the anchors, some of the anchors could be informative. This approach was proposed to Clinical and Biostatistics reviewers; however, after our discussion other issues arose. The Biostatistic reviewer noted that statistical significance was not reached for the primary efficacy period and brought it to our attention that the (b) (4) (b) (4) *attempt of exploring whether the participants on the AT-based dosing regimen achieved the range of change in the Physical Health domain score that was clinically meaningful experienced by the participants on the original dosing regimen seemed futile.*

6. APPENDICES

Appendix A. Hemophilia Quality of Life Questionnaire for Adults Physical Health Domain.
Appendix B. EuroQoL-5 Dimensions-5 Level Questionnaire (EQ-5D-5L) Mobility, Usual Activities, and Pain/Discomfort dimensions.
Appendix C. Treatment Satisfaction Questionnaire for Medication (TSQM-9) item 2.

Appendix A: Hemophilia Quality of Life Questionnaire for Adults Physical Health Domain

Trial ID: Page 1/7

VISIT X

Centre ID/No.: |_|_|_|

Subject No.: |_|_|_|_|_|_|

Visit Date: |_|_|_|_|_|_|_|_| D D M M M Y Y Y Y

HAEM-A- QOL

Questionnaire for Adults

Dear Patient,

We would like to find out how you have been feeling during the past weeks. Please answer the following questions in this questionnaire, which was designed specifically for people with hemophilia.

Please follow the instructions below when answering the questions:

- ⇨ Please read each question carefully.
- ⇨ Think about how things have been for you over the past weeks.
- ⇨ Put an "X" in the box corresponding to the answer that fits you best.
- ⇨ Only mark one box for each question.
- ⇨ There are no right or wrong answers.
- ⇨ It's what you think that matters.
- ⇨ There are some aspects that might not concern you (Sports & Leisure, Family Planning, Work & School, e.g., if you don't work or don't go to school). In such a case, please mark the answer category "not applicable."

All your answers will be treated with the strictest confidence!

Date of completion: __ / __ / __ (month/ day/ year)

1. Here we would like to find out about hemophilia and your PHYSICAL HEALTH

In the past 4 weeks...	never	rarely	sometimes	often	all the time
1. ... my swellings hurt	☐	☐	☐	☐	☐
2. ... I had pain in my joints	☐	☐	☐	☐	☐
3. ... it was painful for me to move	☐	☐	☐	☐	☐
4. ... I had difficulty walking as far as I wanted to	☐	☐	☐	☐	☐
5. ... I needed more time to get ready because of my condition	☐	☐	☐	☐	☐

Appendix B: EuroQoL-5 Dimensions-5 Level Questionnaire (EQ-5D-5L)
Mobility, Usual Activities, and Pain/Discomfort dimensions

Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY
- I have no problems walking ❑
- I have slight problems walking ❑
- I have moderate problems walking ❑
- I have severe problems walking ❑
- I am unable to walk ❑

SELF-CARE
- I have no problems washing or dressing myself ❑
- I have slight problems washing or dressing myself ❑
- I have moderate problems washing or dressing myself ❑
- I have severe problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*
- I have no problems doing my usual activities ❑
- I have slight problems doing my usual activities ❑
- I have moderate problems doing my usual activities ❑
- I have severe problems doing my usual activities ❑
- I am unable to do my usual activities ❑

PAIN / DISCOMFORT
- I have no pain or discomfort ❑
- I have slight pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have severe pain or discomfort ❑
- I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION
- I am not anxious or depressed ❑
- I am slightly anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am severely anxious or depressed ❑
- I am extremely anxious or depressed ❑

2

Appendix C: Treatment Satisfaction Questionnaire for Medication (TSQM-9) Item 2

TSQM-9

Abbreviated Treatment Satisfaction Questionnaire for Medication

Instructions: Please take some time to think about your level of satisfaction or dissatisfaction with the medication you are taking in this clinical trial. We are interested in your evaluation of the effectiveness, side effects, and convenience of the medication *over the last two to three weeks, or since you last used it.* For each question, please place a single check mark next to the response that most closely corresponds to your own experiences.

2. How satisfied or dissatisfied are you with the way the medication relieves your symptoms?

- ☐ 1 Extremely Dissatisfied
- ☐ 2 Very Dissatisfied
- ☐ 3 Dissatisfied
- ☐ 4 Somewhat Satisfied
- ☐ 5 Satisfied
- ☐ 6 Very Satisfied
- ☐ 7 Extremely Satisfied

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SELENA R DANIELS
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Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Medical Policy

PATIENT LABELING REVIEW

Date:	January 27, 2025
To:	Melissa Button, PharmD Regulatory Project Manager **Division of Non-Malignant Hematology (DNH)**
Through:	LaShawn Griffiths, MSHS-PH, BSN, RN Associate Director for Patient Labeling **Division of Medical Policy Programs (DMPP)** Barbara Fuller, MSN, BSN, RN Team Leader, Patient Labeling **Division of Medical Policy Programs (DMPP)**
From:	Susan Redwood, MPH, BSN, RN Patient Labeling Reviewer **Division of Medical Policy Programs (DMPP)** Melissa Khashei, PharmD Regulatory Review Officer **Office of Prescription Drug Promotion (OPDP)**
Subject:	Review of Patient Labeling: Medication Guide (MG) and Instructions for Use (IFUs)
Drug Name (established name):	QFITLIA (fitusiran)
Dosage Form and Route:	injection, for subcutaneous use
Application Type/Number:	NDA 219019
Applicant:	Genzyme Corporation, A Sanofi Company

1 INTRODUCTION

On March 28, 2024, Genzyme Corporation, A Sanofi Company submitted for the Agency's review an original New Drug Application (NDA) 219019 for QFITLIA (fitusiran) injection indicated for the treatment of routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors.

This collaborative review is written by the Division of Medical Policy Programs (DMPP) and the Office of Prescription Drug Promotion (OPDP) in response to a request by the Division of Non-Malignant Hematology (DNH) on April 16, 2024, for DMPP and OPDP to review the Applicant's proposed Medication Guide (MG) and Instructions for Use (IFUs) for QFITLIA (fitusiran) injection. The proposed proprietary name QFITLIA was found conditionally acceptable on June 20, 2024, by the Division of Medication Error Prevention and Analysis (DMEPA).

DMPP conferred with DMEPA and a separate DMEPA review of the IFUs will be forthcoming.

2 MATERIAL REVIEWED

- Draft QFITLIA (fitusiran) injection MG and IFUs received on March 28, 2024, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on January 16, 2025.
- Draft QFITLIA (fitusiran) injection Prescribing Information (PI) received on March 28, 2024, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on January 16, 2025.

3 REVIEW METHODS

To enhance patient comprehension, materials should be written at a 6th to 8th grade reading level, and have a reading ease score of at least 60%. A reading ease score of 60% corresponds to an 8th grade reading level.

Additionally, in 2008 the American Society of Consultant Pharmacists Foundation (ASCP) in collaboration with the American Foundation for the Blind (AFB) published *Guidelines for Prescription Labeling and Consumer Medication Information for People with Vision Loss*. The ASCP and AFB recommended using fonts such as Verdana, Arial or APHont to make medical information more accessible for patients with vision loss.

In our collaborative review of the MG and IFUs we:

- simplified wording and clarified concepts where possible
- ensured that the MG and IFUs are consistent with the PI
- removed unnecessary or redundant information
- ensured that the MG and IFUs are free of promotional language or suggested revisions to ensure that it is free of promotional language
- ensured that the MG meets the Regulations as specified in 21 CFR 208.20

- ensured that the MG meets the criteria as specified in FDA's Guidance for Useful Written Consumer Medication Information (published July 2006)
- ensured that the IFUs meet the criteria as specified in both the FDA Guidance for Useful Written Consumer Medication Information (published July 2006) and Instructions for Use-Patient Labeling for Human Prescription Drug and Biological Products (published July 2022)

4 CONCLUSIONS

The MG and IFUs are acceptable with our recommended changes.

5 RECOMMENDATIONS

- Please send these comments to the Applicant and copy DMPP and OPDP on the correspondence.
- Our collaborative review of the MG and IFUs are appended to this memorandum. Consult DMPP and OPDP regarding any additional revisions made to the PI to determine if corresponding revisions need to be made to the MG and IFUs.

Please let us know if you have any questions.

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/s/

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MELISSA KHASHEI
01/27/2025 01:26:23 PM

BARBARA A FULLER
01/27/2025 02:35:06 PM

LASHAWN M GRIFFITHS
01/27/2025 02:51:23 PM

FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion

****Pre-decisional Agency Information****

Memorandum

Date: January 23, 2025

To: Melissa Button, PharmD, Regulatory Project Manager
Division of Nonmalignant Hematology (DNH)

Virginia Kwitkowski, MS, ACNP-BC, Associate Director for Labeling
(DNH)

From: Melissa Khashei, PharmD, Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

CC: Jina Kwak, PharmD, RAC, Team Leader
(OPDP)

Subject: OPDP Labeling Comments for QFITLIA (fitusiran) injection, for subcutaneous use

NDA: 219019

Background:
In response to DNH's consult request dated April 16, 2024, OPDP has reviewed the proposed Prescribing Information (PI), Medication Guide/Instructions for Use (IFU) and carton and container labeling for the original NDA submission for QFITLIA (fitusiran) injection, for subcutaneous use.

PI/Medication Guide/IFU:
OPDP's review of the proposed PI is based on the draft labeling emailed to OPDP on January 16, 2025, and our comments are provided below.

A combined OPDP and Division of Medical Policy Programs (DMPP) review will be completed for the proposed Medication Guide and IFUs, and comments will be sent under separate cover.

Carton and Container Labeling:
OPDP's review of the proposed carton and container labeling is based on the draft labeling emailed to OPDP on January 16, 2025, and we do not have any comments at this time.

Thank you for your consult. If you have any questions, please contact Melissa Khashei at (301) 796-7818 or melissa.khashei@fda.hhs.gov.

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/s/

MELISSA KHASHEI
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HUMAN FACTORS STUDY REPORT AND LABEL AND LABELING REVIEW
Division of Medication Error Prevention and Analysis 2 (DMEPA 2)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

*** This document contains proprietary information that cannot be released to the public***

Date of This Review:	01/22/2025
Requesting Office or Division:	Division of Non-Malignant Hematology (DNH)
Application Type and Number:	NDA 219019
Product Type:	Combination Product (Drug-Device)
Product Name, Dosage Form and Strength:	Qfitlia (fitusiran) injection, 50 mg/0.5 mL prefilled pen and 20 mg/0.2 mL single-dose vial
Device Constituent:	Prefilled Pen
Rx or OTC:	Prescription (Rx)
Applicant/Sponsor Name:	Genzyme Corporation, a Sanofi company (Genzyme)
FDA Received Date:	03/28/2024; 04/03/2024; 07/18/2024
TTT #:	2024-9027; 2024-8888
DMEPA 2 Safety Evaluator:	Shabana Rauf, PharmD
DMEPA 2 Team Leader:	Millie Shah, PharmD, BCPS
DMEPA 2 Associate Director for Human Factors:	Lolita Sterrett, PharmD
DMEPA 2 Associate Director for Nomenclature and Labeling	Hina Mehta, PharmD

1 REASON FOR REVIEW

This review evaluates the human factors (HF) validation study report and labels and labeling submitted under NDA 219019 for Qfitlia (fitusiran) injection.

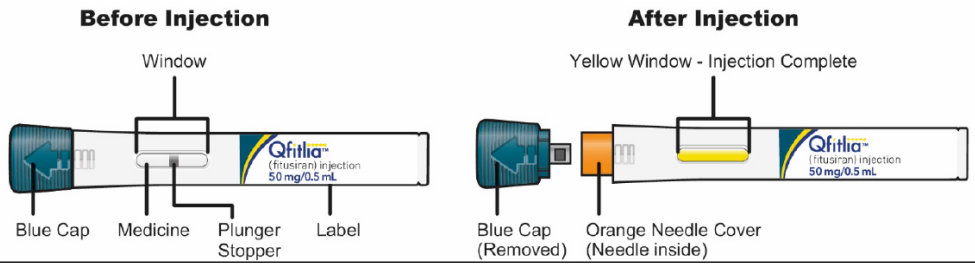
We considered the materials listed in Table 1 for this review.

Table 1. Materials Considered for this Review	
Material Reviewed	Section/Appendix
Product Information	Section 1.1
Relevant Regulatory History Related to the Proposed Product's Human Factors Development Program	Section 1.2
Human Factors (HF) Validation Study Report and HF-Related Supporting Documents	Appendix A
Information Requests Issued During the Review	Appendix B
Labels, Labeling, and Packaging	Appendix C

1.1 PRODUCT INFORMATION

Table 2 presents relevant product information for Qfitlia that Genzyme Corporation, a Sanofi company (Genzyme) submitted on 03/28/2024.

Table 2. Relevant Product Information for Qfitlia	
Initial Approval Date	N/A
Active Ingredient	fitusiran
Indication	routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors.
Route of Administration	Subcutaneous
Dosage Form	Injection
Strength	50 mg/0.5 mL prefilled pen 20 mg/0.2 mL single-dose vial
Dose and Frequency	50 mg once every two months (Q2M). The dose should be adjusted, if needed, to maintain antithrombin (AT) activity between 15-35%. The modified doses include 50 mg once every month (QM), 20 mg Q2M, or 20 mg QM.

	(b) (4)
How Supplied	• It is a clear, colorless to pale yellow solution supplied in a single-dose pre-filled pen (PFP) or a single-dose vial. • Each pre-filled pen is designed to deliver 50 mg of QFITLIA in 0.5 mL (NDC 58468-0348-1). • Each vial is designed to deliver 20 mg of QFITLIA in 0.2 mL (NDC 58468-0347-1). • QFITLIA is available in cartons containing 1 pre-filled pen or 1 vial.
Storage	50 mg Prefilled Pen • Store QFITLIA refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light. • QFITLIA may be stored at (b) (4) room temperature, (b) (4) for a single period of up to 3 months within the expiration date printed on the label. Discard (b) (4) or at the expiration date, whichever comes first. After storage at room temperature, do not return the product to the refrigerator. 20 mg Single-Dose Vial • Store QFITLIA (b) (4) in the original carton to protect from light. • Do not shake QFITLIA at any time. Do not heat QFITLIA. Do not freeze. Do not put into direct sunlight.
Container Closure/ Device Constituent (including figure)	Qfitlia Prefilled Pen (PFP) 

Intended Users	Adult (>18 years of age) and adolescent patients (12-17 years of age), lay caregivers, and healthcare professionals (HCPs)
Intended Use Environment	• At home by patients and lay caregivers • In a clinical setting by HCPs (nurses)

1.2 RELEVANT REGULATORY HISTORY RELATED TO THE PROPOSED PRODUCT'S HUMAN FACTORS DEVELOPMENT PROGRAM

On April 19, 2024, we searched for previous DMEPA reviews and FDA/Applicant interactions relevant to this current review using the terms, "IND 125632", "fitusiran", and "NDA 219019". The identified reviews and FDA/Applicant interactions are provided below.

- On January 30, 2018, we provided written responses for the Applicant's Type C Guidance Meeting that included a question regarding the approach and protocol for the Human Factors Summative study, including proposed user groups, and readability level. We recommended the Applicant submit the updated use-related risk analysis (URRA) and human factors protocol to the IND for review.[a]
- On November 30, 2022, the Applicant submitted a human factors validation study protocol under IND 125632 for fitusiran injection, 50 mg/0.5 mL prefilled pen for Agency review. We determined that the HF validation study protocol is not acceptable. In the Human Factors Validation Study Protocol Advice Letter, we advised that the Applicant implement our recommendations prior to commencing their HF validation study and submit a HF validation study report as part of the marketing application.[b]
- On March 28, 2024, the Applicant submitted human factors validation study report and labeling under NDA 219019, which is the focus of this review.

2 OVERALL ASSESSMENT OF HUMAN FACTORS STUDY DESIGN AND METHODOLOGY

This section provides a summary of the study design, and our evaluation of the study methodology to determine if the study has been appropriately designed to support the safe and effective use of the proposed product.

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[a] McMullen, R. Meeting Request-Written Responses for IND 125632, Qfitlia (fitusiran). Silver Spring (MD): FDA, CDER, OND, DHM1 (US); 2018 Jan 30.

[b] Srivastava, I. Human Factors Validation Study Protocol Review for Qfitlia (fitusiran). Silver Spring (MD): FDA, CDER, OND, DHM1 (US); 2023 Feb 27.

2.1 SUMMARY OF STUDY DESIGN

Table 3 presents a summary of the HF validation study (HFVS) design and our discussion of methodology concerns (as applicable). See Appendix A for more details on the study design.

Table 3. Study Methodology for Human Factors (HF) Validation Study		
Study Design Elements Proposed	Methodology Deviation and Applicant's Rationale (if applicable)	DMEPA Discussion of Methodology Concerns
User Group(s) Total = 76 participants • Adult patients (>18 years of age) diagnosed with hemophilia A or B, (n=15) • Adolescent patients (12–17 years of age) diagnosed with hemophilia A or B, (n=15) • Lay Caregiver (Injection Naïve) adults that provide care for someone with chronic illness that do not have formal medical training or injection experience (including intravenous infusions), (n=16) • Lay Caregiver (Injection Experienced) adults that provide care for patient with hemophilia A or B that do not have formal medical training but do have injection experience (administering intravenous infusions counts as injection experience), (n=15) • Registered Nurses currently licensed and practicing, (n=15)	User Group(s) Due to difficulty recruiting adolescent patients, the study included: • Participants who participated in a PFP study within the last six months. • These participants participated in an autoinjector study for a different product within 5 months of their participation in this validation study. This deviation may have had an impact on the study results; however, based on the Ebbinghaus Forgetting Curve, which estimates people's "Savings" (i.e., memory) of specific tasks as a percentage after a decay period, after a week period, about 90% of all new information is forgotten. • Adolescent patient who was not diagnosed with hemophilia and instead diagnosed with Von Willebrand disease.	We acknowledge the Applicant's rationale regarding difficulty recruiting adolescent patient participants and find this methodology deviation does not preclude our ability to review the HFVS results. We acknowledge the Applicant's rationale regarding participants who were enrolled in a pre-clinical HF study for an alternative presentation of fitusiran and who participated in an autoinjector study for a different product. In this instance, we find the enrollment of these participants does not preclude our review of the HFVS results.

	• While this participant was not diagnosed with hemophilia A or B, patients diagnosed with Von Willebrand disease also typically administer maintenance medications intravenously to treat a blood clotting disease, matching the defined user profile.
	
	• Adolescent patient participant who was eleven years old.
	  • While this participant was outside of the age bracket specified for adolescent patients, this protocol deviation did not negatively impact the ability of the study to meet its goals because this patient had been diagnosed with hemophilia and met all other necessary screening criteria. While the fitusiran PFP is not indicated for patients of 11 years of age, this patient could use this product in the near future to treat their condition.
	
	The study included 4 participants who participated in a pre-clinical HF study assessing an alternative fitusiran prefilled pen in clinical presentation.
	  • As the pre-clinical HF study was assessing an alternative presentation of the product, their participation from that study is not expected to	

	significantly impact performance as part of this HF study.	
Training No training was provided to participants (to approximate higher risk scenario).		
Test Environment & Materials • For adult and adolescent patient and lay caregiver participants, the testing room was configured to resemble a typical home environment. For nurse participants, the testing room was configured to resemble a clinical setting. • Study Materials Include: o PFP device filled with 0.5 mL water and labeled with TRADENAME as the product name. o PFPs that were presented to participants during knowledge task question. o PFP IFU o Attachable injection pads (into which the injections will be performed) o A sink (for hand washing) or hand sanitizer (if no sink is available o Alcohol wipes o Cotton balls o Sharps disposal container o Trash can o Paper towels o Wet wipes o Manikin for injection tasks for lay caregivers and nurses		
Sequence of Study • All participants except for nurses, will complete the Rapid Estimate of Adult Literacy in Medicine (REALM-SF) at the beginning of their study session		

to assess their level of health literacy. Nurses will not complete the REALM-SF as their health literacy is expected to be commensurate with their medical training. • Scenario 1-Injection Task-IFU Optional: Participants will be asked to deliver a dose into the injection pad placed at the injection site of their choosing and will be allowed to use the IFU if they choose to do so. • Root Cause Analysis (RCA) for Scenario 1 • Scenario 2-Knowledge Task Questions: The moderator will ask the participant to answer a set of questions related to "safety-critical" information contained in the IFU. • RCA for Scenario 2 • Scenario 3-IFU Mandatory: Participants will be asked to perform another injection, but the moderator will direct the participant to read and follow the IFU. • RCA for Scenario 3		

3 RESULTS AND ANALYSIS

The HF validation study showed issues with the tasks evaluated by simulated use and knowledge-based assessments (KBAs) listed below in this section.

For each observed issue, we considered the participants' subjective feedback, the Applicant's URRA, RCA, our evaluation of the overall residual risks, the implementation of best practice standards and the Applicant's proposed mitigations (including any post-HFVS changes to the user interface). As a result, in this particular instance we find the proposed user interface has been appropriately designed and we did not identify recommendations to address the identified issues with these tasks.

- User stores carton prior to use

    - KBA-1: How should the product be stored?
    - KBA-2: Who should you keep the medication away from?
- User opens carton.
- User removes device from carton.
- User checks the expiration date.
    - KBA-3: Where should you check for the expiration date?
    - KBA-4: What is the expiration date for this pen?
- User checks for device damage and User checks for proper drug fluid color and clarity
    - KBA-9: Show pens one at a time and ask if the pen is OK to use: Show participant a pen that is: Damaged; Cap off; Drug discolored; Window yellow; OK to use.
- User checks drug name and dosage
    - KBA-10: Where on the product would you check to be sure you have the right drug and dose?
- User warms device prior to injection
    - KBA-6: If the PFP is stored in the refrigerator, what should you do before performing the injection?
    - KBA-7: How should you warm the PFP?
    - KBA-8: What should you not do when warming the PFP?
- User removes the device cap.
- User places device on skin and User starts the injection.
    - KBA-17: How do you know the injection has started?
    - KBA-18: How do you know when the injection is completed?
    - KBA-19: What should you do if the window does not turn completely yellow?
- User holds the device and User lifts the device from skin.
- User disposes of device and cap
    - KBA-20: What should you do with the used PFP and end cap?

In Table 4 below, we provide a summary of the use-related events supplied by the Applicant along with our detailed analysis of use-related events that resulted in recommendations and document our points of dissent with the Applicant's findings.

<table>
<tr><th colspan="4">Table 4. Detailed Analysis of Use Related Events and DMEPA's Recommendations</th></tr>
<tr><td colspan="4">Legend: UE = use error; CC = close call; UD = use difficulty; URRA = use-related risk analysis; RCA = root cause analysis; HFVS = Human Factors Validation Study; KTQ = Knowledge Task Questions; IV = Intravenous; subQ= subcutaneous; AP = adult patient; TP = adolescent patient; CN = caregiver naive; CE = caregiver experienced; H = healthcare professional</td></tr>
<tr><td></td><td colspan="2">Summary of Information Supplied by Applicant</td><td>DMEPA's Identified Areas of Dissent and Recommendations</td></tr>
<tr><td>1.</td><td colspan="2">Task: User selects an appropriate injection site
Scenario 1: IFU (optional)
Scenario 2: KTQs
• KTQ 11: What injection sites can the patient use if they are injecting themself?
[Thigh and stomach]
• KTQ 12: If a caregiver is giving the injection to the patient, what injection sites can they use?
[Outer area of upper arm, thigh, or stomach]
Scenario 3: IFU (mandatory)</td><td></td></tr>
<tr><td></td><td>Reported Issues:</td><td>Participant ID:</td><td></td></tr>
<tr><td></td><td>Scenario 1
UE (n=11)</td><td>AP02; AP08; TP07; TP06; AP06; AP11; AP12; CN11; TP02; TP13; AP05</td><td></td></tr>
<tr><td></td><td>Scenario 2
UE (n=25)</td><td>CE04; CN10; TP12; AP11; CE11; CN01; H01; H14; AP12; TP03; TP06; AP02; AP06; AP12; AP15; CN11; CN14; H09; TP01; TP07; TP12; TP14; CN01; AP11 TP06</td><td></td></tr>
<tr><td></td><td>Scenario 3
UE (n= 2)</td><td>TP03; TP06</td><td></td></tr>
<tr><td></td><td>Scenario 2
CC (n=1)</td><td>CE14</td><td></td></tr>
<tr><td></td><td>UD (n=0)</td><td></td><td></td></tr>
<tr><td></td><td colspan="2">Observed event(s):
Scenario 1: UE
• Chose to inject into or near the antecubital area, into the vein. Read the IFU while performing the task. (AP02, AP08, TP07)</td><td></td></tr>
</table>

	• Chose to inject into the antecubital area, into the vein and the back of their hand. Participants did not read the IFU while performing the task. (TP06, AP06, AP11, AP12, CN11, TP02, TP13, AP05) Scenario 2: UE- For KTQ 11, What injection sites can the patient use if they are injecting themself? Answered that the patient can inject themselves into the arm, thigh, or stomach and antecubital area. (CE04, CN10, TP12, AP11, CE11, CN01, H01, H14, AP12, TP03, TP06) CC- initially answered that the patient can inject themself into the arm, thigh, or stomach and then corrected to stating they can only inject into the thigh or stomach. (CE14) UE-For KTQ 12, If a caregiver is giving the injection to the patient, what injection sites can they use? • Answered that caregivers can only inject into the upper arm and did not include thigh or abdomen in their response. (AP02, AP06, AP12, AP15, CN11, CN14, H09, TP01, TP07, TP12, TP14, CN01, AP11) • Incorrectly answered that the caregiver can give the injection to the patient's antecubital area. (TP06) Scenario 3: UE- Chose to inject into the center of the abdomen, near their navel and antecubital area, into the vein. (TP03, TP06)	
	Harm associated with errors for this task: • Insignificant to modest effect on pharmacokinetics; rupturing of very small vessels (ecchymosis) • Fatal bleeding event or associated fatal events	
	Relevant RCA/Subjective Feedback: Scenario 1:	

	• Negative transfer (*read IFU while performing the task*): chose the injection site based on their experience and stated that when they read the IFU during the session, they were skimming it as they were relying on their injection experience to administer the medication. One participant saw the figure in the IFU and noted that the shoulders could only be done by a caregiver. The other participant referenced the figure in the IFU but only looked at the colors and did not read into what they represented. Six participants chose to inject into or near the antecubital area, into the vein based on their experience (AP02, AP08, TP07). • Study artifact (*did not read IFU while performing the task*): adolescent patient wanted to have fun by choosing a site that they have not given themself a shot in before. They only did this because the injection was "fake." (TP06). • Negative transfer (*did not read the IFU while performing the task*): injected into the antecubital area because that this where they usually give injections at home. One participant stated that is where they would get a vaccine, and another participant indicated that it is easier to see veins on the arm (AP06, AP11, AP12, CN11, TP02, TP13, AP05). Scenario 2: For KTQ 11, What injection sites can the patient use if they are injecting themself? • Amount of information contained in IFU: stated that since pinching is not necessary for this product, they assumed that it would be possible for a patient to inject into their own arm. The participant saw the figure in the instructions but did not see "caregivers only", because the IFU contained a lot of information, it was overlooked (CE04). • Study artifact: stated that they did not look at the instructions close enough.	

	The participant relied on their memory and on looking at the IFU during earlier tasks. Another participant stated that they read the three sites from section B2 of the IFU and accidentally included the arm in their response to the study question (CN10, TP12). • Negative transfer: answered based on their memory and experience. One participant stated that those are acceptable injection sites for the medication, they currently take, and they noticed all three sites mentioned in the IFU when they reviewed it earlier. Another participant indicated that they saw the image prior but did not pay much attention to the caregiver portion of it (AP11, CE11, CN01, H01, H14, TP06). • Ambiguous instructions for use: stated that they had misread the text in B2 pertaining to the upper arm. They had only read the bolded parts of the text and missed the caregiver qualifier. 1 participant noticed (b) (4) in the image depicting the injection sites but did not know what the colors indicated (AP12, TP03). • Negative transfer (CC): stated that, at home, they use the arm to deliver injections to their son exclusively. Initially answered that the patient can inject themself into the arm, thigh, or stomach and then corrected to stating they can only inject into the thigh or stomach (CE14). Scenario 2: For KTQ 12, If a caregiver is giving the injection to the patient, what injection sites can they use? • Ambiguous instructions for use: One participant stated that they interpreted the portion of the graphic in the IFU where it states, " (b) (4) caregiver only," to indicate that is the only acceptable site for the caregiver to inject. The other participant stated that they based their answer off the image and did not read the text part of the	

	instructions. 1 participant had not looked at the diagram previously and only read the written instructions in B2. They initially interpreted the third bullet to mean that the arm is the only spot a caregiver can inject into (AP02, AP06, AP12, AP15, CN11, CN14, H09, TP01, TP07, TP12, TP14, CN01) • Negative transfer: did not refer to the IFU to answer the question and stated that everybody they know gets injections in that area of their arm (TP06). Scenario 3: • Study artifact: chose to inject into the center of the abdomen because they were having trouble placing the injection pad and the center of the abdomen was the easiest place to keep it. • Negative transfer: chose to inject into the antecubital area due to prior experience and, they were rushing through the steps and forgot that is not an appropriate site from when they had read the IFU in the prior scenario.	
	Applicant's Comments and Proposed Post-HFVS Mitigations: • The study observations demonstrate that selection of an appropriate injection site is learnable, and this use error, should it occur due to first use of the product without training or consultation of the instructions, would not be repeated. • In many instances, the distinction between injection sites that are appropriate for self-injection and injection sites appropriate for injection by a caregiver was not made correctly or completely. The potential harm for all these observations is an insignificant effect on pharmacokinetics or the rupturing of very small vessels (ecchymosis).	Our review of the study results identified subjective feedback that indicated the figure in section B2 of the IFU can be improved to clearly identify the self/caregiver vs. caregiver only injection sites on the figure (See figure below). Thus, we provide a recommendation in Table 5 to label the sites as such on the figure. We find this recommendation can be implemented without the submission of additional HF data.

<table>
<tr><td></td><td>

The number of participants who did not choose an appropriate injection site was drastically reduced during Scenario 3.
The overall performance observed demonstrates that the proper injection sites can be learned based on information contained in the instructions.

</td><td>(b) (4)</td></tr>
<tr><td>2.</td><td>
Task: User selects an appropriate injection site
Scenario 2: For KTQ 13, If an adolescent is receiving the medication, should they use the pen to inject themselves without assistance?
[Adolescents 12 – 17 years of age should use the pen to self-inject under the supervision of an adult]
<table>
<tr><td>Reported Issues:</td><td>Participant ID:</td></tr>
<tr><td>UE (n=15)</td><td>AP14; CE12; CE13; TP09; AP09; AP10; TP02; TP06; AP07; AP11; CE06; CE07; H14; TP01; TP08</td></tr>
</table>
Observed event(s):

Incorrectly stated that adolescents could inject without supervision.
Could not find the answer in the instructions after looking and thought adolescents can self-inject without supervision.
Stated that an adolescent age 17 should use the PFP to self-inject, and that provided practice adolescents can inject without supervision.

Harm associated with errors for this task:

Insignificant to modest effect on pharmacokinetics; rupturing of very small vessels (ecchymosis)
Fatal bleeding event or associated fatal events

</td><td></td></tr>
</table>

	Relevant RCA/Subjective Feedback: • Information related to use by adolescents appears in different parts of the instructions: participants referred to the IFU to answer the question and stated that that they were looking in the upper left panel of the IFU and read that the pen was for use by users aged 12 and up. One participant after reading that the product was for users aged 12 and up thought they would be able to use the pen independently. Additionally, thought that their own son (11-years-old) would be able to use the pen independently. Another participant stated that they concluded that the product is for use by adolescents aged 12 and older. They had not read this section when answering the question initially because they were reading through the IFU too quickly (AP14, CE12, CE13). The other participant stated that they thought adolescents could use the pen on their own after reading in the top left panel of the IFU that the device was for use in adolescents above the age of 12. After reading the important information section, they found that adolescents required supervision (T08). • Study artifact: stated that when looking at the image of the appropriate injection sites it stated "self-injection" in the graphic which to them indicated that it is okay to self-administer injections. Also, stated they answered the question without an age in mind and were just answering generally the medication can be self-administered (TP09). The other participant did not expect this kind of information in the IFU. The participant could not find the information in the instructions because they viewed the question as “strange” and was not used to looking for such information (AP10). • Negative transfer: 6 participants stated that an adolescent age 17 should use the PFP to self-inject, and that provided	

	practice adolescents can inject without supervision stated that they assumed a trained adolescent would know what to do to administer the medication. One participant stated that an older adolescent should be able to self-inject if they have performed the injection a couple of times, noting that they have been self-administering their IV infusions since they were 15 years old. The other participant stated that they would want to teach the adolescent independence as there will not always be someone available to supervise them. Two participants stated that because the pen was easy to use, they believed adolescents would be capable of using it themselves (AP07, AP11, CE06, CE07, H14, TP01)	
	Applicant's Comments and Proposed Post-HFVS Mitigations: • No mitigation recommended. • The overall performance observed demonstrate that the proper injection sites can be learned based on information contained in the instructions.	Our review of the study results identified subjective feedback that indicated that the statement in the IFU about adolescent use of the product under adult supervision can be relocated. Specially, some participants who referred to the IFU to answer the question stated that they were looking in the upper left panel of the IFU and read the statement, "This QFITLIA Pre-Filled Pen is only for use in adults and adolescents aged 12 years and older", thereby missing the statement, "In adolescents (12 to 17 years of age), it is recommended that QFITLIA Pre-filled Pen be (b) (4) by or under supervision of an adult." that is located under the "Important information to know before injecting QFITLIA" section (See image of IFU below).

		(b) (4)  We note the information related to use by adolescents appears in different parts of the IFU and find this presentation of information may lead to use error. Therefore, we provide the recommendation in Table 5 to relocate the statement in the Important Information section, so it appears in the same location as the related statement above. We have determined that these changes can be implemented without submitting additional HF validation testing data for Agency review.

4 LABELS AND LABELING

Tables 5 and 6 below include the identified medication error issues with the submitted product samples, packaging, label and labeling, our rationale for concern, and our proposed recommendations to minimize the risk for medication error.

Table 5. Identified Issues and Recommendations for Division of Non-Malignant Hematology (DNH)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
Prescribing Information – General Issues			
1.	The abbreviation "PFP", is used throughout the PI.	Abbreviations may result in confusion.	We recommend replacing the abbreviation "PFP" with "Prefilled Pen".
Highlights of Prescribing Information			
1.	The "Dosage and Administration" section include the error prone abbreviation (b) (4).	Error prone abbreviations may lead to misinterpretation and medication error.	We recommend removing (b) (4) from this section.
Full Prescribing Information – Section 2 Dosage and Administration			
1.	Section 2.1, Table 1, and Table 2 include the following error-prone abbreviations: (b) (4)	Error prone abbreviations may lead to misinterpretation and medication error.	We recommend replacing the abbreviations with their intended meaning: (b) (4)
2.	Section 2.2 contains the statements, (b) (4)	(b) (4) this statement is irrelevant and may lead to confusion.	We recommend removing these statements.
3.	Section 2.2 "Preparation and Administration" includes a statement, "…it is recommended to use a sterile 1 mL syringe (polypropylene or polycarbonate), a sterile	Given the small volume of the 10 mg/0.1 mL dose, we are concerned that the entire volume may not be delivered when switching from the transfer needle to the injection needle,	We recommend removing reference to the transfer needle in the aforementioned statement and revising it, so it reads, "…it is recommended to use a sterile 1 mL syringe

Table 5. Identified Issues and Recommendations for Division of Non-Malignant Hematology (DNH)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
	(b) (4)-gauge (b) (4) to withdraw QFITLIA solution from the vial…."	resulting in underdose errors.	(polypropylene or polycarbonate) and a sterile (b) (4)-gauge ½ inch (13 mm) (b) (4) needle to withdraw QFITLIA solution from the vial and inject subcutaneously…"
4.	Section 2.2 "Preparation and Administration" includes a note which states, (b) (4)	We are concerned (b) (4) medication error.	We recommend removing the statement, (b) (4) to be consistent with IFU.
5.	"Table 1: (b) (4) Dose Modification Based on AT Activity Levels" in Section 2.1 contains error-prone abbreviations and symbols. Additionally, the format of the table can be improved for clarity.	Error-prone abbreviations and lack of clarity may lead to misinterpretation and medication error.	We recommend revising the table by removing error-prone abbreviation "AT" and improving the format as communicated in the draft PI.
6.	Section 2.1 includes the statement, "For subcutaneous use only", which can also be incorporated into the dosage statement.	Incorporating the route of administration statement into the statement with the dosage may improve clarity.	We recommend adding "subcutaneous" into the dosage statement, "The starting dose of QFILTIA is 50 mg once subcutaneously every two months."
Full Prescribing Information – Section 16 How Supplied/Storage and Handling			
1.	Section 16.2 "Storage and Handling" is missing the statement "Store refrigerated" for the PFP.	Not including the "Store refrigerated" statement may result in the risk of the storage information being overlooked and lead to deteriorated drug medication errors.	We recommend revising the storage statement as follows, "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light."

Table 5. Identified Issues and Recommendations for Division of Non-Malignant Hematology (DNH)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
Instructions for Use (Prefilled Pen)			
1.	Based on the results of the human factors validation study (HFVS), participants' subjective feedback and root cause analysis (RCA) indicate that the figure under section B2 "Choose an injection site" can be improved to clearly identify self/caregiver vs. caregiver only administration sites.	Lack of clarity may lead to users missing important information, leading to wrong injection site selection.	We recommend you provide further mitigation to decrease risk of wrong injection site selection. You may consider adding two different figures and text next to the figure to clearly label the injection sites (i.e., one figure labeled "injection by caregiver only" and one figure labeled "Self-injection or Injection by caregiver") or if you choose to retain the existing graphic, consider directly labeling each location.
2.	The statement, "Store refrigerated" is missing from "Storing QFITLIA Pre-filled Pen".	Not including the "Store refrigerated" statement may result in the risk of the storage information being overlooked and lead to deteriorated drug medication errors.	We recommend revising the storage statement as follows, "Store QFITLIA Pre-filled Pen refrigerated between 36°F to 46°F (2°C to 8°C)."
Instructions for Use (Prefilled Pen and Single-Dose Vial)			
1.	In the HFVS, participants' subjective feedback and RCA indicated that the information related to use by adolescents appears in different parts of the Instructions for Use (IFU).	Including related information in different parts of the IFU may result in users' overlooking the information.	We recommend relocating bullet #3 under "Important information to know before injecting QFITLIA" to after the initial statement regarding use in adolescents: • Prefilled Pen: "This QFITLIA Pre-filled Pen is only for use in adults and adolescents aged 12 years and

			older. In adolescents (12 to 17 years of age), it is recommended that QFITLIA Pre-filled Pen be [redacted] by or under supervision of an adult." • Single Dose Vial: "QFITLIA is only for use in adults and adolescents aged 12 years and older. In adolescents (12 to 17 years of age), it is recommended that QFITLIA be [redacted] by or under supervision of an adult."
2.	The expiration date format in Steps A3 (PFP) and Step A2 (single-dose vial) is [redacted] which is inconsistent with the actual expiration date format (i.e., YYYY-MM).	Clearly defining the expiration date will minimize confusion and risk for deteriorated drug medication errors.	Revise the IFU images to be consistent with the expiration date format on the actual container labels and carton labeling.
Instructions for Use (Single-Dose Vial)			
1.	As currently proposed, the figure in the IFU for the single-dose vial under "Choose an injection site" is inconsistent with the prefilled pen (PFP).	Inconsistent use of figures may lead to confusion.	Revise the figure in the IFU for the single dose vial so it is consistent with the PFP (See recommendation #1 above under Instructions for Use-Prefilled Pen).

2.	The Important information section can be improved to direct users to check the prescription label for the dose given the addition of the new dose 10 mg/0.1 mL dose.	We are concerned that overdose errors may result if users do not refer to the prescription label and withdraw the entire contents of the 20 mg/0.2 mL vial if prescribed the 10 mg/0.1 mL dose.	We recommend adding the statement, “Check your prescription label to make sure you draw up the correct medicine dose prescribed by your healthcare provider."
3.	The “Important information to know before injecting QFITLIA” section can be improved by adding information to discard the unused portion of the vial.	We are concerned that lack of important information may mislead the user to believe the vial can be reused for the next dose.	We recommend adding a statement "Only use the vial one time. After you inject your dose, dispose of (throw away) any unused QFITLIA left in the vial. Do not save unused QFITLIA in the vial for later use."
4.	As currently presented, the IFU recommends (b) (4) Additionally, the IFU includes a note in a section (b) (4)	(b) (4)	We recommend removing references and steps associated with (b) (4) Additionally, we recommend revising the injection needle to be 29- or 30-guage (b) (4) . Subsequently, remove the statement, (b) (4) from section A1 and section D.

Medication Guide			
1.	As currently presented, the statement related to the use of QFITLIA in adults and adolescents aged 12 years and older is missing and inconsistent with the IFUs.	Lack of consistency may result in confusion.	We recommend adding the statement, "QFITLIA is only for use in adults and adolescents aged 12 years and older. In adolescents (12 to 17 years of age), it is recommended that QFITLIA be administered by or under supervision of an adult." to be consistent with the IFUs.
2.	The statement, "Store refrigerated" is missing from "Storing QFITLIA Pre-filled Pen" section. Also, the storage instructions for single-dose vial are inconsistent with PI.	Not including the "Store refrigerated" statement may result in the risk of the storage information being overlooked and lead to deteriorated drug medication errors.	We recommend revising the storage statement as follows, "Store QFITLIA Pre-filled Pen refrigerated between 36°F to 46°F (2°C to 8°C)." Additionally, revise the storage instructions for single-dose vial "(b) (4) [redacted] " to be consistent with PI.

Table 6. Identified Issues and Recommendations for Genzyme Corporation, a Sanofi company (Genzyme) (entire table to be conveyed to Applicant)

	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
Carton Labeling (Single-Dose Vial)			
1.	The discard statement "Discard unused portion" is not present next to the package type term "single-dose vial" on the principal display panel (PDP).	Inclusion of this discard statement on PDP next to the package type term rather than side panel helps minimize the risk of the entire contents of the	We recommend revising the statement "One 0.2 mL Single-Dose Vial" to read as "One 0.2 mL Single-Dose Vial. Discard Unused Portion". See *Guidance for Industry: Selection of the*

Table 6. Identified Issues and Recommendations for Genzyme Corporation, a Sanofi company (Genzyme) (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
		vial being given as a single dose.	*Appropriate Package Type Terms and Recommendations for Labeling Injectable Medical Products Packaged in Multiple-Dose, Single-Dose, and Single-Patient-Use Containers for Human Use (October 2018)*.
Carton Labeling (Prefilled Pen)			
1.	The statement, "Store refrigerated" is missing from the storage statement.	Not including the "Store refrigerated" statement may result in the risk of the storage information being overlooked and lead to deteriorated drug medication errors.	We recommend revising the storage statement to read "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light."
2.	The statement, (b) (4) is on the back panel.	(b) (4) Therefore, this statement is irrelevant and may lead to confusion.	We recommend removing the statement from the prefilled pen carton labeling.
3.	The prefilled pen may be stored at room temperature, (b) (4) for a single period of up to 3 months within the expiration date printed on the label. However, as currently presented, there is no space for end-users to write the beyond-use date which is the date the	Since the prefilled pen has a different expiration date after storage at room temperature, the carton labeling should have a designated space and format for end-users to write the beyond-use date to minimize the risk of deteriorated drug medication errors.	We recommend including space for end-users to write the beyond-use date on the carton labeling. For example: Discard after ___/____/____

Table 6. Identified Issues and Recommendations for Genzyme Corporation, a Sanofi company (Genzyme) (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
	product must be discarded 3 months after storage at room temperature.		
Carton Labeling (Prefilled Pen and Single-Dose Vial)			
1.	The terminology within the statement of dosage statement (b) (4) " is inconsistent with the terminology in the Prescribing Information (PI).	To ensure consistency with the terminology in the PI.	We recommend revising the statement of dosage statement to read, "Recommended Dosage: see Prescribing Information."
2.	We note the carton labeling includes the Medication Guide (MG) statement (b) (4) However, the carton labeling does not include how the MG is provided (e.g., accompanied, enclosed, or provided separately).	Per 21 CFR 208.24(d), the label of each container or package, where the container label is too small, of drug product for which a Medication Guide is required under this part shall instruct the authorized dispenser to provide a Medication Guide to each patient to whom the drug product is dispensed and shall state how the Medication Guide is provided. These statements shall appear on the label in a prominent and conspicuous manner.	Ensure the Medication Guide statement appears in accordance with 21 CFR 208.24(d).
Container Label (Single-Dose Vial)			
1.	"Discard unused portion" is bold and may compete in prominence with other more important	The "Discard unused portion" statement should not compete in	We recommend you remove bolding from the "Discard unused portion" statement.

Table 6. Identified Issues and Recommendations for Genzyme Corporation, a Sanofi company (Genzyme) (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
	information on the vial label.	prominence with critical information on the PDP.	
2.	The linear barcode is oriented in a horizontal position on the small container.	Barcodes placed in a horizontal position may not scan due to container curvature. The bending of barcodes around a curved surface affects how light reflects off them, and, if it is distorted in such a way, scanners cannot capture the entire barcode.	We recommend reorienting the linear barcode on the container label to a vertical position to improve the scannability of the barcode.
Container Label (Prefilled Pen)			
1.	The statement, "Store refrigerated" is missing from the storage statement.	Not including the "Store refrigerated" statement may result in the risk of the storage information being overlooked and lead to deteriorated drug medication errors.	We recommend revising the storage statement to read "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light."
2.	The statement, (b) (4) is on the container label.	(b) (4) Therefore, this statement is irrelevant and may lead to confusion.	We recommend removing the statement from the prefilled pen container label.

5 CONCLUSION AND RECOMMENDATIONS

The results of the HF validation study demonstrated use errors, close calls, use difficulties with critical tasks that may result in harm. Based on our review of the available participants' subjective feedback, and root cause analysis, we identified additional risk mitigations to address the use errors with labels and labeling.

Above, we have provided recommendations in Table 5 (for the Division) and Table 6 (for the Applicant). We ask that the Division convey Table 6 in its entirety to the Applicant so that recommendations are implemented within the current review cycle for NDA 217388. These changes can be implemented without submitting additional HF validation testing results for Agency review.

APPENDICES:

APPENDIX A. HUMAN FACTORS (HF) VALIDATION STUDY REPORT AND HF-RELATED SUPPORTING DOCUMENTS

The HF study results report, use-related risk analysis, IFU and background information can be accessed in EDR via: \\CDSESUB1\EVSPROD\nda219019\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hemophilia-a-b\5354-other-stud-rep\hfvsr\study-hfvsr.pdf

APPENDIX B. INFORMATION REQUESTS ISSUED DURING THE REVIEW

On 7/12/2024, we issued an Information Request (IR) to request clarification on the proposed Instructions for Use (IFU) for the single-dose vial that includes a note in section A1) Gather the Supplies and states, "If two needles are not available, the same needle may be used to transfer and inject QFITLIA." We are concerned that if a transfer needle is used to both withdraw and inject the dose, the length of the transfer needle may deliver a dose beyond the subcutaneous tissue and pose risk of wrong route medication error. On 7/18/2024, Applicant provided an acceptable response that can be accessed in EDR via: \\CDSESUB1\EVSPROD\nda219019\0010\m1\us\response-12-jul-2024.pdf

APPENDIX C. LABELS, LABELING, AND PACKAGING

C.1 List of Labels and Labeling Reviewed

Using the principles of human factors and Failure Mode and Effects Analysis,[c] along with post market medication error experiences with similar products, we reviewed the following Qfitlia labels and labeling submitted by Genzyme Corporation, a Sanofi company (Genzyme).

- Container labels received on 03/28/2024.
- Carton labeling received on 03/28/2024.

- Instructions for Use received on 04/03/2024, available from \\CDSESUB1\EVSPROD\nda219019\0002\m1\us\proposedpi-50mg.pdf \\CDSESUB1\EVSPROD\nda219019\0002\m1\us\proposedpi-20mg.pdf

- Medication Guide received on 04/03/2024, available from \\CDSESUB1\EVSPROD\nda219019\0002\m1\us\proposedpi-mg.pdf

- Prescribing Information (Image not shown) received on 04/03/2024, available from \\CDSESUB1\EVSPROD\nda219019\0002\m1\us\annotatedpi.pdf

3 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/ TS) immediately following this page

[c] Institute for Healthcare Improvement (IHI). Failure Modes and Effects Analysis. Boston. IHI:2004.

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CLINICAL INSPECTION SUMMARY

Date	November 1, 2024
From	Anthony Orencia, M.D., Ph.D., F.A.C.P., Senior Physician Min Lu, M.D., M.P.H., Team Leader Jenn Sellers, M.D., Ph.D., F.A.A.P., Branch Chief Good Clinical Practice Assessment Branch Division of Clinical Compliance Evaluation Office of Scientific Investigations
To	Donna Whyte-Stewart, M.D., Sc.M., Clinical Reviewer Carrie Diamond, M.D. Clinical Team Leader Tanya Wroblewski, M.D., Deputy Division Director Ann Farrell, M.D., Division Director Division of Nonmalignant Hematology Office of Cardiology, Hematology, Endocrinology and Nephrology
NDA	NDA 219019
Applicant	Genzyme Corp, A Sanofi Company
Drug	Qfitlia™ (fitusiran)
NME	Yes
Classification	Synthetic small interfering RNA (siRNA)
Proposed Indications	For routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors
Review Type	Priority Review
Consultation Request Date	May 7, 2024
Summary Goal Date	November 20, 2024
Action Goal Date	December 20, 2024
PDUFA Date	March 28, 2025

I. OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

Clinical data from 4 studies (Protocols EFC14768, EFC14769, EFC15110 and LTE15174) were submitted to the Agency in support of an original New Drug Application (NDA) 219019, for fitusiran for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors.

Two clinical investigators [Drs. Savita Rangarajan (India) and Chur-Woo You (South Korea)] were inspected.

Based on the inspection results, the study data derived from the above clinical investigator sites appear acceptable. In general, the clinical data submitted to the Agency for assessment are acceptable in support of the proposed indication.

II. BACKGROUND

Fitusiran was granted Fast Track Designation (FTD) on 23 December 2020 for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in patients with hemophilia A or B with or without factor VIII or IX inhibitors.

The product was also granted Breakthrough Therapy designation for routine prophylaxis to prevent or reduce the frequency of bleeding episodes in patients with hemophilia B with factor IX inhibitors on 06 December 2023.

Fitusiran (SAR439774) is a synthetic small interfering RNA (siRNA) intended to treat the serious conditions of hemophilia A and B, with or without inhibitors. Sponsor proposes the clinical indication fitusiran for bleed control over clotting factor/bypassing agent replacement therapy in patients with hemophilia regardless of subtype or inhibitor status.

Study EFC14768 (ALN-AT3SC-003, ATLAS-INH)
The study was a multicenter, multinational, randomized, open-label, Phase 3 study designed to demonstrate the efficacy and safety of fitusiran in patients with hemophilia A or B with inhibitory antibodies to coagulation factor VIII (FVIII) or coagulation factor IX (FIX) who are currently treated with on-demand bypassing agents. The primary objective was to assess the efficacy of fitusiran on prevention or reduction of bleeding episodes.

Eligible participants were randomized in a 2:1 ratio to the following treatment groups:
1) Fitusiran treatment group: Fitusiran 80 mg administered subcutaneously (SC) as prophylaxis once monthly, with use of on-demand bypassing agent or treatment of breakthrough bleeding episodes.
2) On-demand bypassing agent treatment group: On-demand bypassing agent for treatment of breakthrough bleeding episodes (on-demand use of bypassing agents was defined as the use of these agents, as needed, for episodic bleeding episodes, and not as a regular regimen intended to prevent spontaneous bleeding).

The study was divided into several periods:
1) Onset period: The onset period was Days 1 to 28 (1 month, during which the antithrombin)-lowering capacity of fitusiran was increasing but had not yet reached therapeutic levels).
2) Efficacy period: The efficacy period was Day 29 onward (8 months), during which the antithrombin lowering capacity of fitusiran had achieved its therapeutic target range.
3) Treatment period: The treatment period consisted of both the onset and efficacy periods 9 months total).
4) Follow-up period: The follow-up period lasted from one to six months.

The primary endpoint of the study was annualized bleeding rate in the fitusiran efficacy period (Day 29 to end of study). Annualized bleeding rate was a well-established endpoint that was used as the primary endpoint in global approvals of factor replacement and bypassing agent products.

This study was conducted at 24 centers that randomized participants. The study period started from February 14, 2018 (study initiation date) to June 23, 2021. There were 57 subjects analyzed: 38 participants in the fitusiran arm and 19 participants in the bypass prophylaxis agents on-demand arm. Three participants were treated but not randomized.

Study EFC14769 (ALN-AT3SC-004)

EFC14769 (ALN-AT3SC-004) was a multicenter, multinational, open-label, randomized Phase 3 study designed to evaluate the efficacy and safety of fitusiran in male participants aged 12 years or older with hemophilia A or B, without inhibitory antibodies to FVIII or FIX, who were not receiving prophylactic therapy. The primary objective was to assess the efficacy of fitusiran on prevention or reduction of bleeding episodes.

Eligible participants were randomized in a 2:1 ratio to the following treatment arms:
1)Fitusiran prophylaxis arm: Fitusiran 80 mg administered SC as prophylaxis once monthly, with use of on-demand factor concentrates for treatment of breakthrough bleeding episodes.
2)Factor on-demand arm: On-demand factor concentrates for treatment of breakthrough bleeding episodes.

The study was divided into several periods:
1) Onset period: The onset period was Days 1 to 28 (1 month, during which the antithrombin lowering capacity of fitusiran was increasing but had not yet reached therapeutic levels).
2) Efficacy period: The efficacy period was Day 29 onward (8 months), during which the antithrombin lowering capacity of fitusiran had achieved its therapeutic target range.
3) Treatment period: The treatment period consisted of both the onset and efficacy periods (9 months total).
4) Follow-up period: This period last from one to six months.

The primary endpoint of the study was annualized bleeding rate in the fitusiran efficacy period (Day 29 to end of study).

The study was conducted in 42 countries that randomized patients in 18 centers worldwide. The study period started from March 1. 2018 (first participant screened) to July 14, 2021 (last participant last visit). There were 120 subjects analyzed (80 participants in the fitusiran prophylaxis arm and 40 participants in the factor on-demand arm).

Study EFC15110 (ALN-AT3SC-009)

Study EFC15110 (ALN-AT3SC-009) was a multicenter, multinational, open-label, Phase 3 switching study designed to characterize the efficacy and safety of fitusiran in male participants, aged 12 years or older, with severe hemophilia A or B, previously receiving factor or bypassing agent prophylaxis. Participants were assigned to the same treatment sequence (factor or bypassing agent prophylaxis followed by fitusiran prophylaxis, except a subgroup in Cohort A [factor or bypassing agent prophylaxis could be skipped]). The primary objective was to characterize the efficacy of fitusiran on the prevention or reduction of bleeding episodes with a switch from prophylaxis to fitusiran.

The study consisted of two cohorts: (a) Cohort A consisted of participants with inhibitory antibodies to Factor VIII or Factor IX, and (b) Cohort B consisted of participants without inhibitory antibodies to Factor VIII or Factor IX. A subgroup of Cohort A participants included hemophilia B participants with inhibitory antibodies to Factor IX who were not responding adequately to bypassing agent prophylaxis treatment (historical annualized bleeding rate of at least 20).

The study had 3 main periods defined by the type of prophylaxis regimen:
1) A six-month factor or bypassing agent prophylaxis period in which participants continued the scheduled prophylaxis regimen with factor concentrates or bypassing agents.
The subgroup of Cohort A not responding adequately to bypassing agent prophylaxis treatment, were not to participate in this 6-month bypassing agent prophylaxis period and had to start directly with fitusiran (in the 1-month onset period described below) after the screening period.
2)A one-month onset period in which participants received their first dose of fitusiran while continuing their factor or bypassing agent prophylaxis for up to 7 days.
3)A six-month fitusiran efficacy period in which participants received fitusiran prophylaxis.

The primary endpoint of the study was annualized bleeding rate in the fitusiran efficacy period (Day 29 up to Day 190, or the last day of bleeding follow-up, whichever was the earliest). Annualized bleeding rate was a well-established endpoint that was used as the primary endpoint in global approvals of factor replacement and bypass prophylactic agent products.

This study was conducted at 32 centers that enrolled participants in 15 countries. The study period started from September 21, 2018 (first participant enrolled) to March 25, 2022 (last participant last visit). There were 67 participants who completed the factor/ bypassing agent prophylaxis period [Note: 65 of them started with fitusiran 80 mg QM (before Sponsor initiated pause in dosing), and 2 started with fitusiran 50 mg Q2M (after Sponsor initiated pause in dosing and subsequent protocol amendment)].

Study LTE15174

The ATLAS-OLE trial (LTE15174) was an open-label extension study evaluating the efficacy or long-term safety of fitusiran (SAR439774) in participants with hemophilia A or B, with or without inhibitory antibodies to factor VIII (FVIII) or factor IX (FIX). The primary study objective was to characterize the long-term safety and tolerability of fitusiran.

The primary endpoint of this study was the incidence, severity, relatedness, and seriousness of adverse events, and laboratory assessments. The study enabled participants who had completed any of the Phase 3 studies of fitusiran to continue to be evaluated for long-term safety and efficacy.

The study consists of three following periods:
(1) A Screening Period of up to 60 days.
(2) An Open-label Treatment Period that varied depending on the timing of participant's restart after the dosing pause in October 2020 (i.e., participants are to be dosed up to 48 months on the antithrombin dosing regimen after the re-start). - Participants that first started fitusiran dosing in this study after the dosing pause are had a maximum treatment duration of up to 48 months.

Participants that had started fitusiran before the 2020 dosing pause had maximum treatment duration of up to 76 months.
(3) An approximately 6-month antithrombin Follow-up Period after the last dose of fitusiran.

Some participants underwent two 6-month antithrombin Follow-up Periods, one at the end of the core study (at the 50 mg Q2M dose), i.e., before starting in the low dose cohort (20 mg Q2M), and one at the completion of the whole study.

The study was conducted at 79 centers that enrolled participants in 20 countries/regions. The first study participant enrolled on January 10, 2019. The date of interim data cut-off was June 14, 2023. As of the data cut-off date (14 Jun 2023), there were 281 participants enrolled in this study, including 227 rollover participants who completed a parent Phase 3 clinical study (57 from study EFC14768, 109 from study EFC14769, and 61 from study EFC15110) and 54 de-novo participants from China.

III. RESULTS

1. **Savita Rangarajan, M.D.**
   K. J. Somaiya Hospital and Research Centre
   Somaiya Ayurvihar, Eastern Express Highway
   Sion East, Mumbai-400022
   India

   Inspected studies/sites:
   Study EFC14768 (ALN-AT3SC-003)/Site 9108
   Study EFC14769 (ALN-AT3SC-004)/Site 9108
   Study LTE15174 (ALN-AT3SC-005)/Site 3560007

   Inspection dates: September 16-27, 2024

   For Study EFC14768, 11 subjects were screened. There were 8 subjects enrolled, randomized, and received study treatment.

   For Study EFC14769, 35 subjects were screened. There were 24 subjects enrolled, randomized, and received study treatment.

   For Study LTE1574, 34 subjects were screened. All 34 subjects enrolled and were followed-up in this extension study.

   Source records were reviewed for all 8 enrolled subjects in Study EFC14768, all 24 enrolled subjects in Study EFC14769, and 12 of 34 subjects who were transitioned and enrolled into the extension study, LTE1574.

   Records were reviewed for regulatory documentation, eligibility documentation, subject visit source records, Interactive Response Technology (IRT) drug allocation records, bleed report assessments, SAE reports, and laboratory results.

The primary efficacy endpoint data from Studies EFC14768, EFC14769, and LTE1574 were verifiable.

No discrepancies were noted after a comprehensive review to all reported adverse events and serious adverse events for 3 studies. There was no evidence of under-reporting of adverse events.

At the conclusion of the inspection, a single item of Inspectional Observation was issued to the clinical study site for failure to maintain adequate case histories. On multiple occasions, source documentation in the subject's case histories showed that activities such as removing the investigational product (IP) from the refrigerator to warm the drug, and actual administration of the IP to the subject for treatment occurred, prior to the Interactive Response Technology system providing the subject ID to be used.

Specifically, the following studies, study visits, and patients were affected.

Study Number	Subject ID	Visit	Visit Date
EFC14768 (ALN-AT3SC-003)	(b) (6)	Month 6	(b) (6)
EFC14769 (ALN-AT3SC-004)		Month 7	
		Month 8	
		Month 8	
		Month 7	
		Month 7	
		Month 8	
LTE15174 (ATLAS-OLE)		Month 31	
		Month 27	
		Month 28	

The clinical investigator responded to the FDA's observation on October 17, 2024. The clinical study site acknowledged the source documents indicated removal of the investigational products from the refrigerator and administered to subjects occurred prior to receiving the unit identification allocation from the IRT system. The principal investigator speculated transcription errors by the study staff in some cases, and a delay in the IRT email was suspected as a contributing factor. The site staff, however, followed procedures to confirm the appropriate kit numbers through (b) (4) the IRT vendor, prior to dispensing. The site intermittently experienced significant delays in receiving email communications from the (b) (4) system due to slow hospital server response times, particularly during late 2019, 2020 and 2022.

To mitigate any potential impact on subject care and protocol adherence, the study nurse utilized the kit numbers displayed on the (b) (4) screen, which were consistent with the units allocated by the IRT system. Although the delays were inconsistent, the timestamp of email receipt matched the transaction time, despite the significant lag. Since the transaction timing was displayed in Coordinated Universal Time (UTC), the site did not recognize any discrepancies in the timing, until it was pointed out during the U.S. FDA inspection.

Hence, the evidence such as screenshots of the (b) (4) screen for these transactions had not been collected. The site no longer did encounter issues as of May 2023, after shifting to the new location and email communications were received immediately after the transaction s completed in (b) (4)

Reviewer comments:
The above response by the study site *was considered adequate.*

Despite the above noted regulatory deficiencies, the study data derived from Dr. Rangarajan's site appear acceptable.

2. Chur-Woo You (P.I.)/Ju Young Kim (co-PI)
Department of Pediatrics
Daejeon Eulji Medical Center
Eul Ji University Hospital
95 Dunsanseo-ro,
Seo-gu, Daejeon, 35233
South Korea

Inspected study/site: Study EFC15110 (ALN-AT3SC-009)/Site 8202

Inspection dates: August 5-9, 2024

For Study EFC15110, there were 11 subjects who were enrolled and who received treatment at the site.

Records reviewed for the study comprised Institutional Review Board correspondence and approvals, informed consent forms, source documentation, subject record binders, monitoring, adverse events, subject disposition, primary efficacy data, product accountability, and IP disposition.

The primary efficacy study endpoint was verifiable.

No evidence of under-reporting of adverse events were noted.

The following protocol deviations were discussed at the inspection closeout meeting:

(a) Subject (b) (6) had been enrolled in the study although the screening International Normalized Ratio (INR) result met exclusion criterion #4. The P.I. evaluated the INR result did not deem this as a significant as part exclusion criteria. This protocol deviation was reported in the NDA submission.

(b) Subject (b) (6) had an eligible historical Factor VIII laboratory measurement, but the screening Factor VIII laboratory assay measurement would have been exclusionary.

(c) The laboratory results that should have been reviewed prior to IP administration were not available until after the IP was administered, e.g., Subject (b) (6)'s blood sample at Month #3 was drawn prior to the IP administration at 11:05, but the result was not received until 11:20. The IP administration should have been withheld, pending laboratory result.

Reviewer's comment: The above protocol deviations appear to be isolated and less likely to have significant impact on the primary efficacy endpoint or adverse event outcomes for this study. For Subject (b) (6) screening Factor VIII laboratory assay measurement was exclusionary, but was not acknowledged as part of the protocol violations; however, based upon the principal investigator's clinical assessment, this subject was still enrolled. There is no evidence that this subject's case had an overall effect on the efficacy; neither was any subject safety or serious harms reported. For Subject (b) (6) a 15-minute delay in the blood sampling would unlikely have an impact on the efficacy of the study for this singular occurrence.

{See appended electronic signature page}
Anthony Orencia, M.D., Ph.D., F.A.C.P.
FDA Senior Physician
Good Clinical Practice Assessment Branch
Division of Clinical Compliance Evaluation
Office of Scientific Investigations

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JENN W SELLERS
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Date: August 16, 2024

From: Jane Bai Ph.D., Paula Hyland Ph.D., Wendy Wu Ph.D.,
Division of Applied Regulatory Science (DARS), Office of Clinical Pharmacology (OCP)

Through: Jeffry Florian Ph.D., Associate Director, DARS, OCP

To: Eileen NavarroAlmario MD, Team Lead, Office of New Drugs (OND), Office of Immunology and Inflammation (OII), Division of Hepatology and Nutrition (DHN)

Subject: Potential mechanisms for hepatotoxicity of the siRNA fitusiran in relation to approved siRNAs

EXECUTIVE SUMMARY

DARS received a consult from OND to assess the potential mechanisms of off-target hepatic cell and biliary toxicity that might explain species/product specificity for fitusiran. Fitusiran is a double-stranded small interfering ribonucleic acid (siRNA) and is conjugated to a triantennary N-acetyl galactosamine (GalNAc) ligand for directed delivery to the liver to target antithrombin mRNA. Sponsor reports from the fitusiran submission (NDA 219019), literature on GalNAc-siRNAs (potential for off-target effects), and the labels/chemical structures of approved siRNAs (including one siRNA and five GalNAc-RNAs) were considered in our assessment.

Regarding labeling and chemical structure comparisons, only givosiran labeling has mention of hepatoxicity in the Warning and Precaution section of labeling among the approved GalNAc-siRNAs. Similar chemical modifications to the nucleotides of fitusiran are found in all five approved GalNAc-siRNAs. Though 2'F nucleotides do not occur naturally, the number of 2'F nucleotides in the structure of fitusiran does not stand out in comparison with other approved GalNAc-siRNAs and nothing was identified in the literature regarding potential safety for 2'F nucleotides. Regarding on-target effects, no evidence was identified to suggest that the knockdown of antithrombin, and thus on target effects by fitusiran in humans or rats, might be associated with liver dysfunction or toxicity. Regarding off-target effects, small interference RNAs can cause degradation of other intracellular mRNAs, in addition to its target mRNAs, through complementary matching via its seed sequence of the antisense strand (the nucleotide sequence of 2-8 position from its 5′-end) and cause off-target effects. The sponsor conducted bioinformatics analysis and only selectively studied the off-target effects of fitusiran on eight of nine predicted gene transcripts in vitro using Hep-G2. Among the mRNAs studied, the functions of the corresponding gene products include *INO80D* and *ZBTB40* involved in DNA repairs, *AFAP1* in biliary function, *AKAP12* in liver function, and *ADO* in managing mitochondrial oxidative stress. DARS reviewed the study and noted that while the study adequately addresses a lack of off-target potential for these 8 predicted transcripts, it would have been more informative to cover a broader set of gene transcripts or global RNA effects than those considered in the analysis. In addition, several intermediate metabolites of the fitusiran antisense strand were also detected in human serum and liver S9 fractions. Acknowledging that these metabolites were all less than 10% of fitusiran, the sponsor did not conduct any toxicity assessments of these intermediate metabolites in their assessment of off-target effects.

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Altogether, review of reports from the sponsor's submission (clinical observations and off-target gene analyses), literature, and labels/structure of other approved (or withdrawn) siRNA products did not show any potential mechanism for hepatotoxicity with fitusiran related to structure, on-target effects, or off-target effects. The different severities of fitusiran hepatoxicity in preclinical species may be explained by species differences in fitusiran metabolic stability, biology, and doses studied. However, several gaps in the sponsor's assessment were noted by the team. These include the sponsor not having conducted a broader assessment of impact on all gene transcripts or global gene expression in the off-target analysis and not considering intermediate metabolites as potentially contributing to the observed toxicity. Further exploration of these two gaps through adequate *in vitro* studies (summarized in the appendix) would be one option to better understand the mechanism for the observed nonclinical and clinical fitusiran data.

BACKGROUND

Fitusiran is an antithrombin-directed small interfering ribonucleic acid (siRNA) developed by Sanofi; Sanofi's NDA 219019 submission is under review for approval of the proposed indication of routine prophylaxis to prevent or reduce the frequency of bleeding episodes in adult and adolescent patients aged 12 years and older with hemophilia A or B with or without factor VIII or IX inhibitors. DARS received a consult request from OND/OII/DHN for assessing the potential mechanisms of off-target liver toxicity that might explain species/product specificity for fitusiran for hepatic cell and biliary toxicity. The following background information was provided regarding nonclinical and clinical findings.

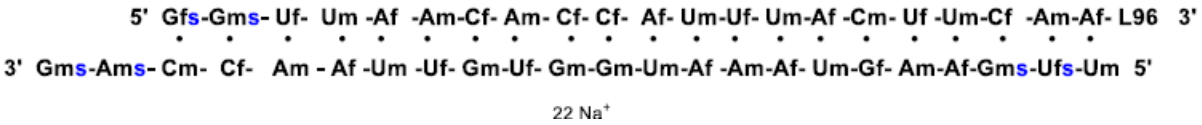
Species differences in liver toxicity are reported for fitusiran. In rats, early mortality was observed in 61% of animals that received 3 mg/kg/week for 7 weeks (>NOAEL)) characterized by hepatic necrosis, biliary hyperplasia and bile duct dilatation. NOAEL in a 26-week toxicity in mice was 30 mg/kg/week. NOAEL for chronic dosing in the 6-month rats and 9-month monkeys' studies was 0.5 mg/kg/week. Reversible transaminase elevations were noted in dogs at 10 mg/kg/week that normalized 12 weeks post dose. Liver cell degeneration and inflammation was seen. All 3 monkeys that received 30 mg/kg/week for 3 weeks died with pleural and gastrointestinal hemorrhage. Monkeys dosed at 1 mg/kg/week showed perivascular/vascular mononuclear cell inflammation in the liver, kidney, stomach, and colon. No liver test abnormalities were noted at 0.5mg/kg in monkeys, although some liver cell vacuolization was noted at 0.3mg/kg.

In clinical study EFC 14768 (N=41), entitled "Study title: ATLAS-INH: A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients with Hemophilia A or B, with Inhibitory Antibodies to Factor VIII or IX," 12 patients with alkaline phosphatase elevations, 13 patients with alkaline aminotransferase elevations, 6 patients with aspartate aminotransferase elevations. In EFC 14769 study (N=79), entitled "ATLAS-A/B: A Phase 3 Study to Evaluate the Efficacy and Safety of Fitusiran in Patients with Hemophilia A or B, without Inhibitory Antibodies to Factor VIII or IX," 10 patients with alkaline phosphatase elevations, 10 patients with alkaline aminotransferase elevations, 16 patients with aspartate aminotransferase elevations. There are 5 Hy's law cases. There was 1 patient with level 1 elevation of alkaline phosphatase in the standard of care group. There were at least 22 cases of cholecystitis and cholelithiasis observed in the fitusiran treatment group compared to the standard of care group. The proposed maximum clinical dose of fitusiran is 50 mg/month, or 0.5 and 0.6 mg/kg/month for an average adult US male (average weight, 91 kg) and female (average weight, 78 kg)."

EVALUATION

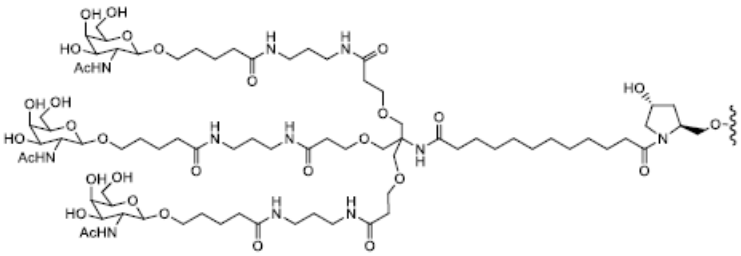
Fitusiran, a double-stranded small interfering ribonucleic acid (siRNA), was developed to target antithrombin (AT) mRNA. The NDA 219019 submission is under review for the proposed indication of routine prophylaxis in adult and adolescent patients aged 12 years and older with hemophilia A or B. In addition to hepatoxicity and biliary effects in preclinical species, excess elevations of transaminase were observed in clinical trials.

Fitusiran is conjugated to a triantennary N-acetyl galactosamine (GalNAc) ligand for directed delivery to the liver. The molecular weight of fitusiran is 17,193 Da and its structural formula is shown below. The upper strand is the sense strand (21 nucleotides) and bottom one the anti-sense strand (23 nucleotides).



Af, Cf, Gf, Uf = 2'-deoxy-2'-fluoro ribonucleotides
Am, Cm, Gm, Um = 2'-OMe ribonucleotides
s = phosphorothioate

L96 =

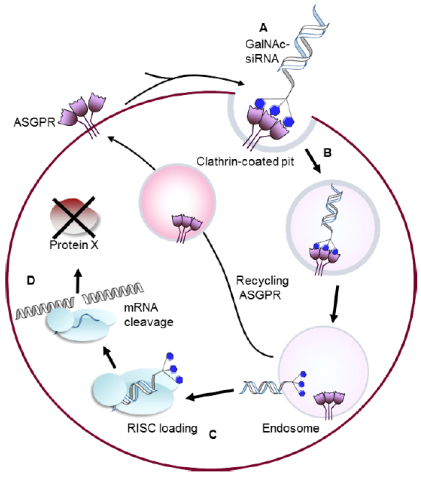


Delivery to hepatocytes and mechanism of action

Attaching a triantennary GalNAc ligand to an siRNA enables hepatocyte binding and subsequent cellular uptake via asialoglycoprotein receptor (ASGPR). ASGPR is a member of the C-type lectin family of receptors that recognizes and binds glycoproteins with terminal, GalNAc residues. Delivery of fitusiran to hepatocytes is illustrated below in Figure 1.

Figure 1-siRNA conjugate delivery mechanism

(Source: 2.7.2 Summary of Clinical Pharmacology studies)

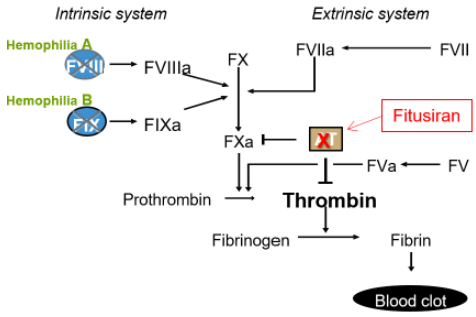


Fitusiran was developed to allow for targeted delivery to hepatocytes and specific silencing of AT. (A) Fitusiran is conjugated at the 3' end of the sense (S) strand with a trivalent GalNAc ligand to allow for targeting to the ASGPR on hepatocytes. (B) Once taken up by receptor- mediated endocytosis, (C) the GalNAc-siRNA loads into a multi subunit protein complex, the RISC, which guides the siRNA to the target mRNA sequence. The siRNA duplex unwinds, and the AS strand that remains bound to RISC (D) directs site specific cleavage of the target complementary mRNA sequence, resulting in mRNA degradation and reduced expression of the target protein. Figure adapted from Cummings and McEver (22). For fitusiran, protein X is antithrombin.

Fitusiran specifically targets degradation of antithrombin messenger RNA (mRNA), resulting in reduction of plasma AT levels (see Figure 2).

Figure 2. Coagulation pathway and fitusiran mechanism of action

(Source: 2.7.2 Summary of Clinical Pharmacology studies)



Potential mechanisms for hepatotoxicity

Potential mechanisms of hepatic toxicity with fitusiran include the GalNAc molecule, the antisense strand and its intermediate metabolites with sufficient number of nucleotides interacting with mRNAs that play a role in hepatic and biliary physiological functions. FDA has approved 6 siRNAs FDA for treating various diseases five of which are GalNAc-siRNAs. Each of these GalNAc-siRNAs has the ligand containing three

N- acetylgalactosamine (GalNAc) for directed delivery to hepatocytes. Among these five approved siRNAs, only givosiran has hepatotoxicity noted in the Warnings and Precautions section of its labeling. Potential mechanisms for the observed hepatotoxicity signal are evaluated below.

Comparisons of structural formulars of fitusiran and the 6 approved siRNAs

To increase stability of synthetic siRNAs, chemical modification strategies include, but do not limit to, replacing 2′-OH with a 2′-O-methyl or 2′-F, and replacing one nonbridging oxygen of a phosphodiester (1). The structural formulas of fitusiran and approved siRNAs including givosiran (the only one in the 5 approved siRANs with hepatoxicity noted in the labeling) are shown in Table S1. Among the final metabolites of fitusiran are 2'OM nucleotides and 2'F nucleotides; 2'OM nucleotides occur naturally while 2'F nucleotide do not. The safety of 2'F nucleotides haves been assessed, and no safety concerns have been raised by 2'F modifications in siRNAs (2). Fitusiran has 18 2'F nucleotide monomers while approved nedosiran and givosiran have 19 and 16 2'F nucleotide monomers, respectively. Nedosiran with an approved dosing of 160mg once monthly does not have liver toxicity noted in the Warning and Precaution section of its labeling but givosiran does.

On-target effects of fitusiran

Fitusiran was designed to target a site in the SERPINC1 mRNA that is primarily conserved across species and in vivo studies confirm dose-dependent activity of fitusiran resulting in durable reduction of antithrombin in mice (WT and hemophilia) rats, dogs, and monkeys. Thus, on target effects are driven by a full sequence match to the target SERPINC1 mRNA. Using categorized literature findings and database information from IPA gene view, we identified only a single study in mice related to liver dysfunction and toxicity following SERPINC1 knockout (3). No evidence was identified to suggest that the knockdown of antithrombin. on target effects by fitusiran in humans or rats might be associated with liver dysfunction or toxicity. However, microvascular thrombosis has reportedly caused hepatic dysfunction (4), low antithrombin resulting from on-target effects may cause microvascular thrombosis, thus indirectly contributing to liver dysfunction.

Off-target effects of fitusiran

It is known that siRNAs can cause sequence-based hybridization off-target effects (see Appendix for more detailed assessment of the sponsor's analysis). Interactions between siRNAs with partially complementary sequences can downregulate mRNAs and cause off-target effects via ARGONAUTE 2 (AGO2) within RNA-induced silencing complex (RISC) and an AGO2 independent degradation mechanism (5). Seed-pairing seems to play a role in off-target effects; seed-pairing destabilization has been utilized to reduce hepatic off-target hepatoxicity of GalNAc-conjugated siRNAs in rats (6, 7). A software tool has recently been developed for identifying potential seed-pairings that could result in knockdown of off-target mRNAs (8).

The sponsor conducted bioinformatic analysis to identify a set of nine transcripts that may potentially be degraded following alignment with the antisense strand of fitusiran, A-116861 (antisense strand), based on sequence homology. Then, they studied inhibition of potential off-target mRNA following exposure to fitusiran using quantitative RT-PCR (qPCR) in HepG2 cells. The highest concentration studied was 10nM which is between the C_{max} values of 4.97 nM (geometric mean) and 28.4 nM (arithmetic mean) following single dose 50mg and 80mg fitusiran subcutaneously, respectively. The off-target mRNAs studied were those of *INO80D*, *ZBTB40*, *AFAP1*, *FRAS1*, *AKAP12*, *PHIP*, *TAPT1*, and *ADO* genes. The biological functions of these gene products (proteins) are summarized in Table 1. Notably, *ADO* is expressed in

mitochondria and indirectly responsible for oxidative stress management. No IC50 value was achieved for eight (INO80D, ZBTB40, AFAP1, FRAS1, AKAP12, PHIP, TAPT1, and ADO) of the 9 off-target predicted transcripts. The absolute IC50 of "off-target" is at least 200-fold higher than the absolute IC50 of "on-target (*SERPINC1).*"

Table 1. List of off target genes and their functions in humans

Gene	Function
INO80D (INO80 complex subunit D)	Predicted to be involved in DNA recombination and DNA repair (https://www.ncbi.nlm.nih.gov/gene/54891)
ZBTB40 (zinc finger and BTB domain containing 40)	Involved in cellular response to DNA damage stimulus (https://www.ncbi.nlm.nih.gov/gene/9923)
AFAP1 (actin filament associated protein 1)	A potential modulator of actin filament integrity in response to cellular signals, and may function as an adaptor protein by linking Src family members and/or other signaling proteins to actin filaments (https://www.ncbi.nlm.nih.gov/gene/60312) Implicated in biliary atresia (9).
FRAS1 (Fraser extracellular matrix complex subunit)	It encodes an extracellular matrix protein that appears to function in the regulation of epidermal-basement membrane adhesion and organogenesis during development (https://www.ncbi.nlm.nih.gov/gene/80144)
AKAP12 (A-kinase anchoring protein 12)	The encoded protein serves as a scaffold protein in signal transduction, and is a cell growth-related protein (https://www.ncbi.nlm.nih.gov/gene/9590) Implicated in ameliorating liver injury (10); its deficiency in mice aggravating thioacetamide-induced liver injury (11).
PHIP (pleckstrin homology domain interacting protein)	The encoded protein may also regulate growth and survival of pancreatic beta cells (https://www.ncbi.nlm.nih.gov/gene/55023)
TAPT1 (transmembrane anterior posterior transformation 1)	This gene encodes a highly conserved protein that localizes to the centrosome and/or ciliary basal body (https://www.ncbi.nlm.nih.gov/gene/202018)
ADO (2-aminoethanethiol dioxygenase)	Is in mitochondria and indirectly responsible for oxidative stress management (12). (https://www.ncbi.nlm.nih.gov/gene/84890) An intracellular oxygen sensor (13).

Metabolism of fitusiran

The in-vitro metabolism of fitusiran was evaluated in serum and liver S9 fractions from mice, rats, dogs, monkeys, and humans. The in-vivo metabolism of fitusiran was evaluated in plasma (mouse, rat, dog, monkey) and liver (mouse and rat) samples collected from selected PK studies.

Figure 3. Sponsor's proposed metabolic pathway of fitusiran

In vitro and in vivo metabolism was generally similar across species (mouse, rat, dog, monkey, human), with AS(N-1)3' identified as the main metabolite in human (<10% of parent molecule) and nonclinical species. From analyzing plasma samples of patients with hemophilia in the Phase 1 study TDR 14767 (ALN-AT3SC-001) who received repeated fitusiran 80mg QM, the N-1 metabolite was less than 10% of intact fitusiran (HPLC analysis). It was concluded that the N-1 metabolite is expected to be the AS (N-1)3' metabolite. AS(N-1)3' was also the main metabolite in human serum and liver S9-fractions. Liver S9-fracitons mainly consist of microsome and cytosol fractions (14).

Though there are several intermediate metabolites of the antisense strand, they may not efficiently enter hepatocytes via ASGR-mediated uptake due to lack of GalNAc (L96; attached to the 3' of the sense strand) to be loaded onto RISC. That is, RISC-mediated targeted degradation of other mRNAs may not be accessible to those intermediate metabolites of the anti-sense strand for them to cause off-target degradation of intracellular mRNAs through seed-based complementary matching. However off target effects via other mechanisms including those of intermediate metabolites have not been assessed.

It has been reported that intracellular concentrations of fitusiran were much higher than the nominal concentrations of incubation media after 24 hours incubation in a sandwich culture human hepatocyte system (15). For instance, when fitusiran concentration in the incubation media was 15.4 nM, its intracellular concentration was 181 nM in hepatocytes. Therefore, assessing potential off-target effects of intermediate metabolites would more informative if studied at their maximal intracellular concentrations.

SUMMARY AND CONCLUSIONS

Fitusiran is a GalNAc-siRNA being developed to target antithrombin (AT) mRNA. It is currently under review as NDA 219019 for the proposed indication of routine prophylaxis in adult and adolescent patients aged 12 years and older with hemophilia A or B.

There have been six siRNAs approved by FDA for various indications including one siRNA and five GalNAc-siRNAs. Among the approved GalNAc-siRNAs, only givosiran has mention of hepatoxicity in the Warning and Precaution section of labeling. Chemical modifications to the nucleotides of fitusiran are used in the five approved GalNAc-siRNAs. Though 2'F nucleotides do not occur naturally, the number of 2'F nucleotides in the structure of fitusiran does not raise safety concerns after comparing it with approved GalNAc-siRNAs and literature reviews. No evidence was identified to suggest that the knockdown or deficiency of antithrombin, and thus on target effects by fitusiran in humans or rats, might be associated with liver dysfunction or toxicity. Small interference RNAs can cause degradation of other intracellular mRNAs, in addition to its target mRNAs, through complementary matching initiated by its seed sequence of the antisense strand (the nucleotide sequence of 2-8 position from its 5′-end) and cause off-target effects. Among the eight predicted off-target gene transcripts that the sponsor studied, several of the proteins encoded by these genes are involved in liver and biliary physiology. DARS reviewed the study and noted that while the study adequately addresses a lack of off-target potential for these 8, it would have been more appropriate to cover a broader set of gene transcripts/mRNAs than those considered in the analysis. In addition, several intermediate metabolites of the fitusiran antisense strand were also detected in human serum and liver S9 fractions; however, no assessments regarding any of the intermediate metabolites have been performed.

Altogether, the available data does not support a potential mechanism for hepatotoxicity with fitusiran related to structure, on-target effects, or off-target effects. However, there are several gaps in the sponsor's assessment including not having considered a broader set of genes or intermediate metabolites in the off-target analyses. Details on additional investigations for exploring these gaps are described in the appendix if the review team is considering asking the sponsor to explore potential mechanisms for fitusiran hepatotoxicity. If such studies are asked of the sponsor, DARS would be willing to assist in informing how the assessments are conducted and in reviewing study results upon submission by the sponsor.

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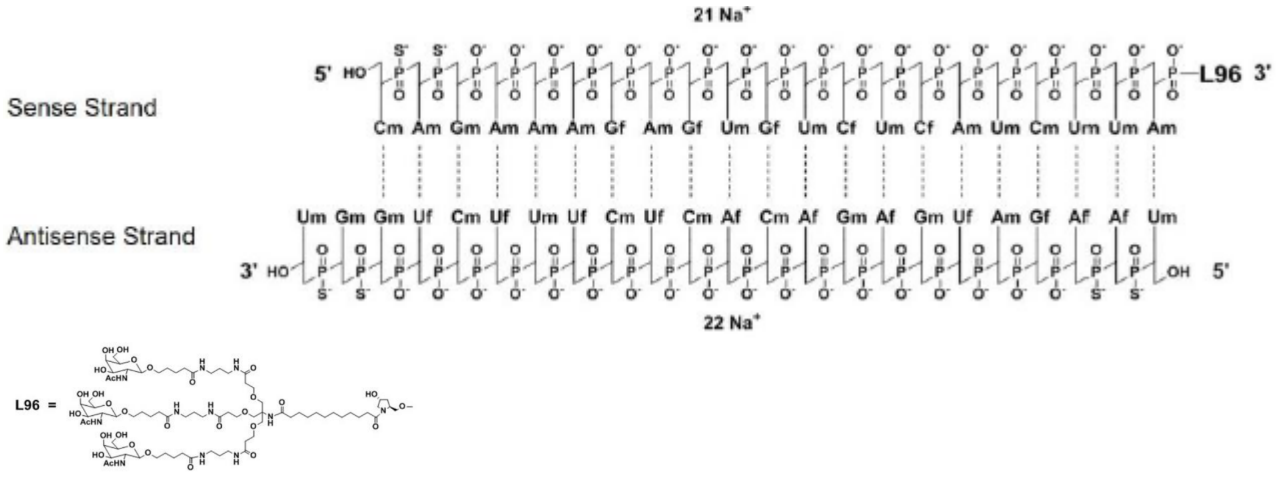
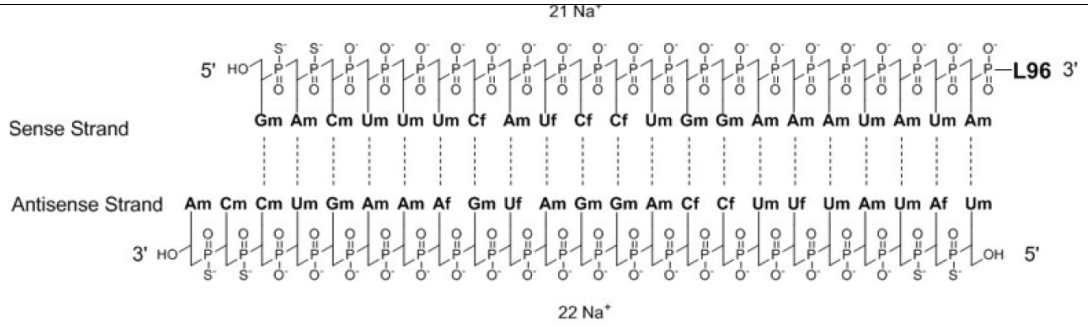
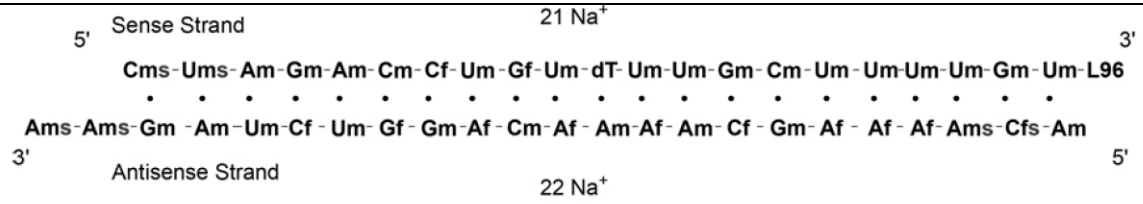
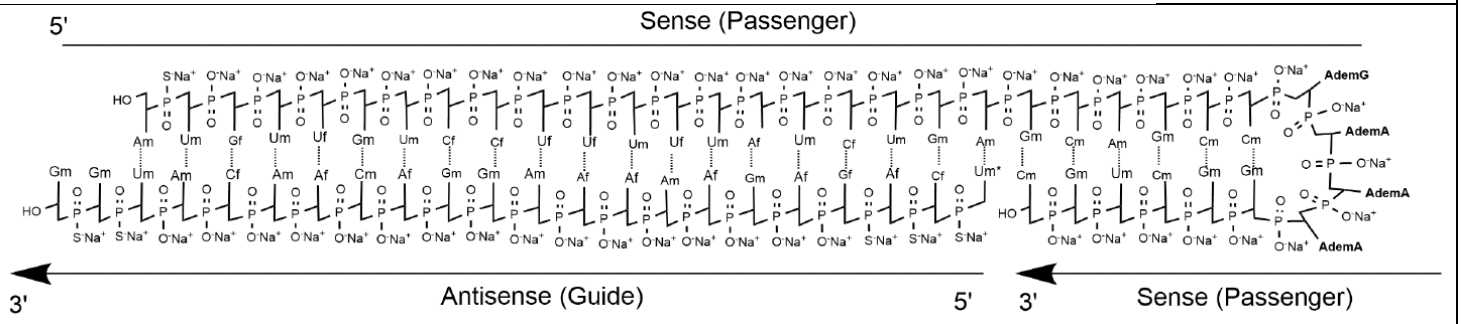
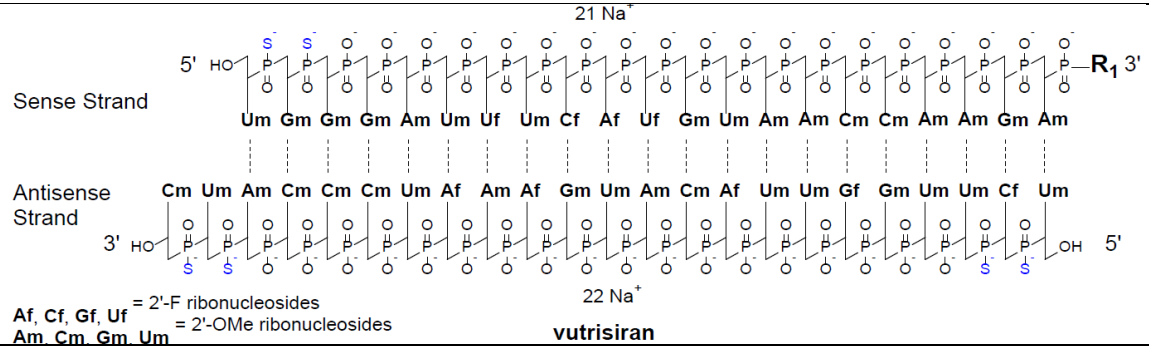
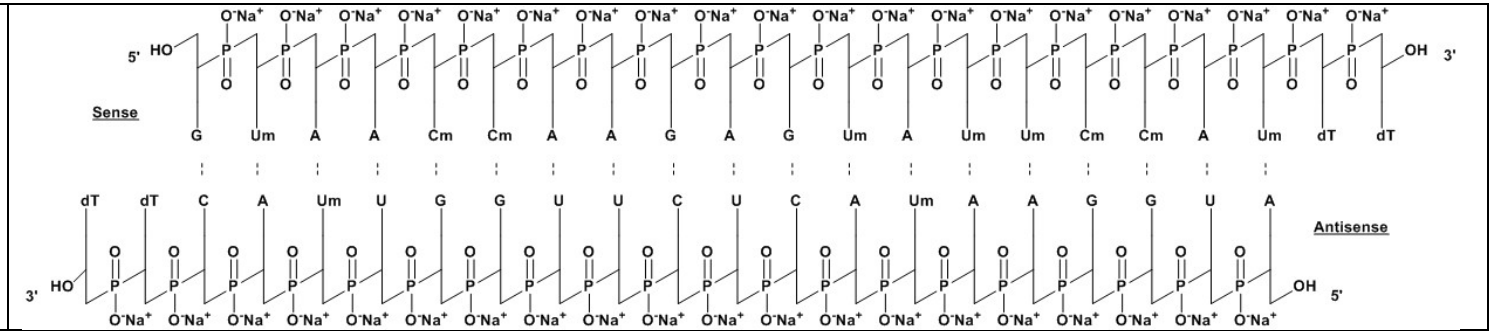
(15) Ramsden, Diane et al. "In Vitro Drug-Drug Interaction Evaluation of GalNAc Conjugated siRNAs Against CYP450 Enzymes and Transporters." Drug metabolism and disposition: the biological fate of chemicals vol. 47,10 (2019): 1183-1194. doi:10.1124/dmd.119.087098

Table S1: the detailed structural formulations of approved individual siRNAs

GalNAc-siRNA	Structure
	
	

	 **Inclisiran Sodium**
Nedosiran	
Vutrisiran	 **Af, Cf, Gf, Uf** = 2'-F ribonucleosides **Am, Cm, Gm, Um** = 2'-OMe ribonucleosides **vutrisiran**

Patisiran	

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Appendix

Detailed reviews of NONCLINICAL PHARMACOLOGY REPORT-EXTERNAL (SAR439774): In Vitro "Off-Target" Analysis of ALN-57213, the SERPINC1-Targeting siRNA Component of ALN-AT3

The sense strand (A-116858) of fitusiran is 21-nucleotides (nts) in length. The 3'-end of the sense strand is conjugated to a triantennary GalNAc moiety (referred to as L96) through a phosphodiester linkage which facilitates direct hepatocyte-specific uptake of the GalNac siRNA (dsRNA) via the asialoglycoprotein receptor (ASGPR) which is specifically expressed on the membrane surface of hepatocytes (~500,000 copies/cell) and barely expressed by other cells with the exception of monocytes which may represent a mobile pool of receptor (1, 2). The receptor comprises two proteins, asialoglycoprotein receptor 1 and 2 (ASGR1 and ASGR2), encoded by the genes *ASGR1* and *ASGR2*. This prevents the siRNA been taken up by non-target cells, such as immune cells which can lead to gene silencing and toxicity. This process should also favor a pharmacokinetic profile with high uptake or endocytic siRNA levels of fitusiran in the liver (3). The extensive 2'-O-Methyl and 2'-F modifications as well as the addition of the GalNAc ligand to the 3' end of the sense strand of fitusiran significantly decreases the likelihood that the sense strand would lead to 'off-target' transcript suppression (Jackson et al., 2006); this strand is also likely to be degraded. Also, while different degradation products of both the sense **(**A-116858) and antisense A-116861) strands are generated by endogenous enzymes the resultant metabolites produced across species are in such low quantities (albeit assessed only after 24 hours at 37°C for both the sense and antisense strands), they are unlikely to contribute to toxicity via a RISC-dependent or -independent mechanism. Also, fitusiran is less than 30 nts and unlikely to trigger an immune response across species following liver uptake. (4)

The antisense strand (A-116861) of fitusiran is 23-nts and contains four phosphorothioate linkages, two at the 3' end and two at the 5' end. The sense strand (A-116858) contains two phosphorothioate linkages at the 5' end. The 21-nt of the sense strand hybridize with the complementary 21 nucleotides of the antisense strand, thus forming 21 nucleotide base pairs and a two-base overhang at the 3'-end of the antisense strand. The seed region (2-8 base from the 5' end) of the antisense strand (A-116861) targets the SERPINC1 transcript (alias, AT3 gene) for cleavage and degradation via siRNA/mRNA RISC-mediated miRNA-like pairing resulting in post-transcriptional gene silencing. While processing of dsRNA and assembly of a functional RISC likely occurs[OBJ][OBJ], whether this occurs in the nucleus, or the cytosol for fitusiran is not known. However, in the absence of off-target RISC independent mechanisms, this specificity is unlikely to contribute to tissue or cell toxicity. In general, siRNAs including fitusiran are considered to have very tight target specificity as they cleave the mRNA before translation.

On-target effect of ALN-57213

Fitusiran was designed to target a site in the SERPINC1 mRNA that is primarily conserved across species and in vivo studies confirm dose-dependent activity of Fitusiran resulting in durable reduction of AT in mice (WT and hemophilia) rats, dogs, and monkeys. Thus, on target effects are driven by a full sequence match to the target SERPINC1 mRNA. Using categorized literature findings and database information from IPA gene view, we identified only a single study in mice related to liver dysfunction and toxicity following SERPINC1 knockout. In 129S2/SvPas * C57BL/6J mouse, mutant embryonic mouse *Serpinc1* gene (allele Serpinc1tm1Sai/Serpinc1tm1Sai) (knockout [homozygous]) increases degeneration of liver (liver dysfunction and toxicity) in mouse (5). Further the depletion of *Serpinc1* favors liver tumorigenesis induced by diethylnitrosamine and CCl4 treatments in mice through neutrophil/IL-8 signaling (6, 7). *Serpinc1* heterozygous knockout rats are associated with increased susceptibility to kidney reperfusion

injury, but not liver toxicity (8) (https://rgd.mcw.edu/rgdweb/report/gene/main.html?id=1309391) While antithrombin levels can be affected by gene mutation, increased consumption, and abnormal liver function, we observed no evidence to suggest that the knockdown or deficiency of AT and thus on-target effects by fitusiran in humans or rats might be associated with liver dysfunction or toxicity.

Off-target effects of ALN-57213

Off-target effects involve the inadvertent downregulation of other transcripts by the siRNA. Off-target effects can be sequence-non-specific off-target effects or mechanisms (also referred to as RISC-dependent off-target effects) and/or non-sequence-non-specific off-target effects or other mechanisms.

RNA-induced silencing complex (RISC)-dependent off-target effects

These sequence-non-specific effects occur due to incomplete base-pair complementarity or mismatching of a transcript with the siRNA antisense strand which then leads to off-target transcript cleavage, degradation, and thus issues with interpretation and potential toxicity. Alternatively, the siRNA antisense and sense strands can also result in seed-based miRNA-like repression of unintended transcripts mediated by RISC (and thus repression of protein translation). For fitusiran, a computational prediction tool against the whole transcriptome was used which would include or should include a potential screen across the 3'UTR regions of all RNAs across the species, however the accurate prediction of this miRNA-like off-target effect remains a challenge. Further, RISC-based off-target effects have been predominantly observed only where sustained high levels of RISC-loaded antisense or siRNA has been achieved. That is, at doses greater than the therapeutic dose *in vivo* (9) and at doses higher than the effective concentration *in vitro* for the intended on-target effect (10).

Many computational *in silico* tools and databases are now available publicly or for download (e.g., BLASTN and RNAhybrid using CLustal W) that may have utility in evaluating sequence-non-specific (or RISC-dependent) off-target effects. However, except for BLASTN, we have no present experience with these tools. Further, many are public web tools and outside the FDA's network. A sequence homology search of the antisense strand (A-116861) of fitusiran would include the seed region (between position 2-8 nt) and nucleotides 2-19 nt. Because the drug is proprietary, we did not examine the antisense strand (A-116861) of fitusiran using these tools.

The HUMAN PROTEIN ATLAS and GTEx analyses suggests that SERPINC1 mRNA (ENSG00000117601.13) is highly expressed in normal liver (600-1200 transcripts per million reads), compared to other tissues (e.g., gall bladder, stomach, and blood macrophages). Murine Serpinc1 mRNA is also highly expressed in mouse liver compared to other tissues (https://www.informatics.jax.org/marker/MGI:88095). And SERPINC1 is highly expressed in Hep-G2 cells and other liver cancer cell lines (see https://www.proteinatlas.org/ENSG00000117601-SERPINC1/cell+line). In the NDA 219019_off target study study-bio-13007Homo, Hep-G2 cells and reverse transcriptase-quantitative PCR (RT-qPCR) detection were used to assess i*n vitro* specificity of the active drug substance ALN-57213 and potential for RISC-dependent off-targets effects based on *in silico* predictions of the antisense strand (A-116861). Of the nine, potential off-target transcripts predicted from *in silico* analysis, TPD52L3 (Off-8) was reported not to be expressed in the Hep-G2 cells. This result was confirmed for Hep-G2 cells and normal hepatocytes cells using RNA sequence data and single-cell sequencing data https://www.proteinatlas.org/ENSG00000170777-TPD52L3/single+cell+type/liver).

Additional exploratory analyses of the remaining eight predicted off-target transcripts together with SERPINC1 using Ingenuity Pathway Analysis knowledge base (Qiagen LLC) suggests that five

transcripts or gene products (AKAP12, FRAS1, SERPINC1, PHIP, and TAPT) are associated with different toxicity endpoints and causal phenotypes including liver cancer, cirrhosis, and degeneration. Further, SERPINC1 and AKAP12 were associated with liver toxicological responses such as fibrosis and necrosis, respectively.

In the dose-response screen carried out by the sponsor only SERPINC1 was inhibited (IC50 of 59.4nM). No IC50 value was achieved for eight (INO80D, ZBTB40, AFAP1, FRAS1, AKAP12, PHIP, TAPT1, and ADO) of the 9 remaining off-target predicted transcripts. However, following additional statistical analysis inhibition of the off-target transcript 2-aminoethanethiol dioxygenase (ADO) was observed (36.49% +/- 14.552 (9=2). However, this level of inhibition was determined not to be therapeutically relevant as the *in vitro* experiment was conducted by transfecting Hep-G2 cells with high concentration of fitusiran, and this high exposure was not achieved in multiple *in vivo* studies with various animal models but was lower than the expected human exposure. In addition, the IC50 of the "off-target/ADO" was at least 200-fold higher than the absolute IC50 of SERPINC1. In animals, ADO has been identified as an important O_2-sensing enzyme and is essential for redox balance and prevention oxidative stress in mice (11, 12). ADO can also regulate the stability of G-protein signaling and interleukin-32 (13). ADO controls the proteasomal degradation of IL-32 in the presence of oxygen (14). While the physiological role of IL-32 remains to be further elucidated (13), strong associations between increased IL-32 expression and the severity of non-alcoholic fatty liver disease have recently been reported (15).

Non-sequence-non-specific off-target effects or other mechanisms

In vitro research also suggests that siRNAs compete with endogenous miRNAs for common cellular machinery or proteins including the RISC complex which can result in machinery saturation. MiRNAs are small non-coding RNAs that also regulate gene expression by repressing protein production via transcript destabilizing and translational silencing. Thus, loss of available RISC (and/or other proteins, e.g., Ago) for miRNA processing through competition with an siRNA will alter the repression of the target genes of endogenous miRNAs and constitutively upregulate the corresponding mRNAs and proteins, and downstream processes in cells/tissue (16, 17). Other off-target mechanisms may include activation of unintended gene expression via interaction with a gene promoters at the DNA level (18-21) and alterations in splicing regulation (22). We are not aware if these potential mechanisms were evaluated. For the latter, this would require additional computational screening of both strands (reverse transcribed) to the relevant reference genome and delivery of antisense and sense strands (or dsRNA) to the nucleus. Also, it is not known to what extent, therapeutic siRNAs participate in these phenomena. However, these effects should be considered in the analysis of the potential non-specificity of fitusiran in relation to potential liver toxicity and dysfunction, and differences across species.

Summary and suggestions for consideration:

The above information highlights several potential off-target effects or mechanisms that may be related to the non-specificity of fitusiran in the liver leading to tissue dysfunction and toxicity. To better evaluate both "sequence"-non-specific effects (RISC-dependent) and "non-sequence" or "other" off-target effects of fitusiran at a global comprehensive level (that will complement previous and/or future *in silico* prediction), you may consider requesting the sponsor to use total RNA-sequence analyses to better assess the potential hepatocyte or liver toxicity of fitusiran. Total RNA sequencing will allow a more comprehensive assessment of the whole transcriptome including mRNA transcripts and non-coding RNAs (including miRNAs) as well as inform on the indirect effect of altered mRNAs and gene

expression regulators (like miRNAs) on other transcripts. This will allow an agnostic, unbiased, and quantitative assessment of the potential off-target effects of fitusiran and allow confirmation of previous *in silico* prediction results and/or RT-qPCR results using an absolute measure rather than relative. For *in vitro* evaluation, the transcriptomic profile of the Hep-G2 cell line should be considered with respect to what genes are not expressed (and what genes are mutated in this cell line). Six liver cancer cells lines are known to express SERPINC1 mRNA (and ASGRP protein), albeit Hep-G2 cell has the largest transcript level (90.8 nTPM) of SERPINC1 (>8 fold change in number of transcript per million) compared to other cell lines (https://www.proteinatlas.org/) and is likely the best model to reflect the high abundance SERPINC1 in normal primary hepatocytes (single cell seq average= 2400 nTPM; https://www.proteinatlas.org/).

It is also noteworthy, that transcript levels by themselves are not sufficient to predict protein change. Changes in mRNA transcript levels do not definitively translate into or indicate a similar magnitude of protein level change for all mRNAs. Careful consideration of tissue-specific expression or regulation of a transcript (and its translation efficiency in that tissue) as well as the impact of a quantitative change in transcript levels (fold-change or expression difference) on protein level and function should also be considered. For example, INO80D is only lowly expressed at the transcript level in hepatocytes (e.g., RNA-seq [using HPA, GTEx and Fantom5 data] average nTPM = 3.6; single cell sequencing [The Human Protein Atlas] average nTPM = 8.6) but is highly expressed at the protein level indicating the potential for cell/tissue-specific translational differences across different transcripts. In these instances what might be considered a modest or insignificant change in transcript levels could have a significant impact on protein levels and thus downstream pathways and processes in the tissue or organ of interest.

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