CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

218614Orig1s000

OTHER REVIEW(S)

Division of Hepatology and Nutrition Consultation

Drug-Induced Liver Injury (DILI) Team

NDA (IND)	NDA 218614 (IND 136692)
Consultation Issue	Drug-induced liver injury (DILI)
Drug Product	Olezarsen (TRYNGOLZA)
Indication	Familial Chylomicronemia Syndrome
Applicant	Ionis Pharmaceuticals, Inc
Requesting Division	Division of Diabetes, Lipid Disorders and Obesity (DDLO)
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Signatory Authority	Paul H. Hayashi, MD, MPH Associate Director for DILI, OND/DHN
Assessment Date	Dec 13, 2024

Context: Olezarsen (OLZ) is a subcutaneously delivered antisense oligonucleotide (ASO) that targets apolipoprotein C-III (apoC3) mRNA. The Sponsor seeks NDA approval to treat familial chylomicronemia syndrome (FCS), a rare autosomal recessive disorder, also known as type I hyperlipoproteinemia or lipoprotein lipase (LPL) deficiency. The disease leads to severe hypertriglyceridemia with substantial morbidity and mortality. There are no approved therapies. ApoC3 inhibits lipoprotein lipase (LPL) hydrolysis of triglycerides. OLZ's ASO anneals to ApoC3 mRNA marking it for degradation, thereby decreasing ApoC3 synthesis and disinhibiting LPL, which should increase LPL hydrolysis (i.e., degradation) of triglycerides. Unlike volanesorsen (VLN), an ASO which also targets LPL mRNA and was not approved in the US (b) (4), OLZ has a N-acetyl-galactosamine-conjugate (GalNAc) conjugate that binds the asialoglycoprotein receptor (ASGR) on hepatocytes thereby delivering high levels of ASO specifically to liver cells where much of ApoC3 is expressed. The cell specific delivery is expected to overcome the thrombocytopenia risk of VLN.

Mean aminotransferase levels increased on OLZ compared to placebo in the FCS phase 1 and phase 3 trials as well as one prevalent hypertriglyceridemia (PHTG) trial under IND 136692. The Division of Diabetes, Lipid Disorders and Obesity (DDLO) requested the

DHN DILI Team assess DILI risk and need for liver enzyme monitoring. They also requested recommendations on labeling and the need for any post-market requirements or commitments.

Executive Summary: We did not see a substantial risk for acute liver injury that would hold up approval for treatment of FCS assuming efficacy is acceptable in meeting this unmet need. There were no subjects who developed jaundice and only two subjects that had ALT levels over 5x ULN. However, the number of FCS and PHTG subjects exposed to OLZ is small (<200) so cases of more substantial liver test elevations could occur post-market. Also, the frequent, chronic, and modest elevations in aminotransferases (AT) that occurred in healthy volunteers, FCS and PHTG subjects while on OLZ creates concerns for chronic liver injury. Possible explanations for the chronic AT elevations include fat deposition in the liver and the high levels of ASO delivered to hepatocytes. It is unclear whether any such effects on hepatocytes could lead to long-term, clinically important liver disease such as development of cirrhosis or chronic liver failure. Therefore, a PMR or PMC study should be considered to monitor for chronic liver disease development. Such a study would need annual liver imaging (e.g., ultrasound or MRI based imaging for hepatic fat and scar development) and periodic blood tests. Labeling should include description of AT increases, checking of baseline liver tests, and periodic rechecks that could coincide with standard of care triglyceride monitoring. Our full assessment and recommendations follow.

Consultation Sections:

Abbreviations:
ADA: anti-drug-antibody
ADME: absorption, distribution, metabolism, excretion
ALP or AP: alkaline phosphatase
ALT: alanine aminotransferase
ApoC3 (or ApoCIII): apolipoprotein C-3 (or C-III)
ASGR: asialoglycoprotein type 1 receptor
AST: aspartate aminotransferase
ASO antisense oligonucleotide
AT: aminotransferase (ALT and/or AST)
BMI: body mass index
CPK or CK: creatine phosphokinase
CT: computerized tomography
CVD cardiovascular disease
DB: direct bilirubin
DDI: drug-drug interaction
DILI: drug-induced liver injury

FCS: Familial Chylomicronemia Syndrome
GalNAc: N-acetyl galactosamine
GGT: gamma-glutamyl transferase
HTG: hypertriglyceridemia
IP: investigational product
IDL: intermediate-density lipoproteins
ISIS 678354: Olezarsen (OLZ)
MRE: magnetic resonance elastography
MRI: magnetic resonance imaging
MR-PDFF: Magnetic Resonance-Proton Density Fat Fraction
LPL: lipoprotein lipase
MOE: methoxyethyl
mRNA: messenger ribonucleic acid
OLZ: Olezarsen
PHTG: prevalent hypertriglyceridemia
SC: subcutaneous
TB: total bilirubin
(b) (4)
UDP: uridine 5'-diphospho-glucuronosyltransferase
US: ultrasound
ULN: upper limit of normal
VLN: volanesorsen

1.0 Target Disease and Rationale

1.1 Target Disease: Familial Chylomicronemia Syndrome (FCS) is a rare monogenic autosomal recessive endogenous lipid disorder, also known as type I hyperlipoproteinemia or lipoprotein lipase (LPL) deficiency (LPLD). Located in the vascular endothelium, LPL hydrolyzes triglycerides in peripheral circulation leading to their degradation. Thus, LPLD leads to the accumulation of chylomicrons and very-low density lipoprotein and severe hypertriglyceridemia (e.g., >1000 mg/dL) which manifests in a variety of ways including pancreatitis, xanthomas, cutaneous papules, lipemia retinalis and hepatosplenomegaly. It is suspected when such symptoms arise in younger patients with normal body mass index (BMI).[1] FCS is linked to least six gene mutations, only one of which involves the LPS gene.[2] While genetic testing is definitive, it is not widely available. The estimated prevalence of FCS varies from one in 300,000 to one in a million, bringing the totals to 300 to 1000 patients in the US.[3] In contrast, 26.8 million in

[1] Baass A et *al*. Familial chylomicronemia syndrome: an under-recognized cause of severe hypertriglyceridaemia. *J Intern Med* 2020; 287: 340–348.
[2] https://link.springer.com/article/10.1007/s40259-022-00520-2
[3] Orpha.net https://www.orpha.net/en/disease/detail/444490 (accessed Dec 1, 2024).

the US have prevalent hypertriglyceridemia (PHTG)[4,5,6] which is typically associated with other factors such as obesity, alcohol, insulin resistance or diabetes and has moderate hypertriglyceridemia (150-499 mg/dL). About 3.4 million PHTG patients have levels >500 mg/dL.

There are no FDA approved medications for FCS treatment. Strict dietary restriction of fat is needed to reduce pancreatitis risk. Statins, fibrates, niacin and proprotein convertase subtilisin-kexin type 9 inhibitors are ineffective as they do not impact chylomicron synthesis or release into circulation.

1.2 Rationale for Drug Use: OLZ is a third generation N-acetyl-galactosamine-conjugated (GalNAc), antisense oligonucleotide (ASO) targeting hepatic apolipoprotein C-III (apoC3) mRNA.[7] ApoC3 inhibits LPL hydrolysis and has other detrimental effects on cardiovascular health (**Figure 1**). OLZ has a ten-base, deoxyribonucleic acid (DNA), gapmer. The OLZ gapmer has flanking 2'-MOE (2'-o-(2-methoxyethyl) modified five nucleotide sequences (modified RNA).[8] The OLZ gapmer is then linked to a GalNAc amino sugar via a 5'-(b) (4) linker (**Figure 2**). GalNAc binds the hepatocyte asialoglycoprotein receptor (ASGR) which leads to intracellular ASO delivery. The ASO then anneals to the apoC3 mRNA triggering RNase H1 enzyme degradation of the mRNA, after which the ASO can bind another apoC3 mRNA.[9] Decline in apoC3 synthesis leads to disinhibition of LPL, which leads to increased TG hydrolysis and decline in serum TG levels (**Figure 1**)

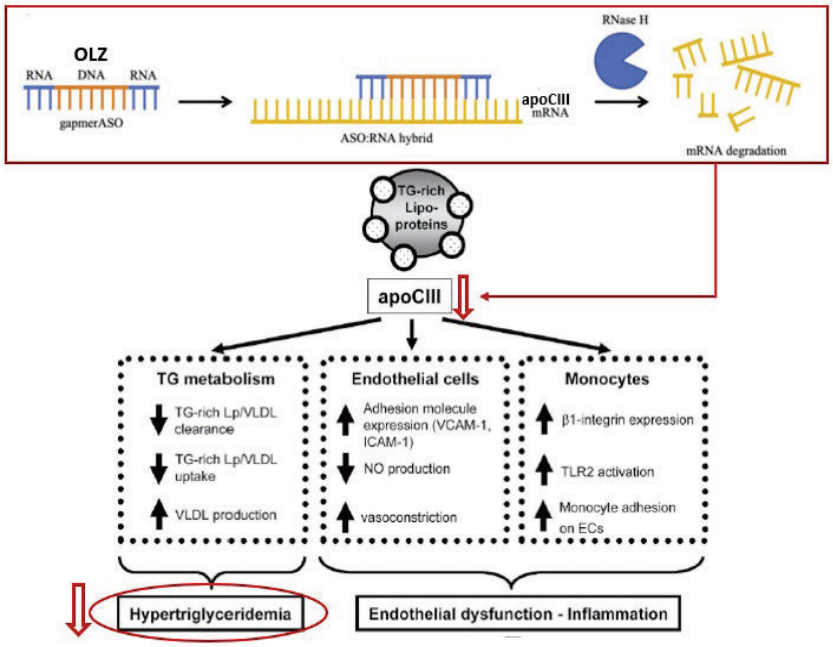

Figure 1: OLZ mechanism of action and downstream effects

[4] Gurevitz C, Chen L, Muntner P, Rosenson RS. Hypertriglyceridemia and Multiorgan Disease Among U.S. Adults. *JACC Adv*. 2024 Mar 25;3(5):100932. doi: 10.1016/j.jacadv.2024.100932. PMID: 38939631; PMCID: PMC11198666.
[5] Christian JB, Bourgeois N, Snipes R, Lowe KA. Prevalence of severe (500 to 2,000 mg/dl) hypertriglyceridemia in United States adults. Am J Cardiol. 2011 Mar 15;107(6):891-7. doi: 10.1016/j.amjcard.2010.11.008. Epub 2011 Jan 19. PMID: 21247544.
[6] https://doi: 10.1016/j.amjcard.2010.11.008.
[7] DOI: 10.1093/eurheartj/ehab820
[8] NDA218614 (218614 - 0018 - (18) - 2024-08-05 - TRIAGE-1 /Electronic Submission/Gateway) - Summary of Clinical Pharmacology Studies (#114)
[9] https://doi.org/10.1038/s43856-023-00419-1.

of ApoC3 decreased synthesis (red lines and arrow).[10]

OLZ's ASO DNA sequence is similar to the volanesorsen (VLN) ASO. The European Union conditionally approved VLN in 2019 for FCS, but the FDA did not due to safety concerns, particularly thrombocytopenia.[11] The Sponsor believes the OLZ GalNAc specific binding to hepatocytes provides more targeted deliver, longer efficacy at a lower dose (80 mg monthly versus 285 mg weekly), and lower thrombocytopenia risk compared to VLN. VLN does not use GalNAc. The **Appendix, Table A** compares these two compounds in more detail. (b) (4)

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2.0 Human ADME and DDI data pertinent to DILI

2.1 Structure and Dose: OLZ structural formula is in **Figure 2**. Proposed dose is 80 mg subcutaneous, every four weeks.

Antisense oligonucleotide

Figure 2: Chemical structure of olezarsen.

[10] Adapted from Investigational Brochure, p. 27 (IND 136692) and Shengging L, Hong L, Wei D-Q, et al. Deep learning facilitates efficient optimization of antisense oligonucleotide drugs. *Molecular Ther Nuc Acids*. 2024. https://doi.org/10.1016/j.omtn.2024.102208

[11] Esan O and Wierzbicki AS. Volanesorsen in the Treatment of Familial Chylomicronemia Syndrome or Hypertriglyceridaemia: Design, Development and Place in Therapy. *Drug Des Devel Ther*. 2020 Jul 6;14:2623-2636. doi: 10.2147/DDDT.S224771.

2.2 Absorption: Following subcutaneous (SC) injection, OLZ was rapidly absorbed into the systemic circulation with a Tmax of about 2 hours post dose. OLZ bioavailability has not been determined. The predicted accumulation was two to three-fold. Anti-drug antibodies (ADA) formed in one-third of healthy volunteers after 12 weeks of 60 mg given every 4 weeks. The sponsor reported no ADA impact on the peak plasma exposures, but higher C_{trough} levels occurred in ADA-positive subjects. The Sponsor reported no clinically relevant impact of ADA efficacy, safety, and pharmacodynamics. Thus, OLZ is rapidly absorbed and there is a prediction of mild to moderate accumulation. ADAs developed but are not predicted to have clinical impact.

2.3 Distribution: OLZ distributed primarily to the kidney and liver. Generally, ASO therapeutics readily distribute to tissues with discontinuous or fenestrated capillaries such as the liver and kidneys. Distribution to tissue was the dominant mechanism for plasma clearance. Human plasma protein binding was 99%.

2.4 Metabolism: Like other ASOs, OLZ is not expected to be metabolized by CYP enzymes. The unconjugated OLZ is slowly metabolized via endo- and exonucleases in tissue to shorter oligonucleotides which are excreted in urine, a process that usually takes 3-5 weeks. The GalNAc sugar rapidly undergoes endogenous carbohydrate catabolism, and the (b) (4) linker was extensively metabolized via oxidation and excreted more slowly. There were no human unique metabolites compared to animals.

2.5 Excretion: Following SC administration, time to steady state was about 20 weeks, consistent with a $T_{1/2}$ of 4 weeks. Thus, OLZ is slowly eliminated from tissues via urinary excretion of fragmented oligonucleotide metabolites which form via endo-and exonucleases. The GalNAc moiety is expected to be eliminated within 24 hours after each dose. In healthy volunteers, full-length OLZ was hardly detectable in urine (<1%) in the first 24 hours post dose, likely due to the rapid tissue uptake. Thus, OLZ rapidly taken up by tissue and slowly eliminated.

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

Table 1: ADME summary table[12]

Item	Finding
Absorption	Rapid tissue uptake from subcutaneous delivery
Distribution	Highly plasma protein binding
Metabolism	Via peripheral (vascular) endo- or exonucleases
Elimination	Urine elimination with estimated $T_{1/2}$ of 4 weeks

2.6 Drug-Drug Interactions (DDI): As an ASO, OLZ is not expected to have clinical important DDIs.

3.0 Non-clinical data

[12] Table made by DILI Team

3.1 In vitro data: Being an ASO, in vitro data specific to DILI risk and mechanisms of liver injury are limited. The Sponsor did not do neutralizing ADA assays because OLZ achieves its biological activity intracellularly. Anti-OLZ antibodies developed in 28% of animals receiving 30 mg/kg/week and 25% to 63% in those dosed with 2 to 12 mg/kg/week. There were no notable changes in exposures (C_{max} and AUC_{0-48}) or efficacy.

3.2 Animal data: For mice, seven mg/kg/week dosing for 13 weeks resulted in mononuclear cell infiltration, karyomegaly, nuclear degeneration with inclusions, and single cell necrosis in the liver along with 2-fold and 1.7-fold increases in ALT and AST, respectively. 26-week dosing produced hepatocellular hypertrophy, karyomegaly, mitotic figures, and vacuolated, granular macrophages along with reversible 6.5-fold and 2.6-fold increases in ALT and AST, respectively. One mouse had focal hepatocellular necrosis attributable to OLZ at 20 mg/kg/week dose. The Sponsor suggests the liver changes were in part due to the increased ASO delivery to hepatocytes via the GalNAc binding ASGR entry mechanism. There was partial resolution of the liver changes at the recovery period.

In monkeys, ≥ 6 mg/kg/week dosing for 13 to 39-week resulted in hepatocyte and Kupfer cell basophilic granules with Kupffer cell hypertrophy and glycogen vacuolation. After 13 weeks off drug, these findings partially reversed in the recovery compared to interim necropsies. The Sponsor attributed the slow recover to delayed product elimination. [25] Perivascular mononuclear infiltration was not as prominent compared to volanesorsen (VLN) treated monkeys. Following 39-week exposures, OLZ treated monkeys had small vascular infiltrates "*consisting of 1-2 cell layers in thickness"* that were not associated with *"pathologic alterations in the blood vessels."* In contrast, 39-week VLN exposure in monkey showed perivascular infiltration by neutrophils, eosinophils, macrophages, and lymphocytes, often 20 or more cell layers thick, surrounded blood vessels in organs including liver.[13]

3.3 Safety Margins: Sponsor reported safety margins relative to the (b) (4) OLZ clinical dose in **Table 2**.

Table 2: Non-clinical safety margins for OLZ at planned clinical dose (based on plasma AUC).[14]

NOAEL[a] 6 mg/kg/wk (24 mg/kg/month)	Plasma AUC_{0-48h} (µg*h/mL)	Estimated Therapeutic Index[b]	
		(b) (4)	80 mg/month (5.5 µg*h/mL)[c]
Mouse	14.3 (57.4)[d]		2.6-fold (10.4-fold)[f]
Monkey	141 (564)[e]		25.6-fold (103.0-fold)[f]

[13] IND115063 (115063 - 0218 - (218) - 2022-10-21 - Multiple Submissions /Multiple Categories/Subcategories) - 304801-as11--monkey-39wk-repeat-dose-sc-20wk-interm-necropsy-26wk-recovery (#286)
[14] Investigator brochure, page 59.

Our summary of non-clinical findings related to DILI risk is in **Table 3**.

Table 3: Summary of non-clinical data pertaining to DILI risk [15]

Item	Findings
In Vitro Studies	
Major CYPs or UGTs	N/A
Reactive metabolites (i.e., glutathione trapping)	N/A
Mitochondria studies/inhibition	N/A
Time dependent inhibition	N/A
LogP (lipophilicity) values >3 associated with increased DILI risk	N/A
Covalent binding	N/A
Transporter inhibition (BSEP or MRP2 inhibition may increase risk of DILI)	No evidence of such inhibition
Animal Studies	
Elevation in liver analytes (e.g., ALT, AP, TB)	Transaminase increased
Liver histopathology findings (animal species)	Single cell necrosis and mononuclear cell infiltration in mice. accumulation of basophilic granules in hepatocytes and Kupffer cells in monkeys

3.4 Division of Applied Regulatory Science (DARS) Consult

The DILI Team consulted DARS regarding the potential for OLZ hepatotoxicity both acute and chronic. See the Attached for the complete DARS consult, but we give a brief overview here.

Overall, DARS did not find evidence of OLZ having "on-target or non-specific off-target sequence effects on mRNAs in the liver that may be related to hepatic/hepatobiliary dysfunction and toxicity." Volanesorsen decreased hepatic fat presumably by APOC3 production. Their review did not suggest hepatotoxicity from the (b) (4) linker. They found no evidence of acute hepatotoxicity due to GalNac-ASGR mediated high uptake or slow release of ASOs from the liver. Nevertheless, DARS found little information on the chronic effects that ASO use may have on the liver stating, "*Whether increased tissue concentrations of conjugated-ASO and non-conjugated ASOs in endosomes may have implications for malfunction of the endosomal-lysosome systems is not known, but malfunction of this system(s) by other mechanisms is associated with liver disease such as non-alcoholic fatty liver disease.*" Indeed, hepatocyte endosomes may serve as substantial depots for ASO like OLZ (**Figure 3**) allowing for long drug duration with a "*rate of endosomal escape (or maximal response in humans) of Olezarsen … not defined*."

[15] Table made by DILI Team

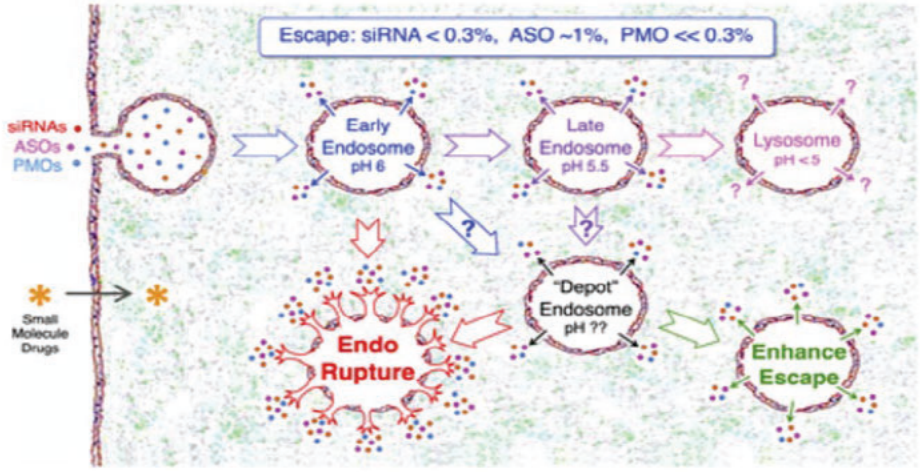


Figure 3: Potential "Depot Endosome" formation for siRNAs (small interfering RNAs), ASOs and PMOs (phosphorodiamidate morpholino oligomer) versus small molecule drugs that get to the cytoplasm without endosome processing.[16]

4.0 Clinical data:

4.1 In class or near class data:
The liver toxicity profile of marketed ASOS based on labeling and Liver Tox® are summarized below (**Table 4**).

Table 4: Approved ASO drugs with DILI information by label and LiverTox®[17]

Drug (Brand), Target Disease(s)	**ASO structure & target**	**Approval year(s),[18,19] Indication**	**Labeling regarding DILI in** Box Warning (BW) Warnings/Precautions, Highlights (W/P, HL) Adverse Reactions, Highlights (AR, HL) Sections 5 & 6 (S5 & S6)	**LiverTox® category[20]**
Eplontersen **(WAINUA)** Hereditary Transthyretin Amyloidosis (HTA)	GalNAc conjugate degradation of TTR mRNA	2023 (HTA)	**BW**: No W/P, HL: No AR, HL: No S5: No S6: No	N/A
Casimersen **(AMONDYS 45)** Duchenne Muscular Dystrophy (DMD)	Binds to exon 45 of dystrophin pre-mRNA	2021 (DMD)	**BW**: No W/P, HL: No AR, HL: No S5: No S6: No	E: unlikely cause of clinically apparent liver injury
Viltolarsen **(VILTEPSO)** Duchenne Muscular Dystrophy (DMD)	Binds to exon 53 of dystrophin pre-mRNA.	2020 (DMD)	**BW**: No W/P, HL: No AR, HL: No S5: No S6: No	E: unlikely cause of clinically apparent liver injury

[16] Dowdy SF. Endosomal escape of RNA therapeutics: How do we solve this rate-limiting problem? *RNA*. 2023; 29: 396-401
[17] LiverTox® https://www.ncbi.nlm.nih.gov/books/NBK547852/
[18] https://www.centerwatch.com/directories/1067-fda-approved-drugs/listing/4197-skyrizi-risankizumab-rzaa
[19] FDA Approved Oligonucleotide Drugs (bocsci.com)
[20] LiverTox® https://www.ncbi.nlm.nih.gov/books/NBK547852/

Golodirsen **(VYONDYS 53)** Duchenne Muscular Dystrophy (DMD)	Binds to exon 53 of dystrophin pre-mRNA on the DMD gene.	2019 (DMD)	**BW**: No W/P, HL: No AR, HL: No S5: No S6: No	E: unlikely cause of clinically apparent liver injury
Inotersen **(TEGSEDI)** Hereditary Transthyretin Amyloidosis, Polyneuropathy (HTA)	Targets TTR mRNA	2018 (HTA)	**BW**: No W/P, HL: Yes, Liver injury, monitor liver tests. AR, HL: No S5: Yes, liver injury, monitor liver tests S6: Yes, Liver injury	NA
Nusinersen **(SPINRAZA)** Spinal Muscular Atrophy (SMA)	Increases exon 7 inclusion in SMN2 (mRNA) protein production	2016 (SMA)	**BW**: No W/P, HL: No. AR, HL: No S5: No S6: No	NA
Eteplirsen **(EXONDYS 51)** Duchenne Muscular Dystrophy (DMA)	Binds to exon 51 of dystrophin pre-mRNA.	2016 (DMA)	**BW**: No W/P, HL: No. AR, HL: No S5: No S6: No	E: unlikely cause of clinically apparent liver injury
Mipomersen **(KYNAMRO)** Homozygous Familial Hypercholesterolemia (HFH)	Targets (mRNA) for apo B-100	2013 (HFH)	Voluntarily withdrawn by Sponsor[21]; Hepatotoxicity (fatty liver) risk labeled	NA Off the market
Fomivirsen **(VITRAVENE)** Cytomegalovirus retinitis (CMVr)	Inhibits replication of human cytomegalovirus	1998 (CMVr)	Off the market due to the prevalence of highly active antiretroviral therapy (HAART) that significantly reduced the incidence of AIDS-related CMVR. [22]	NA Off the market

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
HTA: Hereditary Transthyretin Amyloidosis,
DMD: Duchenne Muscular Dystrophy,
SMA: Spinal Muscular Atrophy, HFH: Homozygous Familial Hypercholesterolemia

4.2 Study Level Data

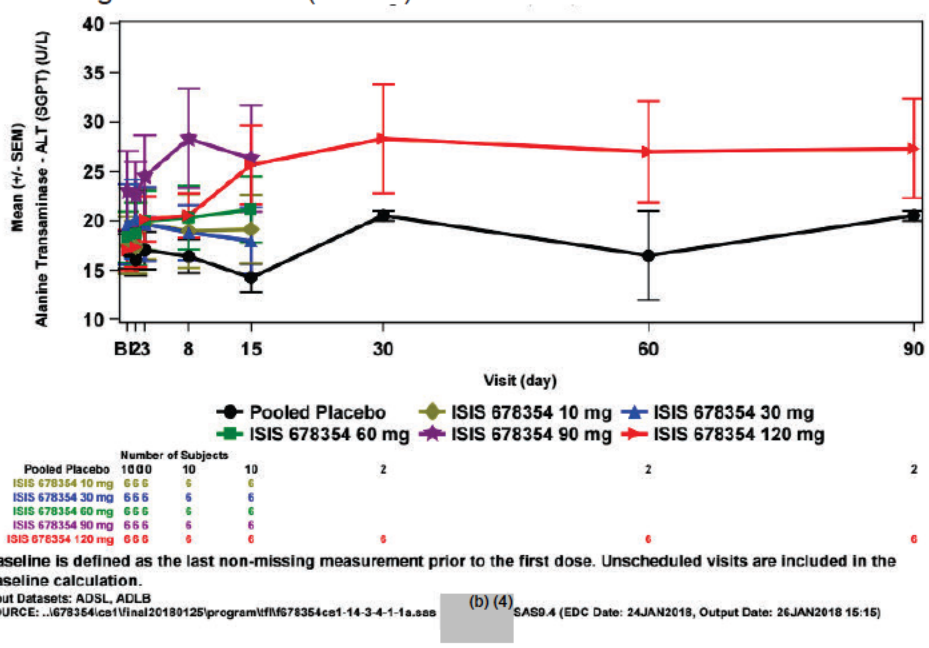
4.2.1 *Phase 1 Studies*: We focused on two phase 1 studies that enrolled "healthy" volunteers. Study 678354-CS1[23] subjects had hypertriglyceridemia while Study AKCEA-

[21] https://www.govinfo.gov/content/pkg/FR-2019-07-03/pdf/2019-14219.pdf
[22] Bege M and Borbas A. Rise and fall of fomivirsen, the first approved gene silencing medicine – A historical review. *Acta Pharmaceutica Hungarica*. 2022; 92:38-44. DOI: 10.33892/aph.2022.92.38-44
[23] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 16.1.1 Protocol and Protocol Amendments

CS1[24] had subjects with and without hypertriglyceridemia. The latter study occurred in Japan. Schematics for both studies are in the **Appendix, Figures A and B**.

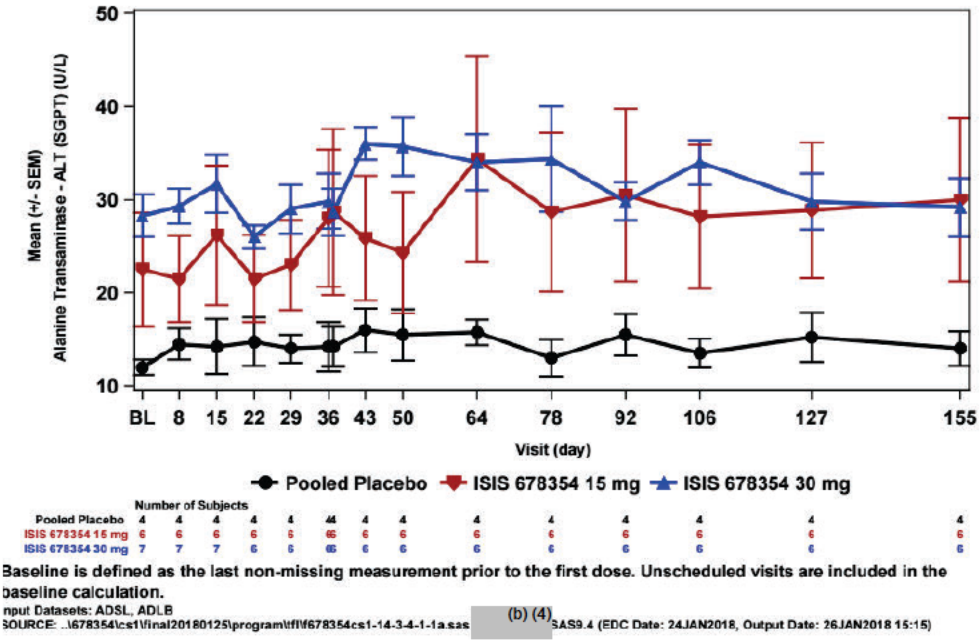
4.2.1.1 *Study 678354-CS1 (phase 1)*: Mean ALT levels increased in the single, weekly, and monthly dosing cohorts on OLZ compared to placebo (**Figure 4**). Mean AST changes were similar (data not shown). While mean ALT values on OLZ were typically <40 U/L, they were often two to three times the mean values on placebo, and the standard deviation whiskers suggest some subjects had values >50-60 U/L. We presume the baseline higher mean ALT and AST for those on OLZ in the weekly dosing data is due to subjects in the single dose cohort moving on to weekly dosing (**Figure x, (b)**). The protocol does not explicitly exclude such enrollment from the single dose cohort to the multiple dose cohorts. Otherwise, the different mean baseline values in these weekly dosing data are unexplained, and the values remained elevated over time. No obvious difference was seen with either alkaline phosphatase, total and direct bilirubin (data not shown).

a Single dose cohort (N = 40)[25]



(b) Weekly dose multiple dose cohorts (N = 17)[26]

[24] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 16.1.1 Protocol and Protocol Amendments (#300)
[25] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 14 Tables, Figures and Graphs (#2756)
[26] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 14 Tables, Figures and Graphs (#2776)



(c) Monthly multiple dose cohorts (N=10)[27]

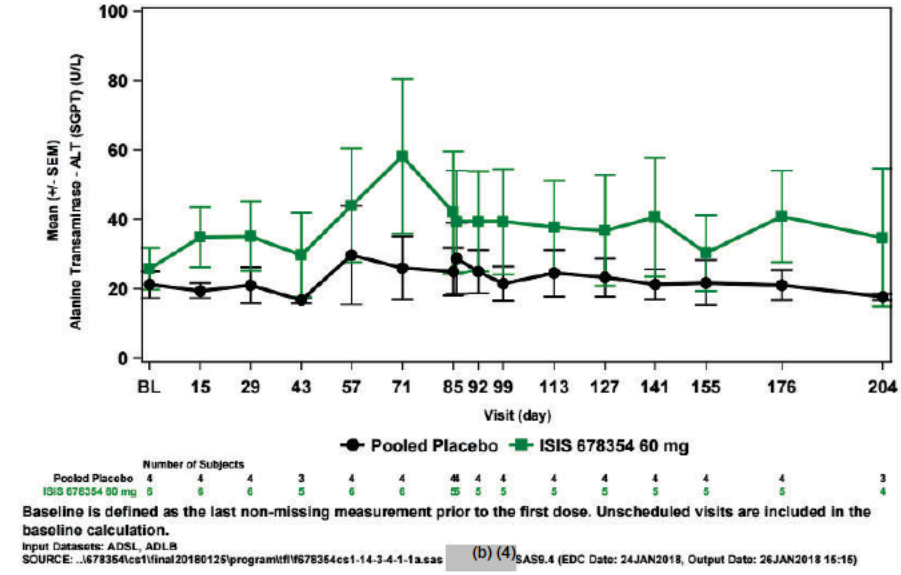


Figure 4: Mean change in ALT for Phase 1 studies, (a) Single dose, (b) weekly, multiple dose and (c) monthly multiple dose cohorts.

4.2.1.2 *Study AKCEA-CS1* (phase 1) (Japan): We did not find figures for mean liver blood test levels over time in the "Study Report Body Chapter." However, tabular data on mean and percent change in ALT were available. Like Study 678353-CS1, mean ALT values for the single dose cohort were similar between OLZ and placebo groups, but mean baseline ALT values were higher for the OLZ group in the multiple dose data (**Appendix, Tables B and C**). Thereafter, the percent change from baseline varied over time but tended to

[27] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 14 Tables, Figures and Graphs (#2796)

increase and increase more for OLZ group compared to placebo in the multiple dose data. For example, at Day 99, those on OLZ, 60 mg every four weeks had mean percent change in ALT from baseline of +55%, compared to -27% for those on placebo (**Table x**).

Table 5: Mean ALT change from baseline (absolute and percent) for subjects treated with placebo versus OLZ 60 mg every four weeks.[28] (ISIS 678354 = OLZ)

Test: Alanine Aminotransferase (U/L)

Visit/Time Point	Value	Statistic	Placebo Every 4 Weeks (N=2)	ISIS 678354 60 mg Every 4 Weeks (N=6)	Overall (N=8)
Day 99, 336 h	Change from Baseline	n	2	6	8
		Mean	-6.0	19.2	12.9
		SD (SEM)	5.66 (4.00)	44.80 (18.29)	39.68 (14.03)
		Median (Q1, Q3)	-6.0 (-10.0, -2.0)	9.0 (-17.0, 46.0)	-1.0 (-13.5, 32.0)
		Minimum	-10	-26	-26
		Maximum	-2	94	94
	Percent Change from Baseline	n	2	6	8
		Mean	-26.9	55.2	34.7
		SD (SEM)	16.32 (11.54)	149.12 (60.88)	131.79 (46.59)
		Median (Q1, Q3)	-26.9 (-38.5, -15.4)	20.0 (-63.0, 83.6)	-7.7 (-50.7, 61.8)
		Minimum	-38	-65	-65
		Maximum	-15	336	336

4.2.2 *Phase 2 and 3 studies*: We focused on the phase 3 study ISIS 67835-CS3 (CS3) and phase 2 study ISIS 67835-CS8 (CS8) in alignment with the primary review team's safety analyses. Both studies had completed the treatment periods at the data cut date. CS8 enrolled 154 subjects with prevalent chylomicronemia syndrome (CS), aka prevalent hypertriglyceridemia. The CS8 study population was older, had higher BMI, and more cardiovascular disease compared to the CS3 enrollees (**Appendix, Table D**).

4.2.2.1 *Study CS3 (Phase 3, FCS subjects)*: *A randomized, double-blind, placebo-controlled, phase 3 study of OLZ administered subcutaneously to patients with FCS*.[29]

Study Design: The study included two OLZ treatment arms, 50 mg and 80 mg given every four weeks for 53 weeks and a placebo arm (**Figure 5**). The study enrolled 66 subjects.

[28] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 14.3 Safety Data Tables (#482)
[29] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS3 - 16.1.1 Protocol and Protocol Amendments (#3)

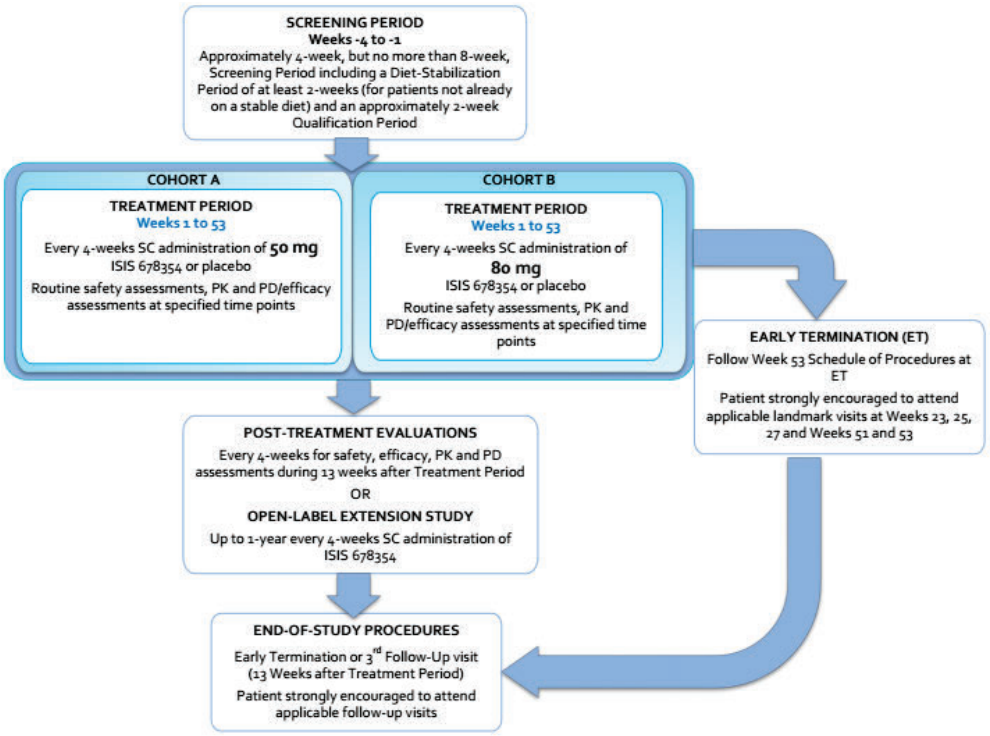


Figure 5: Study CS3 schematic.[30]

Schedule for liver blood tests (chemistry panel) assessments are shown in **Table 6**,

Table 6: Liver blood test schedule (chemistry panel). For footnote explanations please see reference.[31]

	Screening		Treatment Period																	Follow-up Period		
Study Week	Run-in[A] -4 to -2	Qual -2 to -1[O]	1	5[O]	9[O]	13[O]	17[O]	21[O]	23/ET -L1[O,R]	25/ET -L2[O,R]	27/ET -L3[O,R]	29[O]	33[O]	37[O]	41[O]	45[O]	49[O]	51/ET -L4[O,R]	53/ET-L5[O,R] or Tx ET[O]	4[O,P]	8[O,P]	13[O,P] / Post-Tx ET
Chemistry Panel[I,J]	X		EVERY 14 DAYS (± 2 days)[O]				X	X		X		X	X	X	X	X	X		X	X	X	X

Liver blood test data capture: The proportion of subjects with ALT was adequate through week 53 (**Figure 6**). Proportions with AST, ALP, and TB data were similarly adequate.

[30] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS3 - 16.1.1 Protocol and Protocol Amendments (#130)
[31] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS3 - 16.1.1 Protocol and Protocol Amendments (#301)

Figure 6: proportions of subjects with ALT data by study week and treatment arm for Study C3.[32]

Mean change in liver blood tests: Similar to phase 1 data, the mean ALT levels increased compared to placebo while subjects were on OLZ, but the mean change from baseline were less prominent (up to 10 U/L) and only evident at the 80 mg dose (**Figure 7**). AST changes had a similar pattern, but the increases did not appear until week 33 (data not shown). There were no differences in mean changes for ALP and TB.

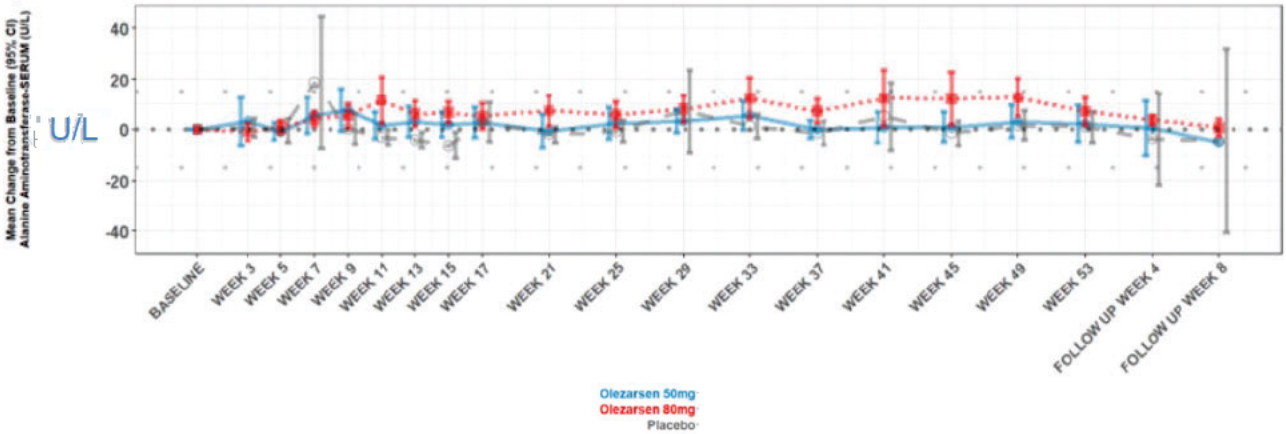


Figure 7: ALT mean changes from baseline in Study CS3 by study week and treatment arm.[33]

Hepatocellular (eDISH) and cholestatic DILI screening plots: There were no subjects with jaundice (TB >2x ULN) and therefore, no subjects plotting to potential Hy's Law (**Figure 8**). While there was a modest shift toward higher ATs in the 80 mg group compared to placebo, there was only one subject on OLZ in Temple's Corollary compared to two on placebo, and the two placebo subjects had the higher peak AT levels. No subjects on OLZ

[32] CDS Safety Table and Figures.
[33] CDS Safety Tables and Figures

plotted outside the left lower quadrant on the cholestatic DILI screening plot (Figure not shown).

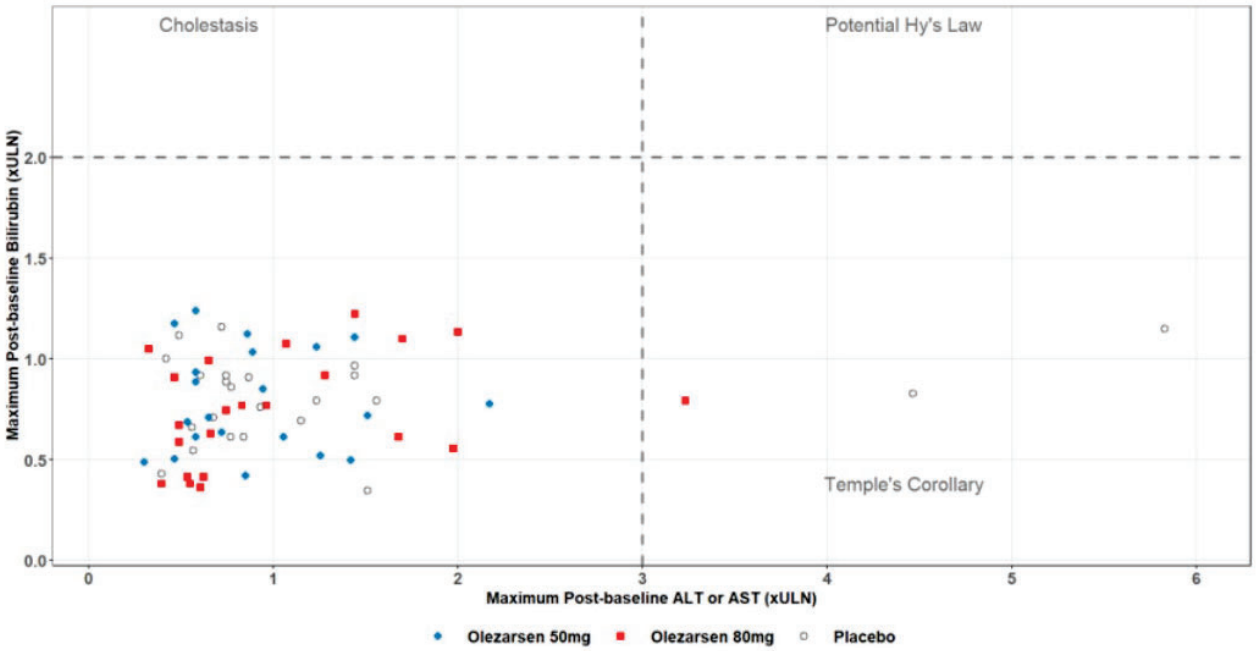


Figure 8: Hepatocellular DILI screening plot (eDISH) for CS 3.[34]

Serious adverse events (SAE): There was one subject on OLZ with an SAE of cirrhosis arising during Study CS3 while one subject on placebo had gastric varices as an SAE (**Appendix, Table E**).

4.2.2.2 *Study CS8 (Phase 2, prevalent HTG): A Randomized, Double-blind, Placebo-Controlled, Phase 2b Study of ISIS 678354 in Patients with Hypertriglyceridemia and Atherosclerotic Cardiovascular Disease (Established or at Increased Risk for), and/or with Severe Hypertriglyceridemia*

Study Design: The trial randomized subjects 1:1 to Cohorts A (50 mg weekly) and B (80 mg weekly). Within each Cohort, subjects were randomized 3:1, OLZ to placebo (**Figure 9**).

[34] CDS Safety Tables and Figures

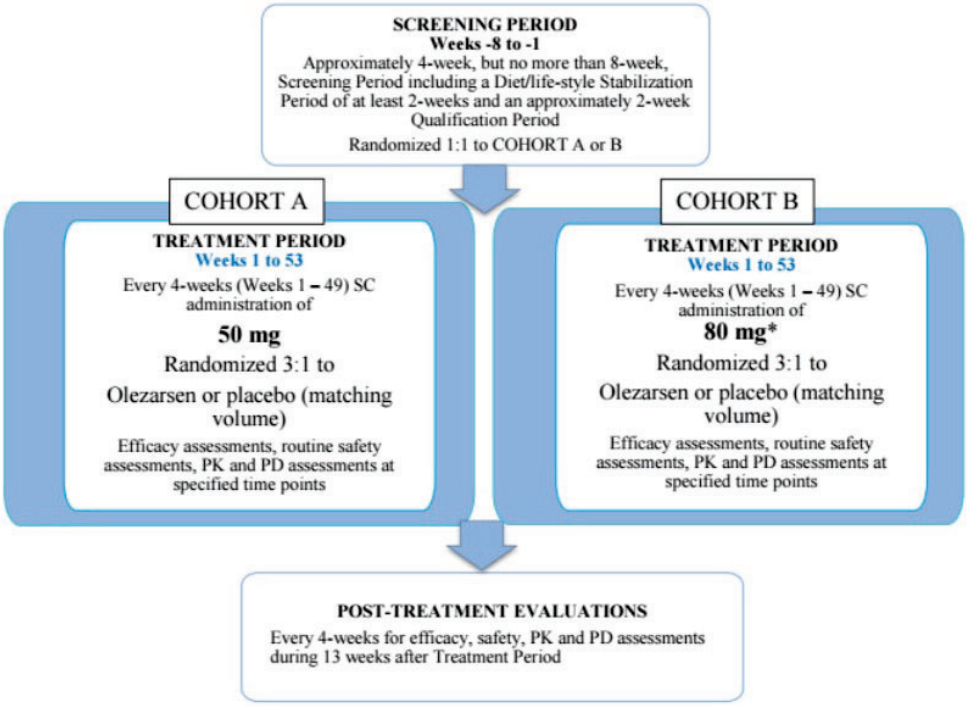


Figure 9: Schematic for Study CS8.[35]

Schedule for liver blood tests (chemistry panel) assessments are shown in **Table 7**. The DILI Team did not find proportions of subjects with liver blood test data over time in the CDS Tables and Figures report for CS8.

Table 7: Liver blood test schedule (chemistry panel). For footnote explanations please see reference.[36]

	Screening[A]		Treatment Period																Follow up Period		
Study Week	Run-in	Qual[M]	1	5[M]	9[M]	13[M]	17[M]	21[M]	25/ET-L1[M,N]	27/ET-L2[M,N]	29[M]	33[M]	37[M]	41[M]	45[M]	49[M]	51/ET-L3[M,N]	53/ET-L4[M,N] or Tx ET[M]	4[M,O]	8[M,O]	13[M,O] or Post-Tx ET[M]
	-4 to -2	-2 to -1																			
Study Day	-28 to -15	-14 to -1	1[B]	29	57	85	113	141	169	183	197	225	253	281	309	337	351	365	28	56	91
Visit and Testing Window ± Days	-28	0	-3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Chemistry[G,H]	X		X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X

Mean change in liver blood tests: Similar to the phase 1 and CS3 studies, mean change in ALT from baseline was higher for subjects on OLZ compared to those on placebo (**Figure 10**). The increase in mean ALT tended to be higher in the 50 mg group but this difference dissipated through the trial.

[35] NDA218614 (218614 - 0012 - (12) - 2024-07-22 - ORIG-1 /Quality/Response To Information Request) - ISIS 678354-CS8 - 16.1.1 Protocol and Protocol Amendments (#17)
[36] Adapted from NDA218614 (218614 - 0012 - (12) - 2024-07-22 - ORIG-1 /Quality/Response To Information Request) - ISIS 678354-CS8 - 16.1.1 Protocol and Protocol Amendments (#75)

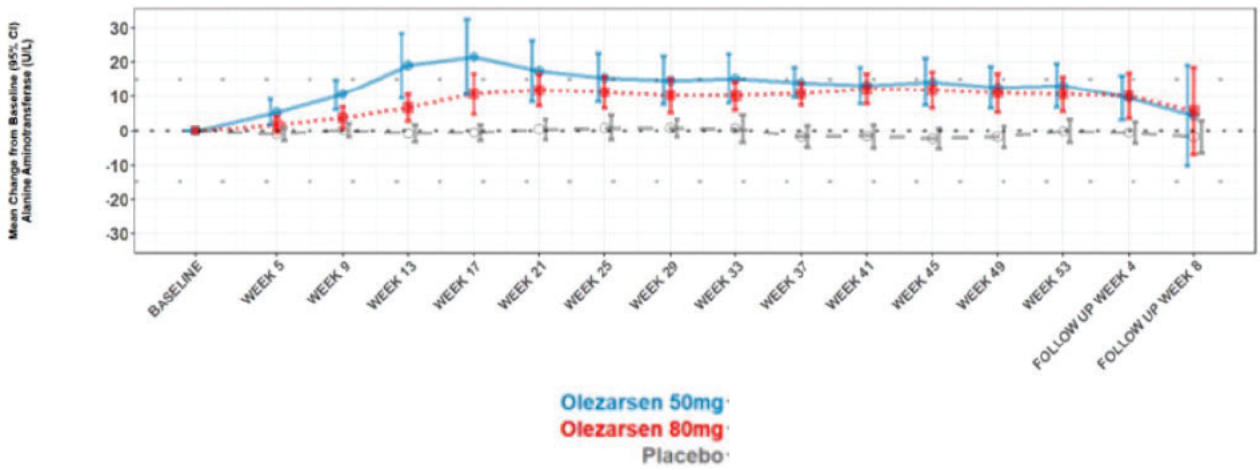


Figure 10: ALT mean changes from baseline in Study CS8 by study week and treatment arm.[37]

Hepatocellular (eDISH) and cholestatic DILI screening plots: On eDISH, AT levels tended to be higher on OLZ compared to placebo (**Figure 11**). There were four subjects plotting to Temple's Corollary quadrant compared to one subject on placebo. We assessed the three with the highest ALT levels as probable DILI due to OLZ (See **Section 4.3.2** for details), but there were no subjects with jaundice and therefore no Hy's Law cases. Indeed, there were no subjects on OLZ with TB >1.5x ULN. There

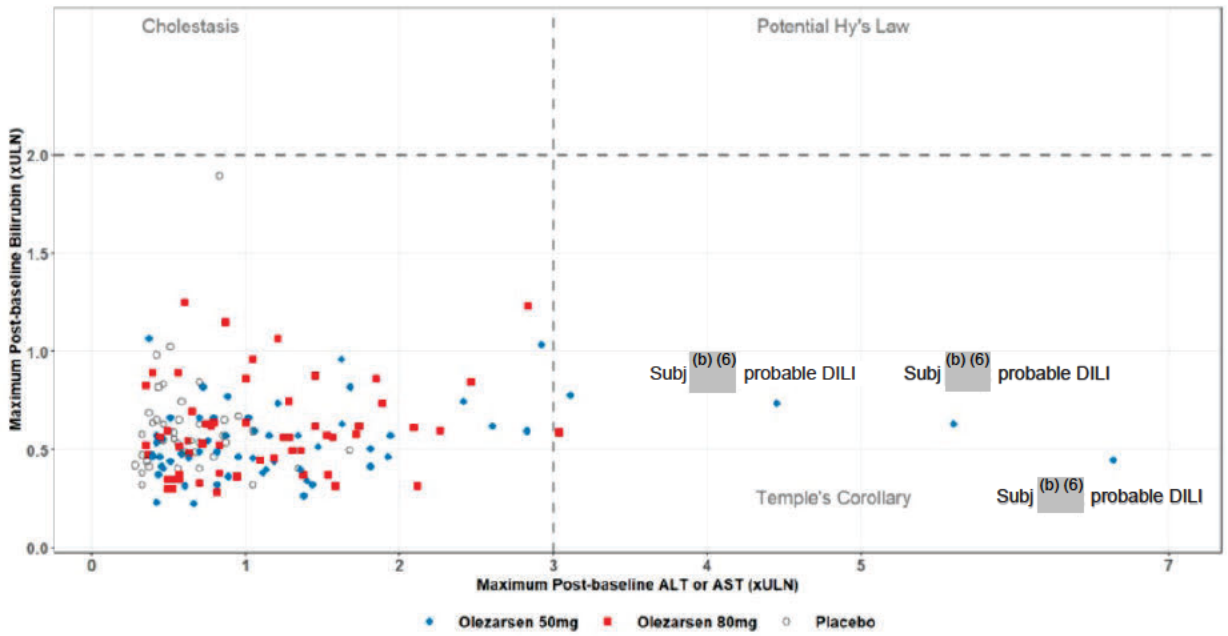


Figure 11: Hepatocellular DILI (eDISH) screening plot for CS8.[38]

Adverse events: There were no liver related serious AEs or AEs leading to drug discontinuation. There were higher rates of hepatitic injury AEs in the OLZ groups (6.9% for 50 mg; 5.3% for 80 mg) compared to placebo (2.6%). Under common adverse events,

[37] CDS Tables and Figures
[38] CDS Tables and Figures, Jul 17, 2024

ALT increased also had a higher rate in the OLZ groups (5.2% for 50 mg; 5.3% for 80 mg) compared to placebo (0%).[39]

4.3 Case level data: We assessed seven subjects who discontinued OLZ for liver test abnormalities in the two phase 1 and 2 studies. There were no subjects in the FCS phase 3 study that discontinued OLZ or had ALT or AST elevations >5x ULN. Phase 1 studies enrolled "healthy" volunteers with or without prevalent HTG. Narrative details and evaluation testing were sparse for the phase 1 volunteer cases.

4.3.1 *Phase 1 cases*:

1. *Subject* (b) (6) *(Study 678353-CS1)*: Probable hepatotoxicity due to OLZ.

Summary: This subject was a 50-year-old obese white male who had a normal ALT at baseline, but then developed ALT elevations starting on Day 15. ALT peaked at just over 150 U/L on Day 70 shortly after his third and last injection of OLZ 60 mg. ALT improved but remained elevated through last study visit despite dose discontinuation (**Figure 12**, brown line).

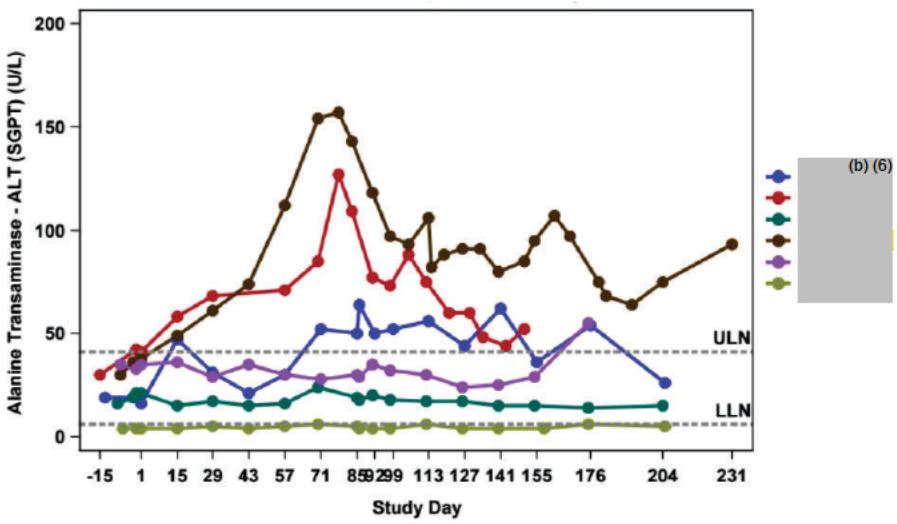


Figure 12: ALT levels for OLZ 60 mg treatment group (n = 6), Study ISIS 678354. Subject (b) (6) (brown line) had OLZ stopped for ALT elevations.[40]

Assessment: Based on latency, partial dechallenge washout of ALT, and elevation in mean ALT levels in the phase 1 studies, we assessed this as probable hepatotoxicity due to OLZ.

2. *Subject* (b) (6) *(Study 678353-CS1)*: Unlikely hepatotoxicity due to OLZ.

Summary: This subject was a 47-year-old white male, BMI of 27.5 kg/M^2 had ALT elevations on Day 15, peaking on Day 70 at 125 – 130 U/L (**Figure 12 above, red line**). However, maximum ALT elevations with a CPK elevation to >5000 U/L. AST was modestly elevated 80-90 U/L. The enzyme elevations were attributed to exercise and alcohol use.

[39] CDS Tables and Figures, Jul 17, 2024
[40] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 14 Tables, Figures and Graphs (#3362)

Assessment: Based on the concurrent CPK elevations, we assessed this case as unlikely DILI due to OLZ.

3. *Subjects* [redacted] *and* [redacted] *(Study AKCEA-CS1):* We found no narrative or clinical details for these two subjects with modest ALT and AST elevations but assessed them both as possible DILI due to OLZ based on latency and dechallenge decline in liver tests. OLZ was discontinued for both subjects due to enzyme elevations (**Table 8**).[41]

Table 8: Liver enzymes by study day for Subjects [redacted] & [redacted], Study AKCEA-CS1

	Subject [redacted]				Subject [redacted]			
Dose	Study Day	ALT, U/L (ULN 55)	AST, U/L (ULN 39)	ALP U/L	Study Day	ALT, U/L (ULN 55)	AST, U/L (ULN 39)	ALP U/L
	0	65	34	89	0	28	20	60
Dose 1	Predose	55	31	85	Predose	28	20	61
	15	50	28	90	15	32	19	57
Dose 2	29	61	27	89	29	44	29	63
	43	139	42	75	43	157	33	60
Dose 3 held	60							
	71	134	56	99	71	136	74	59
Dose 4 held	90							
	86	105	45	102	86	121	52	60
	113	105	43	104	113	121	77	71
	127	133	64	99	127	90	71	70
	141	81	32	109	141	111	61	59
	176	38	20	94	176	62	41	57
	204	86	39	101	204	43	30	67

4.3.2 Phase 2 (CS8) cases: We assessed two subjects with ATs >5x ULN and one subject with chronic AT elevations as probably related to OLZ. OLZ was discontinued in all cases.

1. *Subject* [redacted] *(Study CS8): Probable DILI due to OLZ.*

Summary: This is a 73-year-old female, white, with hypertriglyceridemia and atherosclerotic cardiovascular disease, who developed elevated aminotransferases approximately 88 days after starting OLZ. Initial dose was 50 mg SC every four weeks.

At baseline, the subject's BMI was 25 kg/m^2. Relevant medical history, besides the target disease, included hypothyroidism, atrial fibrillation, and cardiac pacemaker insertion. Alcohol history was not provided. Concurrent medications relevant to DILI risk were unremarkable as all of them continued through the liver injury event, except for a COVID19 booster (see below). The subject's ALT, AST, AP, and TB were 18 U/L, 26 U/L, 54 U/L, and 0.47 mg/dL, respectively. Prior to OLZ start she had COVID19 on [redacted] (Day -27); she was asymptomatic and treated with nirmatrelvir/ritonavir.

[41] Table made by DILI Team

The subject started study drug (OLZ) 50 mg Q 4 weeks on [redacted] (b) (6) (Day 1). She did well but on [redacted] (b) (6) (Day 63), ALT was up to 44 U/L; AST was also modestly increased at 36 U/L. AP and TB were stable. No symptoms were mentioned. The study drug continued without change. On [redacted] (b) (6) (Day 83), she received a COVID-19 vaccine booster. On [redacted] (b) (6) (Day 94), ALT, AST, AP, and TB were 199 U/L, 134 U/L, 75 U/L and 0.51 mg/dL, respectively. Still no symptoms were mentioned. There was no mention of study drug change. Her fourth dose of OLZ was given that day, but by [redacted] (b) (6) (Day 99), she had fatigue and by [redacted] (b) (6) (Day 105), ALT was up 205. The study drug was held. Liver tests would peak at ALT of 352 U/L and AST 240 U/L on [redacted] (b) (6) (Day 117). TB and ALP remained normal. Thereafter, liver enzymes improved with >50% decline from peak for ALT, AST occurring within 8 to 16 days. Return to baseline occurred within 38-44 days after peak values. (**Figure 13**) No further OLZ was given. Evaluation testing included negative HBsAg, HAV IgM, CMV and HCV antibody. HCV RNA testing was not done. Autoimmune markers (ANA, ASMA) were negative. IgG level was not checked. Liver imaging was done by US and CT; results showed possible fatty liver without acute changes.

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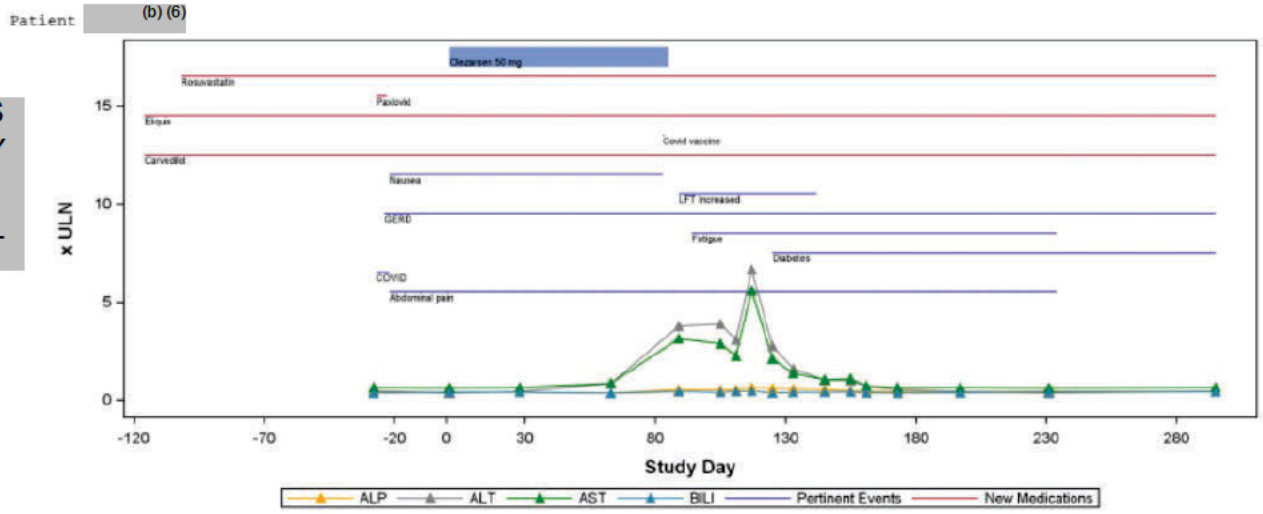

Figure 13: Liver test line graph by study day for Subj [redacted] (b) (6). [42]

Assessment: We assessed this case as a probable to strong possible DILI due to OLZ; Latency and final dechallenge washout consistent with DILI. COVID 19 booster competes, but ALT had already increased to 44 U/L from baseline of 18-23 on [redacted] (b) (6), before the booster shot. COVID-19 vaccine injury is reported by quite rare considering the number of vaccines given worldwide. A population-based study from Hong Kong suggests no higher liver injury incidence above baseline, before vaccination.[43] This modest elevation would be consistent with the elevation in mean ALT for those on OLZ with a subsequent resolution off drug. Evaluation testing partially complete; no HCV RNA or

[42] NDA218614 (218614 - 0016 - (17) - 2024-08-02 - ORIG-1 /Clinical/Response To Information Request) - Response to Information Request of 25July2024 - Clinical Safety (#10)

[43] Wong CKH, et al. Risk of acute liver injury following the mRNA (BNT162b2) and inactivated (CoronaVac) COVID-19 vaccines. *J of Hep*. 2022; 77:1339-1348.

EBV testing was done, but there were HCV risk factors identified, and no fever or systemic symptoms to suggest EBV other than fatigue.

2. *Subject* (b) (6) *(Study CS8): Probable DILI due to OLZ.*

Summary: This is a 44-year-old male, white, ethnic Hispanic, with hypertriglyceridemia and atherosclerotic cardiovascular disease; he developed elevated aminotransferases approximately 28 days after starting OLZ.

At baseline, the subject's BMI was 32 kg/m^2. Relevant medical history, besides the target disease, included hypertension and prediabetes. Alcohol history was not provided. There were no concurrent medications relevant because all were started more the two years prior and/or continued through and after the liver injury event. The subject's ALT, AST, AP, and TB were 31 U/L, 25 U/L, 59 U/L, and 0.5 mg/dL, respectively.

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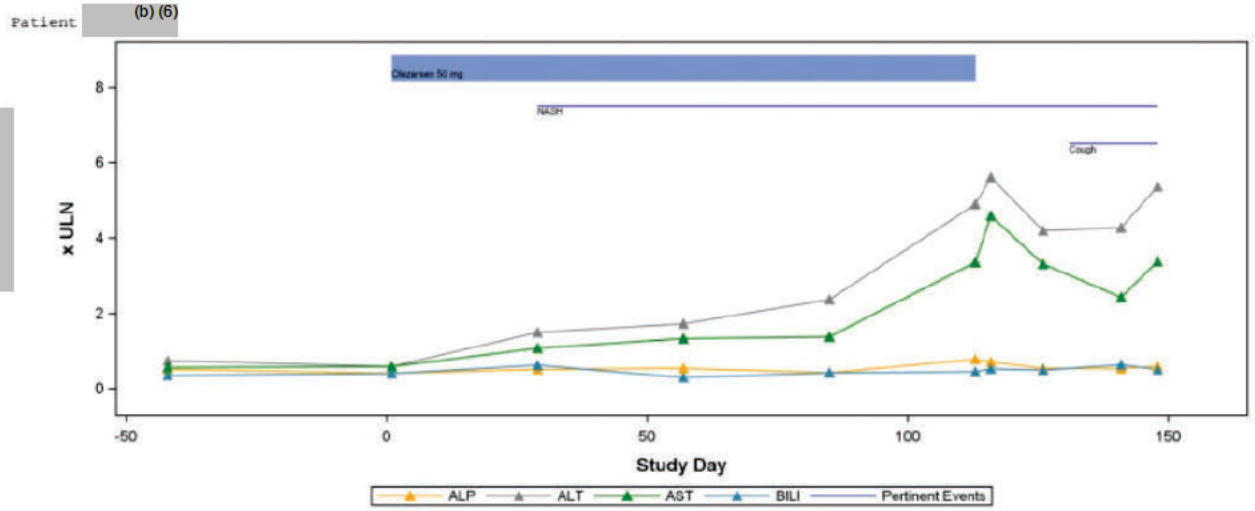

Figure 14: Liver test line graph by study day for Subj (b) (6).[44]

He started OLZ 50 mg SC q 4 weeks on (b) (6) (Day 1). By (b) (6) (Day 28), ALT and AST had increased to 79 U/L and 46 U/L, respectively. No symptoms were mentioned, and study drug continued. ATs continued to rise through the next three doses. On (b) (6) (Day 115), ALT, AST, AP, and TB were 297 U/L, 197 U/L, 103 U/L and 0.6 mg/dL, respectively. Still no symptoms were mentioned. There was no mention of study drug change, but on (b) (6) (Day 141), ALT and AST were still abnormal at 226 U/L and 104 U/L, respectively. Now, the subject had a cough, and the study drug was held. So last dose was on (b) (6) (Day 112). Thereafter, ALT and AST rose again modestly and at last follow-up ((b) (6), Day 148) had not declined (**Figure 14**). Evaluation testing included negative HBsAg, HAV IgM, CMV and anti-HCV antibody. HCV RNA, HEV tests and anti-HBc were not done. ANA was (+) at 2 (ULN 0.6), ASMA 1:20, and anti-LKM negative. IgG level was not checked. Liver imaging was not done.

[44] NDA218614 (218614 - 0016 - (17) - 2024-08-02 - ORIG-1 /Clinical/Response To Information Request) - Response to Information Request of 25July2024 - Clinical Safety (#21)

Assessment: We assessed this case as probable to possible DILI due to OLZ because of appropriate latency. Lack of complete follow-up and incomplete evaluation testing hurt the argument for DILI, but there was no definite competing diagnosis. ANA and ASMA were only weakly positive in this male subject. There were no imaging tests to rule out gallstone disease, but the subject had no abdominal complaints.

3. *Subject* (b) (6) *(Study CS8): Probable DILI due to OLZ.*

Summary: This is a 55-year-old male, Asian, with hypertriglyceridemia and atherosclerotic cardiovascular disease, who developed elevated aminotransferases approximately 89 days after starting study drug (OLZ).

At baseline, the subject's BMI was 27 kg/m^2. Relevant medical history, besides the target disease, included metabolic dysfunction associate steatohepatitis (MASH), and type 2 diabetes. Alcohol history was not provided. Concurrent medications were irrelevant to DILI risk because all were started over a year prior to and continued through the liver event. ALT, AST, AP, & TB were 90 U/L, 44 U/L, 40 U/L, & 0.6 mg/dL, respectively.

He started OLZ at 50 mg SC 4 weeks on (b) (6) (Day 1). ALT and AST began a gradual rise by (b) (6) (Day 33). On (b) (6) (Day 89), ALT, AST, AP, and TB were 157 U/L, 71 U/L, 47 U/L and 0.9 mg/dL, respectively. No symptoms were mentioned; study drug continued. On (b) (6) (Day 196), ALT and AST were still elevated at 155 U/L and 86, respectively. Still no symptoms were mentioned, but the study drug was held. OLZ restarted on (b) (6) (Day 224) after ALT fell to 128 U/L, but on that day the ALT was back up to 159 U/L.The last dose of study drug was taken on (b) (6) (Day 281) because liver enzymes increased again to peak at 236 U/L on (b) (6) (Day 302), and study drug was discontinued. Thereafter, ATs gradually fell to baseline (**Figure 15**) falling by >50% from peak within 119-132 days and to baseline within 153 days.

Evaluation testing included negative HBsAg, HAV IgM, anti-HCV, CMV, and EVB serologies. HCV RNA testing was not done. Autoimmune markers were all negative. IgG level was not checked. Liver imaging was not done.

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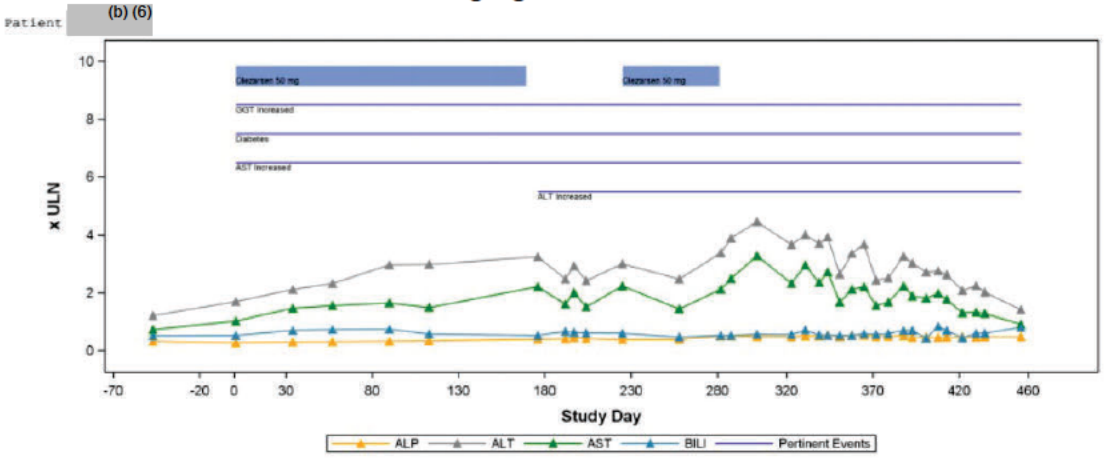

Figure 15: Liver test line graph by study day for Subj (b) (6) [45]

[45] NDA218614 (218614 - 0016 - (17) - 2024-08-02 - ORIG-1 /Clinical/Response To Information Request) - Response to Information Request of 25July2024 - Clinical Safety (#16)

Assessment: We assessed this case as probable DILI due to OLZ. The pattern of ALT and AST elevation while on OLZ is remarkably like the overall change in mean ALT levels in the OLZ subjects as a group. There is a hint of positive rechallenge and gradual washout of enzymes after stopping OLZ. While MASH can have AT fluctuations, there was no MASH related explanation for the gradual AT rise (e.g., weight gain), and then resolution (e.g., weight loss). In contrast, the rise and fall coincided well with OLZ start and stop.

5.0 Assessment and Recommendations:

5.1 **Assessment**: OLZ is a N-acetyl-galactosamine-conjugated (GalNAc), antisense oligonucleotide (ASO), targeting apolipoprotein C-III (apoC3) mRNA. ApoC3 inhibits LPL hydrolysis of triglycerides and is mainly in the liver and enterocytes. OLZ's DNA sequence binds the ApoC3 mRNA triggering RNase H1 degradation of the mRNA. Decreasing ApoC3 synthesis is expected to disinhibit LPL leading to lower blood triglyceride levels. Volanesorsen (VLN) has the same ASO sequence as OLZ but failed in development (b) (4). Unlike VLN, the OLZ ASO is linked to GalNAc which binds the highly expressed asialoglycoprotein receptor (ASGR) on the hepatocyte cell surface. GalNAc-ASGR binding delivers high levels of OLZ specifically to hepatocytes which the Sponsor believes will overcome the VLN's thrombocytopenia side effect. The higher intra-hepatocyte delivery may also enhance efficacy, overcoming the endosomal withholding of ASOs from the cytoplasm where the target mRNA resides.

The Sponsor seeks approval for treatment of familial chylomicronemia syndrome (FCS), a rare inherited lipid disorder due to inadequate LPL hydrolysis of triglycerides. FCS causes severe hypertriglyceridemia leading to substantial morbidity and mortality. There are no approved therapies. The labeled OLZ dose is 80 mg subcutaneous injections, (b) (4)

Non-clinical data do not suggest a DILI risk through typical reactive metabolite formation because degradation of the ASO is via endonucleases and not cytochromes or UDP. Mitochondrial toxicity or transporter inhibition studies were not available. The Division of Applied Regulatory Science (DARS) did not find evidence for liver injury with the ASO to GalNac linker nor the GalNac-ASGR deliver mechanism. However, they found little data on whether high delivery of ASOs with deposition in endosomes may have deleterious effects on hepatocyte liposome and endosome trafficking. In mice, there were transaminase elevations with focal hepatocellular necrosis; in monkeys there was hepatic Kupfer cell hypertrophy and vascular inflammation, though the latter was less prominent compared to VLN.

Clinical experience regarding liver risk with other ASOs is mixed. Mipomersen was removed from the market voluntarily by the Applicant in part due to increased fat deposition in the liver, but it targeted apolipoprotein B-100 which facilitates cholesterol trafficking. Inotersen for hereditary transthyretin amyloidosis polyneuropathy is labeled for liver injury in warning and precautions. In contrast, at least six other ASOs remain on

the market unlabeled for hepatotoxicity. [redacted] (b) (4) GalNAc linker (eplontersen), and it is unlabeled for liver injury risk.

The FCS and PHTG trial data do not suggest a substantial risk for acute liver injury, though the number exposed remains low (<200). No case met Hy's Law, and only one FCS subject plotted just barely into Temple's Corollary quadrant (i.e., ALT >3x ULN). There were three PHTG subjects with probable DILI plotting to Temple's quadrant, but none had TB >ULN nor ATs greater than 7x ULN. One subject was lost to follow-up before injury resolution, and the other two resolved after stopping OLZ. While substantial acute injuries did not occur, chronic injury while on drug is a concern. VLN lowered hepatic fat, but whether the same will occur with OLZ is unclear. Whether high OLZ ASO delivery to the liver may cause chronic damage with long-term clinical consequences is also unknown. Mean ALT consistently increased with OLZ compared to placebo across the phase 1 (healthy volunteers), phase 3 (FCS) and phase 2 (PHTG) studies. The increases in mean ALT were modest (10-20 U/L) but persisted, falling only when OLZ stopped. The risk of liver inflammation and scarring with these chronic modest elevations is unknown.

Thus, there were no acute liver injuries that would hold up approval for FCS. However, the risk of chronic liver injury is not defined and a post-market requirement (PMR) or commitment (PMC) looking at long-term outcomes may be warranted. We do not recommend a placebo-controlled trial particularly if efficacy is substantial for this rare disease without other therapies. However, a single-arm study gathering liver blood tests and imaging for liver fat and fibrosis over several years could define a chronic liver injury risk. FCS patients are generally not at risk for liver scarring from fatty liver, so development of liver fibrosis or portal hypertension may be attributable to the drug. Baseline and annual MRI-Proton Density Fat Fraction (MR-PDFF) and magnetic resonance elastography (MRE) would be best, but ultrasound imaging for fat and fibrosis may suffice. Periodic liver related blood tests and platelet counts could be added to standard of care triglyceride checks without additional venipuncture. Labeling language should include description of liver enzyme elevations. Labeling for liver blood test monitoring should reflect any PMR or PMC. In the absence of a PMR or PMR, we recommend baseline liver blood tests and periodic testing as clinically indicated thereafter. Repeat testing could coincide with triglyceride checks decreasing additional venipunctures.

5.2 **Recommendations**

1. Do not hold up approval for DILI risk.
2. Consider a PMR or PMC to assess the risk of chronic liver injury.
    a. For example: single arm study assessing changes in liver blood tests, platelet counts, and liver imaging for hepatic fat and fibrosis over 3 to 5 years.
        i. Imaging with MRI-PDFF and MRE preferred.
        ii. Ultrasound imaging for fat and fibrosis acceptable.
3. Considerations for labeling, Sections 5 or 6.
    a. Description of chronic modest aminotransferase elevation.

b. Description of occasional acute aminotransferase elevations requiring OLZ discontinuance in PHTG subjects.
c. Recommend checking liver blood tests (ALT, AST, ALP, and TB) at baseline and periodically thereafter. Periodic testing may coincide with triglyceride checks decreasing additional venipunctures.

Eileen E. Navarro Almario -S

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Date: 2024.12.16 07:45:16 -05'00'

Eileen Navarro Almario MD
Team Lead, DILI Team, DHN
CDER/OND

Paul H. Hayashi -S

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Date: 2024.12.16 11:03:04 -05'00'

Paul H. Hayashi, MD, MPH
Associate Director for DILI,DHN
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Appendix: Additional Tables and Figures.

Tables

Table A: OLZ versus VLN comparison.[46]

	Olezarsen (ISIS 678354) NDA 218614 IND 136692	**Volanesorsen** NDA 210645 IND 115063
Sponsor	Ionis Pharmaceuticals, Inc	Akcea therapeutics, Inc
Status	Present NDA	Marketed AU and Brazil
Class[4]	3rd generation GalNAc ASO	2nd generation ASO
Indication	Familial chylomicronemia syndrome (FCS)	Familial chylomicronemia syndrome (FCS)
MOA: Target	hepatic APOC3 mRNA	hepatic APOC3 mRNA
Sequence/ Structure	2'-MOE 2'-Deoxy 2'-MOE (RNase H1 sensitive) (b) (4) Note the central core of 10 nucleotides are common to both olezarsen and volanesorsen however the flanking nucleotides on either end contain **thymine** in olezarsen GalNAc3 (b) (4) 2'-MOE = 2'-methoxylethyl RNA 2'-Deoxy = DNA (b) (4)	5'-AGMeCMeUMeUMeCTTGTMeCMeCAGMeCMeUMeUMeUAMeU-3' Vs **uracil** in volanesorsen[5] (underlined residues are 2"-o-(2-methoxyethyl)nucleosides, the rest are 2"-deoxynucleosides)
Ligand		none
Receptor affinity	Higher	Low
Dosage	80 mg every 4 weeks	285 mg weekly
Side effects	Low risk of thrombocytopenia Low risk bleeding vs placebo	Severe thrombocytopenia- from study C56 [7] 76% VLN thrombocytopenia vs 27% placebo 36% VLN bleeds vs 6% placebo

Table B: Study AKCEA-CS1 ALT data at screenin and baseline, sin le dose cohorts.[47]

Test: Alanine Aminotransferase (U/L)

Visit/Time Point	Value	Statistic	Placebo (N=4)	ISIS 678354 30 mg (N=5)	ISIS 678354 60 mg (N=5)	ISIS 678354 90 mg (N=6)	Overall (N=20)
Screening	Observed	n	4	5	5	6	20
		Mean	32.0	18.2	16.6	19.2	20.9
		SD (SEM)	22.18 (11.09)	8.61 (3.85)	3.36 (1.50)	11.30 (4.61)	12.77 (2.85)
		Median	25.0	16.0	18.0	17.5	17.5
		(Q1, Q3)	(16.0, 48.0)	(11.0, 24.0)	(13.0, 19.0)	(13.0, 20.0)	(13.0, 22.0)
		Minimum	15	10	13	7	7
		Maximum	63	30	20	40	63
Baseline	Observed	n	4	5	5	6	20
		Mean	22.8	16.4	15.6	20.0	18.6
		SD (SEM)	5.91 (2.95)	5.13 (2.29)	6.27 (2.80)	9.53 (3.89)	7.15 (1.60)
		Median	25.0	16.0	14.0	20.0	17.0
		(Q1, Q3)	(19.5, 26.0)	(12.0, 20.0)	(14.0, 15.0)	(14.0, 24.0)	(14.0, 24.5)
		Minimum	14	11	9	7	7
		Maximum	27	23	26	35	35

ISIS 678354 = OLZ.

Table C: Study AKCEA-CS1 ALT data at screening & baseline, single dose cohorts.[48]

[46] Made by DILI Team.
[47] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 14.3 Safety Data Tables (#172)
[48] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 14.3 Safety Data Tables (#468)

Test: Alanine Aminotransferase (U/L)

Visit/Time Point	Value	Statistic	Placebo Every 4 Weeks (N=2)	ISIS 678354 60 mg Every 4 Weeks (N=6)	Overall (N=8)
Screening	Observed	n	2	6	8
		Mean	17.5	37.3	32.4
		SD (SEM)	3.54 (2.50)	21.55 (8.80)	20.44 (7.23)
		Median	17.5	32.5	24.5
		(Q1, Q3)	(15.0, 20.0)	(21.0, 61.0)	(17.5, 49.0)
		Minimum	15	12	12
		Maximum	20	65	65
Day -1	Observed	n	2	6	8
		Mean	18.0	36.7	32.0
		SD (SEM)	5.66 (4.00)	14.47 (5.91)	15.13 (5.35)
		Median	18.0	31.5	29.5
		(Q1, Q3)	(14.0, 22.0)	(28.0, 53.0)	(21.0, 42.5)
		Minimum	14	20	14
		Maximum	22	56	56

ISIS 678354 = OLZ

Table D: Baseline characteristics of subjects in CS3 and CS8.[49]

	FCS CS3			Prevalent CS CS8		
	Olezarsen		Placebo	Olezarsen		Placebo
Characteristic	50 mg N=21	80 mg N=22	N=23	50 mg N=58	80 mg N=57	N=39
Country -United States	5 (23.8)	7 (31.8)	7 (30.4)	55 (94.8)	56 (98.2)	35 (89.7)
Genetic confirmation, n (%)	21 (100)	22 (100)	23 (100)	NA	NA	NA
LPL gene status, n (%)						
Compound heterozygous	3 (14.3)	5 (22.7)	5 (21.7)	NA	NA	NA
Double heterozygous	0	1 (4.5)	0	NA	NA	NA
Hemizygous	0	1 (4.5)	0	NA	NA	NA
Homozygous	14 (66.7)	10 (45.5)	16 (69.6)	NA	NA	NA
Missing	4 (19.0)	5 (22.7)	2 (8.7)	NA	NA	NA
Sex, n (%) Male	6 (28.6)	11 (50.0)	11 (47.8)	34 (58.6)	40 (70.2)	15 (38.5)
Age, years, Mean (SD)	43.2 (12.1)	47.7 (13.3)	44 (14.7)	62.3 (10.1)	60.4 (10.8)	63.3 (9.5)
BMI Mean (SD)	22.4 (3.5)	25.1 (6)	24.2 (4.1)	not	presented	
>25 to ≤30	3 (14.3)	6 (27.3)	10 (43.5)	15 (25.9)	14 (24.6)	7 (17.9)
>30	1 (4.8)	2 (9.1)	1 (4.3)	42 (72.4)	38 (66.7)	31 (79.5)
Diabetic status, n (%)	3 (14.3)	7 (31.8)	6 (26.1)	37 (63.8)	38 (66.7)	30 (76.9)
Pancreatitis history, n (%)	6 (28.6)	5 (22.7)	8 (34.8)	NA	NA	NA
NO chronic pancreatitis, n (%)	18 (85.7)	14 (63.6)	17 (73.9)	NA	NA	NA
Coronary artery disease, n (%)	0	1 (4.5)	1 (4.3)	12 (20.7)	10 (17.5)	5 (12.8)
Stroke (CVA)/TIA, n (%)	0	0	1 (4.3)	3 (5.2)	0	1 (2.6)
From CDS Table 2						

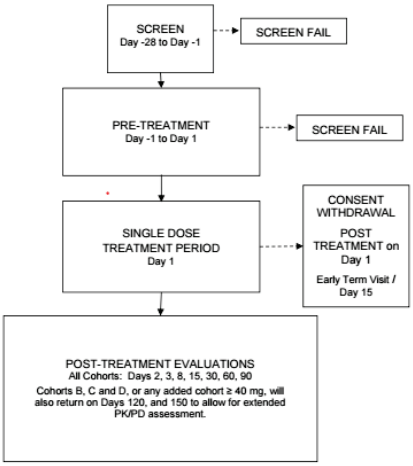
Table E: Liver related SAEs for Study CS3.[50]

System Organ Class Preferred Term	Olezarsen 50 mg N=21 n (%)	Olezarsen 80 mg N=22 n (%)	Placebo N=23 n (%)	Olezarsen 50 mg vs. Placebo Risk Difference % (95% CI)	Olezarsen 80 mg vs. Placebo Risk Difference % (95% CI)
Any SAE	3 (14.3)	2 (9.1)	6 (26.1)	-11.8 (-35.5, 13.3)	-17.0 (-39.6, 6.3)
Gastric varices	0	0	1 (4.3)	-4.3 (-21.3, 11.8)	-4.3 (-21.3, 11.1)
Gastric varices haemorrhage	0	0	1 (4.3)	-4.3 (-21.3, 11.8)	-4.3 (-21.3, 11.1)
Hepatobiliary disorders (SOC)	0	1 (4.5)	1 (4.3)	-4.3 (-21.3, 11.8)	0.2 (-17.4, 18.4)
Hepatic cirrhosis	0	1 (4.5)	0	0.0 (-14.6, 15.8)	4.5 (-10.4, 22.1)
Cholangitis	0	0	1 (4.3)	-4.3 (-21.3, 11.8)	-4.3 (-21.3, 11.1)

[49] Table made by DILI Team from CDS Essential Safety Analyses documents.
[50] Adapted from CDS Tables and Figures

Figures:

(a) Single ascending dose



(b) Multiple dose

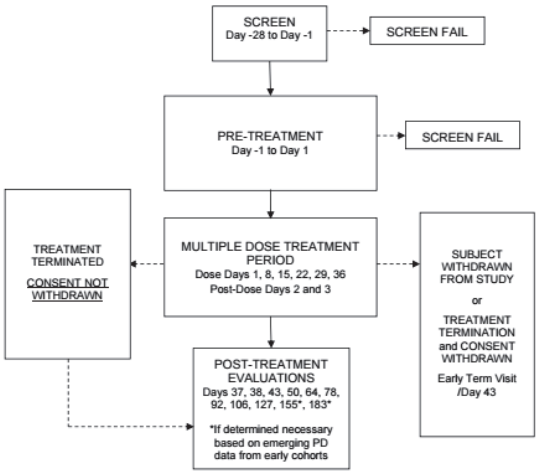
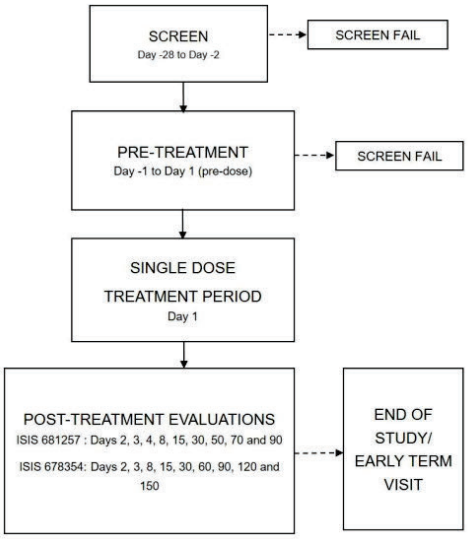


Figure A: Schema for Study 678354-CS1, (a) single dose[51] and (b) multiple doses.[52]

(a) Single ascending dose



(b) Multiple dose

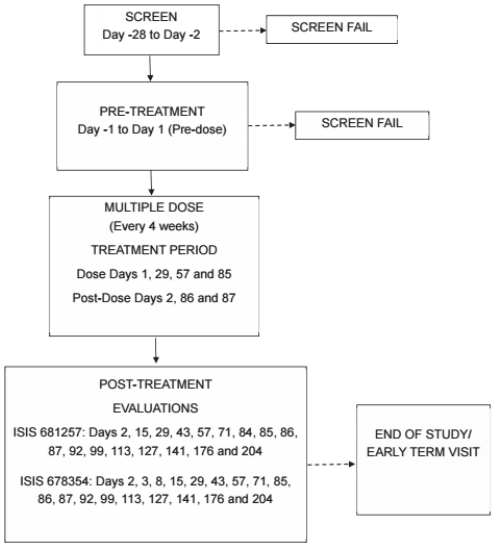


Figure B: Schema for Study AKCEA-CS1, (a) single ascending dose [53] and (b) multiple doses.[54]

[51] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 16.1.1 Protocol and Protocol Amendments (#14)
[52] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - ISIS 678354-CS1 - 16.1.1 Protocol and Protocol Amendments (#15)
[53] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 16.1.1 Protocol and Protocol Amendments (#314)
[54] NDA218614 (218614 - 0001 - (1) - 2024-04-19 - ORIG-1 /Multiple Categories/Subcategories) - AKCEA-CS1 - 16.1.1 Protocol and Protocol Amendments (#315)

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/s/

PAUL H HAYASHI
12/16/2024 12:33:33 PM

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING

Division of Medication Error Prevention and Analysis 1 (DMEPA 1)
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:	December 10, 2024
Requesting Office or Division:	Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number:	NDA 218614
Product Name, Dosage Form, and Strength:	Tryngolza (olezarsen) injection, 80 mg/0.8 mL
Applicant Name:	Ionis Pharmaceuticals, Inc. (Ionis)
FDA Received Date:	December 9, 2024
TTT ID #:	2024-9221-3
DMEPA 1 Safety Evaluator:	Vraj Patel, PharmD
DMEPA 1 Team Leader:	Damon Birkemeier, PharmD, FISMP, NREMT

1 PURPOSE OF MEMORANDUM

Ionis Pharmaceuticals, Inc. submitted a revised container label received on December 9, 2024 for Tryngolza. The Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container label for Tryngolza (Appendix A) to determine if it is acceptable from a medication error perspective.

2 DISCUSSION

Ionis wanted to update the container label stating,

> *"We need to make a change to the container label specifically removing* (b) (4)*. It looks like olezarsen doesn't require* (b) (4) *per the relevant regulations and currently* (b) (4) *is creating issues at the contract site. So we would like to remove it."*

However, we notified Ionis that, (b) (4) must appear on the drug's label as defined by section 201(k) of the Federal Food, Drug, and Cosmetic Act. Thus, to address issues with their contracting site, Ionis submitted an updated container label.

3 CONCLUSION

We find the updated container label acceptable and we have no additional recommendations at this time.

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

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This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

VRAJ K PATEL
12/10/2024 02:56:02 PM

DAMON A BIRKEMEIER
12/10/2024 03:18:29 PM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Division of Pediatrics and Maternal Health
Office of Rare Diseases, Pediatrics, Urologic
and Reproductive Medicine
Office of New Drugs
Center for Drug Evaluation and Research
Food and Drug Administration
Silver Spring, MD 20993
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Division of Pediatrics and Maternal Health Review

Date: 12/6/2024 **Date consulted:** 11/18/2024

From: Katherine Kratz, MD, Medical Officer, Maternal Health
Division of Pediatrics and Maternal Health (DPMH)

Through: CDR Miriam Dinatale, DO, Team Leader, Maternal Health, DPMH

Lynne P. Yao, MD, OND, Division Director, DPMH

To: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)

Drug: Tryngolza (olezarsen) injection

NDA: 218614

Applicant: Ionis Pharmaceuticals

Subject: Pregnancy and Lactation Labeling

Proposed Indication:
For treatment as an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS)

Materials Reviewed:

- DPMH consult request dated 11/18/24. DARRTS Reference ID: 5480610
- Applicant's submitted background package and proposed labeling for NDA 218614, Sequence Number (SN) 0001, dated 4/19/24.

Consult Request: "Review of Section 8 of the PI for review of the PLLR language."

I. INTRODUCTION AND BACKGROUND

On April 19, 2024, the Applicant, Ionis Pharmaceuticals, submitted a 505(b)(1) New Drug Application (NDA) for priority review for olezarsen, a new molecular entity (NME) and anti-sense oligonucleotide (ASO), for the proposed indication of treatment as an adjunct to diet to reduce triglycerides in adults with FCS. DDLO consulted DPMH on November 18, 2024 to assist with the Pregnancy and Lactation subsections of labeling.

Relevant Regulatory History

- FDA has not approved any drug products to treat FCS.
- One other ASO, volanesorsen (NDA 210645), indicated as an adjunct to diet for the treatment of patients with FCS with the same active component as olezarsen, was reviewed by FDA but not approved. Since volanesorsen was not approved, olezarsen is considered an NME. Olezarsen is volanesorsen linked to N-acetylgalactosamine (GalNAc). The GalNAc delivers the drug to the liver where the GalNAc is cleaved, allowing the active component to work.
    - A complete response was issued for volanesorsen due to (b) (4)
- DPMH was consulted to review volanesorsen.[1]
- Volanesorsen is approved in the European Union.
- FDA has approved ASOs for indications other than FCS as follows:
    - 1998: fomivirsen for the treatment of cytomegalovirus (CMV) retinitis
    - 2013: mipomersen for the treatment of homozygous familial hypercholesterolemia
    - 2016: eteplirsen for the treatment of Duchenne muscular dystrophy (DMD)
    - 2016: nusinersen for the treatment of spinal muscular atrophy (SMA)
    - 2018: inotersen for the treatment of hereditary transthyretin amyloidosis and polyneuropathy
    - 2019: golodirsen for the treatment of DMD
    - 2020: viltolarsen for the treatment of DMD
    - 2021: casimersen for the treatment of DMD
    - 2023: eplontersen for polyneuropathy of hereditary transthyretin-mediated amyloidosis
- DPMH was not consulted to review these other ASOs.
- Fomiversen and mipomersen were withdrawn from the market, not for safety or efficacy reasons, in 2011 and 2019, respectively.
- Postmarketing requirements (PMRs) for a descriptive pregnancy safety studies (DPSS) were issued for nusinersen, inotersen and eplontersen. In January 2024, DPMH reviewed the interim report for the DPSS for inotersen.[2] (b) (4)

[1] DPMH Review of volanesorsen (NDA 210645) by Carrie Ceresa, PharmD, MPH, dated 6/2/2018, DARRTS Reference ID: 4270433.
[2] DPMH Memo for IND 113968 (NDA 211172) for TEGSEDI (inotersen) by Catherine Roca, MD, dated 1/30/24, DARRTS Reference ID: 5319447.

(b) (4)

- ASOs are part of a broader class of oligonucleotide drug products that includes small interfering mRNAs (siRNAs). Five siRNAs have been approved by FDA: patisiran, givosiran, lumasiran, inclisiran, and nedosiran. DPMH was consulted to review labeling for lumasiran, inclisiran and nedosiran.

Drug Characteristics[3]

Drug class	Antisense oligonucleotide-GalNAc3 conjugate
Mechanism of action	Binds to apoC-III mRNA, resulting in the reduction of apoC-III protein. ApoC-III protein regulates triglyceride metabolism and hepatic clearance of chylomicrons.
Dosage	80 mg
Administration	80 mg subcutaneous injection once monthly
Molecular weight	9124.48 Daltons
Half-life	4 weeks
% protein bound	> 99%
Bioavailability	Not specified
Warnings and Precautions	Hypersensitivity reactions

Reviewer comment:
DPMH discussed the GalNAc attachment that delivers olezarsen to the liver with the Pharmacology/Toxicology (P/T) team. Per the P/T team, the GalNAc conjugate has been added to multiple drug products to target delivery to the liver. GalNAc has not demonstrated toxicity. To assess potential toxicity of the linker between the ASO and the GalNAc, the Applicant was asked to perform studies with various breakdown products to which the linker was attached. These studies did not reveal safety concerns.

II. REVIEW

PREGNANCY

FCS and Pregnancy

FCS and pregnancy have been previously reviewed by DPMH.[4] Briefly, FCS is a rare autosomal recessive disorder resulting from mutations in the genes encoding lipoprotein lipase (LPL) or one of its regulators (APOC2, APOA5, GPIHBP1, and LMF1),[5] which leads to non-function or very low function of LPL. LPL is an enzyme located on the endothelial surface of adipose and muscle tissues that plays an important role in triglyceride lipolysis and clearance of chylomicrons from

[3] NDA 218614, SN 0001, Module 1.14.1.3, Draft Labeling Text, under review by DDLO.
[4] DPMH Review, see ref 1.
[5] Goldberg RB, Chait A. A Comprehensive Update on the Chylomicronemia Syndrome. Front Endocrinol (Lausanne). 2020 Oct 23;11:593931. doi: 10.3389/fendo.2020.593931. PMID: 33193106; PMCID: PMC7644836.

the plasma.[6] In FCS, a lack of LPL functionality impairs clearance of chylomicrons, which are triglyceride-rich lipoprotein molecules, from the plasma.[7] Clinical features of FCS include triglyceride values exceeding 1,000 mg/dl[8] or 10 mmol/L (885 mg/dl),[9] eruptive xanthomas, lipidemia retinalis, abdominal pain, hepatosplenomegaly, and recurrent hypertriglyceride-induced acute pancreatitis (HTG-AP).[10] FCS typically presents in infancy or childhood. FCS affects between 1:100,000 to 1:1,000,000 patients globally[11] and occurs more frequently in males than females.[12,13]

Many cases of FCS syndrome in women are discovered in the third trimester of pregnancy.[14] Due to a physiological reduction in LPL activity during the third trimester, individuals with LPL deficiency have an exceptionally high risk of HTG-AP in the third trimester and peripartum.[15] Management of FCS in pregnancy involves a low-fat diet, exercise, avoidance of excessive weight gain, and lipid-lowering agents, such as niacin and omega-3 fatty acids.[16,17] Even with

[6] Wanninayake S, Ochoa-Ferraro A, Patel K, Ramachandran R, Wierzbicki AS, Dawson C. Two successful pregnancies -in patients taking Volanesorsen for familial chylomicronemia syndrome. JIMD Rep. 2024 Jun 16;65(4):249-254. doi: 10.1002/jmd2.12435. PMID: 38974616; PMCID: PMC11224504.

[7] Moulin P, Dufour R, Averna M, Arca M, Cefalù AB, Noto D, D'Erasmo L, Di Costanzo A, Marçais C, Alvarez-Sala Walther LA, Banach M, Borén J, Cramb R, Gouni-Berthold I, Hughes E, Johnson C, Pintó X, Reiner Ž, van Lennep JR, Soran H, Stefanutti C, Stroes E, Bruckert E. Identification and diagnosis of patients with familial chylomicronaemia syndrome (FCS): Expert panel recommendations and proposal of an "FCS score". Atherosclerosis. 2018 Aug;275:265-272. doi: 10.1016/j.atherosclerosis.2018.06.814. Epub 2018 Jun 18. PMID: 29980054.

[8] Berglund L, Brunzell JD, Goldberg AC, Goldberg IJ, Sacks F, Murad MH, et al. Evaluation and treatment of hypertriglyceridemia: an Endocrine Society clinical practice guideline. Endocrine Soc J Clin Endocrinol Metab (2012) 97:2969–89. doi: 10.1210/jc.2011-3213

[9] Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Ma7nagement of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Atherosclerosis (2016) 253:281–344. doi: 10.1016/j.atherosclerosis.2016.08.018

[10] Pećin I, Leskovar D, Šabić M, Perica D, Šućur N, Godan Hauptman A, Merćep I, Borovečki F, Premužić V, Jelaković B, Reiner Ž. Prophylactic therapeutic plasma exchange in pregnant woman with Familial Chylomicronemia Syndrome - A case report. Transfus Apher Sci. 2022 Jun;61(3):103346. doi: 10.1016/j.transci.2021.103346. Epub 2021 Dec 15. PMID: 34924316.

[11] Brahm AJ, Hegele RA. Chylomicronaemia--current diagnosis and future therapies. Nat Rev Endocrinol. 2015 Jun;11(6):352-62. doi: 10.1038/nrendo.2015.26. Epub 2015 Mar 3. PMID: 25732519.

[12] Pallazola VA, Sajja A, Derenbecker R, Ogunmoroti O, Park J, Sathiyakumar V, Martin SS. Prevalence of familial chylomicronemia syndrome in a quaternary care center. Eur J Prev Cardiol. 2020 Dec;27(19):2276-2278. doi: 10.1177/2047487319888054. Epub 2019 Nov 13. PMID: 31718261.

[13] Davidson M, Stevenson M, Hsieh A, Ahmad Z, Roeters van Lennep J, Crowson C, Witztum JL. The burden of familial chylomicronemia syndrome: Results from the global IN-FOCUS study. J Clin Lipidol. 2018 Jul-Aug;12(4):898-907.e2. doi: 10.1016/j.jacl.2018.04.009. Epub 2018 Apr 26. PMID: 29784572.

[14] Moulin, see ref 7.

[15] Wanninayake, see ref 6.

[16] Coronado Arroyo JC, Concepción Zavaleta MJ, García Villasante EJ, Kcomt Lam M, Concepción Urteaga LA, Zavaleta Gutiérrez FE. Familial Chylomicronemia Syndrome-Induced Acute Necrotizing Pancreatitis during Pregnancy. Rev Bras Ginecol Obstet. 2021 Mar;43(3):220-224. doi: 10.1055/s-0040-1722173. Epub 2021 Feb 18. PMID: 33601464; PMCID: PMC10183904.

[17] Wanninayake, see ref 6.

these measures, however, pregnant individuals with FCS have an increased risk of HTG-AP.[18] Treatment of HTG-AP in pregnancy involves parenteral nutrition, intravenous hydration, insulin, heparin and plasmapheresis.[19] In severe cases, support in an intensive care unit may be needed. HTG-AP is associated with adverse pregnancy outcomes, including preterm labor and birth, gestational diabetes mellitus, preeclampsia, and maternal and fetal mortality.[20]

Four case reports and one case series describing pregnant individuals with HTG-AP were previously reviewed by DPMH.[21] Additional case reports were identified for this review.[22,23,24,25] From all of these publications, one maternal death and 20 pregnancy outcomes were reported. The pregnancy outcomes appear in the list below. Pregnant individuals were treated with the measures noted in the preceding paragraph in addition to one individual being treated with antibiotics for sepsis.

Pregnancy outcomes:

- Eight full-term infants: six healthy, and two with unknown status
- Five pre-term infants: two healthy, two with unknown status, and one with cerebral palsy
- Four unknown outcomes
- One intrauterine fetal demise at 22 weeks
- Two neonatal deaths after pre-term delivery: one at 32 weeks of gestation without further details; and one at 29 weeks of gestation due to necrotizing enterocolitis

Nonclinical Experience

The Applicant and FDA agreed that Embryofetal Development (EFD) and Pre- and Post-Natal Development (PPND) studies did not need to be conducted with olezarsen on the basis of reproductive and developmental toxicity studies in mice and rabbits with volanesorsen, which has the same sequence and same chemistry as the active moiety of olezarsen.[26] Briefly, these are the nonclinical findings with volanesorsen: In animal reproduction studies conducted with the unconjugated antisense oligonucleotide (lacking GalNAc) in rabbits and mice, no adverse effects on development or pregnancy were observed at doses 21 times or 20 times, respectively, the maximum recommended clinical dose.[27]

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[18] Haiyan Z, Na P, Jialin H, Qingjian L, Jianying B, Xiumei B. Acute Pancreatitis in Pregnancy: A Ten-Year Noninterventional, Retrospective Cohort Experience. Gastroenterol Res Pract. 2022 Jun 9;2022:3663079. doi: 10.1155/2022/3663079. PMID: 35721824; PMCID: PMC9203233.

[19] Coronado, see ref 16.

[20] Coronado, see ref 16.

[21] DPMH Review, see ref 1.

[22] Pećin, see ref 10.

[23] Coronado, see ref 16.

[24] Lin MH, Tian XH, Hao XL, Fei H, Yin JL, Yan DD, Li T. Management of a pregnant patient with chylomicronemia from a novel mutation in GPIHBP1: a case report. BMC Pregnancy Childbirth. 2020 May 6;20(1):272. doi: 10.1186/s12884-020-02965-1. PMID: 32375710; PMCID: PMC7201967.

[25] Gupta N, Ahmed S, Shaffer L, Cavens P, Blankstein J. Severe hypertriglyceridemia induced pancreatitis in pregnancy. Case Rep Obstet Gynecol. 2014;2014:485493. doi: 10.1155/2014/485493. Epub 2014 Jun 3. PMID: 24995138; PMCID: PMC4065762.

[26] NDA 218614, SN 0001, Module 2.6.6, Toxicology Written Summary, pp. 25-27.

[27] NDA 218614, SN 0001, Module 1.14.1.3, Draft Labeling Text, reviewed by DDLO Pharmacology/Toxicology team.

A Fertility and Early Embryonic Development (FEED) study was conducted for olezarsen because of nonclinical findings with volanesorsen that included decreased prostate/seminal vesicle and sperm count in a combined mouse study. In the FEED study, there were no olezarsen-related adverse effects on early embryonic development up to 20 mg/kg; therefore, the Applicant reported that the NOAEL in mice was 20 mg/kg every other week (i.e., 40 mg/kg/month), providing an approximately 18 (b) (4) fold safety margin over expected human exposure based on monthly clinical dose at (b) (4) 80 mg/month.[28]

The reader is referred to the full Pharmacology/Toxicology review by Dr. Njwen Anyangwe.

> ***Reviewer comment:***
> *The P/T team explained that FDA agreed that a full series of additional animal reproduction studies were not necessary given that the active substance of volanesorsen is the same as that of olezarsen. Per the P/T team, in the volanesorsen development program, there were no effects on reproductive endpoints. Of note, apo-CIII mRNA is present in rabbits and mice; therefore, volanesorsen was able to act on its target.*

Review of Pregnancies during Drug Development[29]
Across all individual clinical studies, one partner pregnancy was reported. Given that olezarsen is not genotoxic (see the Males and Females of Reproductive Potential section below), this pregnancy is not relevant.

Review of Literature
Applicant's review:
The Applicant did not conduct a literature review related to pregnancy exposure to ASOs.

DPMH review:
DPMH conducted an updated search of published human studies in PubMed from the time of the DPMH review of volanesorsen in 2018 to the present, using the search terms "antisense oligonucleotide" OR "volanesorsen" AND "pregnancy," "pregnancy outcomes," "birth defects," "malformations," "stillbirth," and "spontaneous abortion." One publication was identified as follows:

- Wanninayake et al.[30] published a case series from the United Kingdom in which two patients were treated with volanesorsen during pregnancy for FCS. In one case, a patient was treated from the ages of 18-21 years old with volanesorsen. The patient was taking volanesorsen when she discovered that she was pregnant. An ultrasound demonstrated that she was 38 weeks pregnant. When pregnancy was diagnosed, volanesorsen was discontinued. She underwent cesarean section at 39 weeks of gestation and delivered a healthy infant. At 24 months of age, the child was developing normally. The second case involved a patient who was treated with volanesorsen for 5 years and discontinued it approximately 6 months before conception. She resumed volanesorsen at 22 weeks of gestation. She delivered a healthy infant at 35 weeks of gestation.

[28] NDA 218614, SN 0001, Module 2.4, Nonclinical Overview, p. 38.
[29] NDA 218614, SN 0001, Module 2.5, Clinical Overview, p. 95.
[30] Wanninayake, see ref 6.

DPMH also searched Micromedex[31] for volanesorsen, and no information was found.

> ***Reviewer comment:***
> *Nonclinical studies conducted with volanesorsen and olezarsen did not demonstrate embryofetal toxicity. There are no clinical data related to olezarsen use during pregnancy. Clinical data are limited to two case reports of exposure during pregnancy to volanesorsen, which has the same active moiety as olezarsen, and no adverse pregnancy outcomes were reported; however, the data are too limited to inform a drug-associated risk.*

LACTATION

Nonclinical Experience

The Applicant did not provide nonclinical lactation data; however, volanesorsen is present in the milk of pregnant mice at low levels (≤ 0.7 µg/mL at subcutaneous doses up to 87.5 mg/kg/wk).[32]

Review of Lactation Cases during Drug Development

There were no cases involving lactation during drug development.

Review of Literature

Applicant's review:

The Applicant did not conduct a literature review related to lactation and ASOs.

DPMH review:

DPMH conducted a search for published human studies in PubMed, using the search terms: terms "antisense oligonucleotide" OR "volanesorsen" AND "lactation" and "breastfeeding." No publications were found.

In addition, DPMH conducted a search for volanesorsen in Micromedex, Hale's *Medications and Mothers' Milk*,[33] Reprotox, the Drugs and Lactation Database (LactMed),[34] and Briggs *Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk.*[35] No information was found.

> ***Reviewer comment:***
> *There are no available data about the presence of olezarsen in animal or human milk. There are no data related to the effects of olezarsen on the breastfed infant or on milk production. Volanesorsen is known to be present in low levels in the milk of mice.[36] Given that olezarsen has the same sequence and same chemistry as the active moiety of*

[31] Truven Health Analytics information, http://www.micromedexsolutions.com. Accessed 11/27/24.
[32] DPMH Review, see ref 1.
[33] Hale, Thomas W. Hale's Medications & Mothers' Milk 2021: A Manual of Lactational Pharmacology. 19th ed. New York: Springer Publishing Company, 2020. www.halesmeds.com
[34] Drugs and Lactation Database (LactMed). Accessed 11/27/24.
[35] Briggs, Gerald G., Craig V. Towers, and Alicia B. Forinash. Briggs Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk. 12th edition. Philadelphia, PA: Lippincott Williams & Wilkins, 2021. Print.
[36] DPMH Review, see ref 1.

volanesorsen, olezarsen may be present in low concentrations animal milk. Although drugs that are present in animal milk are likely to be present in human breast milk, olezarsen is likely to have poor oral bioavailability as was seen with volanesorsen.[37] *Another drug product that falls within the broader class of oligonucleotides is the siRNA inclisiran, which was found in the milk of lactating rats. Labeling for inclisiran states, "Oligonucleotide-based products typically have poor oral bioavailability; therefore, it is considered unlikely that low levels of inclisiran present in milk will adversely impact an infant's development during lactation." DPMH recommends that lactation labeling include information about the poor bioavailability of oligonucleotides such that it is unlikely that potential exposure via breast milk will lead to clinically relevant levels in breastfed infants.*

FEMALES AND MALES OF REPRODUCTIVE POTENTIAL

Nonclinical Experience

Olezarsen was administered at doses of 0, 5, 10, or 20 mg/kg given every other week to male and female mice prior to mating, followed by every other day dosing after mating and until gestation day 6 in females. There was no effect on fertility in mice administered olezarsen at doses up to 20 mg/kg (approximately 2-times the monthly maximum recommended human dose based on body surface area).[38] Olezarsen was negative for genotoxicity in vitro (bacterial reverse mutation assay and chromosome aberration assay in Chinese hamster lung cells) and in vivo (mouse bone marrow micronucleus assay).[39]

Carcinogenicity studies were not conducted with olezarsen. The rationale for not conducting carcinogenicity studies with olezarsen was based on the aggregate results of carcinogenicity studies conducted with volanesorsen in mice and rats. The findings for volanesorsen are as follows: when the unconjugated ASO lacking GalNAc was administered weekly in mice and rats at subcutaneous doses of 0, 6, 25, 40 mg/kg/week (along with a mouse-specific surrogate ASO at 25 mg/kg/week) and 0, 0.2, 1, 5 mg/kg/week, respectively, for 2 years, there were statistically significant increases in the incidences of hepatocellular adenomas and carcinomas at ≥25 mg/kg/week and hemangiomas and hemangiosarcomas at all doses. In female mice, there were statistically significant increases in the incidences of histiocytic sarcomas at all doses (including the mouse-specific surrogate) and pituitary gland adenomas at 25 mg/kg/week. In rats, the incidence of malignant fibrous histiocytoma at the injection site was increased in both sexes at doses ≥1 mg/kg/week. These tumors are considered a response to chronic tissue irritation and inflammation caused by repeated subcutaneous injection. The clinical significance of these findings is uncertain.[40]

The reader is referred to the full Pharmacology/Toxicology review by Dr. Njwen Anyangwe.

[37] DPMH Review, see ref 1.
[38] NDA 218614, SN 0001, Module 1.14.1.3, Draft Labeling Text, reviewed by DDLO Pharmacology/Toxicology team.
[39] NDA 218614, SN 0001, Module 1.14.1.3, Draft Labeling Text, reviewed by DDLO Pharmacology/Toxicology team.
[40] NDA 218614, SN 0001, Module 1.14.1.3, Draft Labeling Text, reviewed by DDLO Pharmacology/Toxicology team.

Drug-Drug Interactions (DDIs)
The Applicant reported that olezarsen has a very low potential for involvement in CYP- or transporter-mediated drug-drug interactions either as a perpetrator or victim.[41]

Review of Fertility Cases during Drug Development
There were no cases related to fertility that were reported by the Applicant.

Review of Literature
Applicant's review:
The Applicant did not conduct a literature review related to fertility and ASOs.

DPMH review:
DPMH conducted a literature search for human studies in PubMed, using the search terms "antisense oligonucleotide" OR "volanesorsen" AND "fertility," "contraception," "oral contraceptives," and "infertility." No information was found. DPMH also conducted a search in Micromedex. No information was found.

> ***Reviewer comment:***
> *Nonclinical studies did not show adverse effects of olezarsen on fertility. Olezarsen was not found to be genotoxic in in vitro and in vivo mouse studies. DDIs were discussed with the Clinical Pharmacology team, and they agreed with the Applicant that olezarsen has a very low potential for involvement in CYP- or transporter-mediated drug-drug interactions either as a perpetrator or victim; therefore, DDIs with hormonal contraceptives are unlikely. As there are no human reproductive or fertility concerns to convey to prescribers, DPMH recommends omitting 8.3 from labeling. Animal findings should be included in labeling in subsection 13.1.*

III. DISCUSSION AND CONCLUSIONS

Pregnancy
EFD and PPND studies were not conducted for olezarsen since these studies had been conducted for volanesorsen, which has the same active moiety as olezarsen. The nonclinical data for volanesorsen did not demonstrate embryofetal toxicity. Per the P/T team, the GalNAc component of olezarsen, which delivers it to the liver and is not a component of volanesorsen, has been studied for other drug products and has not demonstrated toxicity. The linker that attaches GalNAc to the active moiety of olezarsen was studied by the Applicant and did not demonstrate any safety concerns. Importantly, apo-CIII mRNA is present in rabbits and mice; therefore, volanesorsen was able to act on its target. A FEED study conducted with olezarsen did not demonstrate any adverse effects on early embryonic development or fertility in mice. The nonclinical embryofetal development data related volanesorsen should be included in subsection 8.1 of labeling. The fertility data should appear in subsection 13.1.

In terms of clinical data, there are no available data in published literature on olezarsen exposure during pregnancy. There are two published cases of exposure to volanesarsen during pregnancy,

[41] NDA 218614, SN 0001, Module 2.6.4, Pharmacokinetics Written Summary, p. 13.

which are not adequate to inform labeling. The Risk Summary in subsection 8.1 should state that there are no available data on use of olezarsen during human pregnancy to inform a drug-associated risk.

Given that there are significant maternal and fetal risks of HTG-AP associated with FCS, DPMH recommends including a Clinical Considerations section under 8.1. Per the Pregnancy and Lactation Labeling Rule (PLLR), a subsection titled Disease-Associated Maternal and/or Embryo-Fetal Risk should be including in labeling to describe the serious known risk of pancreatitis to the pregnant woman and the fetus associated with the underlying disease.

DPMH does not recommend issuing a postmarketing requirement (PMR) to conduct a pregnancy safety study. Given that FCS is a very rare disease, neither a pregnancy exposure registry nor a descriptive pregnancy safety study (DPSS) is likely to enroll an adequate number of patients to be informative. (b) (4) DPSS for inotersen, which is indicated to treat hereditary transthyretin amyloidosis and polyneuropathy and is more common that FCS, occurring in 1:100,000.[42] Additionally, the likelihood of a pregnancy safety study uncovering a safety concern is low given that the nonclinical embryofetal toxicity studies were negative and FCS is often diagnosed in pregnancy with the onset of HTG-AP during the third trimester after organogenesis is complete.

Lactation

There are no nonclinical or clinical data on the presence of olezarsen in breast milk or its effects on the breastfed infant or milk production. There are data, however, on the presence of low levels of volanesorsen in the milk of lactating mice, which should be included in subsection 8.2 of labeling. Information about the poor bioavailability of oligonucleotides should appear in subsection 8.2 because it is unlikely that low levels present in milk will lead to clinically relevant levels in breastfed infants. Given that there are no data available for prescribers specific to olezarsen, the standard Pregnancy and Lactation Labeling Rule (PLLR) risk-benefit statement should appear in labeling.

DPMH does not recommend issuing a PMR for a lactation study for the following reasons: 1) FCS is a very rare disease and is unlikely to enroll an adequate number of patients who are taking the drug for the indicated condition, 2) it is unlikely that olezarsen will be present in high concentrations in breast milk, and based on the drug's poor oral bioavailability, it is unlikely that it would lead to clinically relevant levels in breastfed infants, and 3) the half-life of olezarsen is long at 4 weeks, which would likely preclude healthy women recruited for a lactation study from continuing to breastfeed after receiving a dose of olezarsen.

[42] Hereditary Transthyretin Amyloidosis. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwj0tvOik_2JAxXOEmIAHZdzFsUQFnoECBgQAw&url=https%3A%2F%2Farci.org%2Fwp-content%2Fuploads%2F2021%2F03%2FDisease-Overview_hATTR.pdf&usg=AOvVaw1QXdQpLE82qwS6-OnEQSRe&opi=89978449

Females and Males of Reproductive Potential

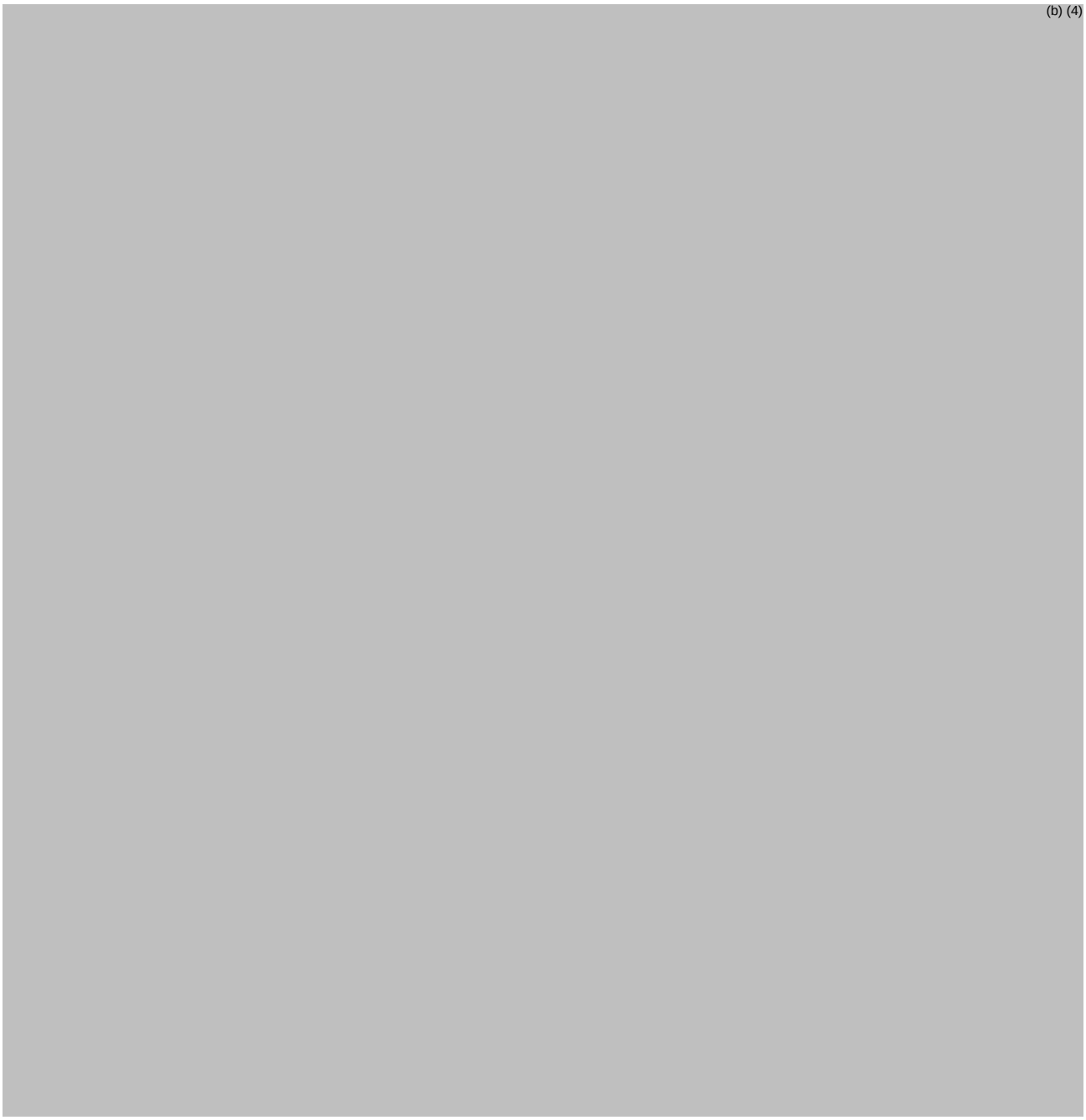
DPMH recommends omitting subsection 8.3 of labeling as there are no human fertility data or genotoxicity data to convey to prescribers, and olezarsen does not pose a risk for DDIs with hormonal contraceptives.

LABELING RECOMMENDATIONS

DPMH revised subsections 8.1 and 8.2 of labeling (see below). DPMH refers to the final NDA action for final labeling.

DPMH Proposed Pregnancy and Lactation Labeling

(b) (4)



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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:	November 26, 2024
To:	Ron Picking III Regulatory Project Manager **Division of Diabetes, Lipid Disorders, and Obesity (DDLO)**
Through:	LaShawn Griffiths, MSHS-PH, BSN, RN Associate Director for Patient Labeling **Division of Medical Policy Programs (DMPP)** Marcia Williams, PhD, LCSW, BCD Team Leader for Patient Labeling **Division of Medical Policy Programs (DMPP)**
From:	Sharon Williams, MSN, BSN, RN Senior Patient Labeling Reviewer **Division of Medical Policy Programs (DMPP)** Ankur Kalola, PharmD Regulatory Review Officer **Office of Prescription Drug Promotion (OPDP)**
Subject:	Review of Patient Labeling: Patient Package Insert (PPI) and Instructions for Use (IFU)
Drug Name (established name):	TRYNGOLZA (olezarsen)
Dosage Form and Route:	injection, for subcutaneous use
Application Type/Number:	NDA 218614
Applicant:	Ionis Pharmaceuticals, Inc.

1 INTRODUCTION

On April 19, 2024, Ionis Pharmaceuticals Inc. submitted for the Agency's review a New Drug Application (NDA) for olezarsen. Olezarsen is indicated as an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS). Olezarsen was granted Orphan Drug Designation for the treatment of FCS. A conditional approval of the proposed proprietary name, Tryngolza was granted on March 11, 2024.

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 Diabetes, Lipid Disorders, and Obesity (DDLO) on May 3, 2024 for DMPP and OPDP to review the Applicant's proposed Patient Package Insert (PPI) and Instructions for Use (IFU) for Tryngolza (olezarsen) injection, for subcutaneous use.

2 MATERIAL REVIEWED

- Draft TRYNGOLZA (olezarsen) PPI and IFU received on April 19, 2024, and received by DMPP and OPDP on November 19, 2024.

- Draft TRYNGOLZA (olezarsen) Prescribing Information received on April 19, 2024, revised by the review division and received by DMPP and OPDP on November 19, 2024.

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 review of the PPI and IFU we:

- simplified wording and clarified concepts where possible
- ensured that the PPI and IFU are consistent with the PI
- ensured that the PPI and IFU meet the criteria as specified in FDA's Guidance for Useful Written Consumer Medication Information (published July 2006)
- ensured that the IFU meets 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 PPI and IFU are acceptable with our recommended changes.

5 RECOMMENDATIONS

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

Please let us know if you have any questions.

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11/26/2024 02:11:22 PM

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

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

Memorandum

Date: November 25, 2024

To: Ronald Picking, Regulatory Project Manager, Division of Diabetes, Lipid Disorders, and Obesity (DDLO)

Melinda Wilson, Associate Director for Labeling, DDLO

From: Ankur Kalola, Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

CC: Sapna Shah, Team Leader, OPDP

Subject: OPDP Labeling Comments fo TRYNGOLZA (olezarsen) injection, for subcutaneous use

NDA: 218614

Background:
In response to DDLO's consult request dated May 3, 2024, OPDP has reviewed the proposed Prescribing Information (PI), Patient Package Insert (PPI), and Instructions for Use (IFU) for Tryngolza.

PI/PPI/IFU:
OPDP's review of the proposed PI is based on the draft labeling emailed to OPDP on November 19, 2024, and our comments are provided below.

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

Thank you for your consult. If you have any questions, please contact Ankur Kalola at 301-796-4530 or Ankur.Kalola@fda.hhs.gov.

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ANKUR S KALOLA
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Clinical Outcome Assessment Review Memorandum

From	Yasmin Choudhry, M.D. Clinical Outcome Assessment (COA) Reviewer Division of Clinical Outcome Assessment (DCOA) Selena Daniels, PharmD, Ph.D. Deputy Division Director DCOA
To	Division of Diabetes, Lipid Disorders and Obesity
COA tracking number	C2024156
NDA#; Referenced IND#; (Drug name)	NDA 218614; IND 136692 (olezarsen)
Drug Sponsor	Ionis Pharmaceuticals
Indication:	Treatment of familial chylomicronemia syndrome (FCS) Please check all that apply: ☒ Rare Disease/Orphan Designation
Instrument(s) reviewed:	1. FCS Symptoms and Impacts Scale ☒ Patient-reported outcome (PRO)
	2. FCS-Symptoms (2-Week recall) ☒ Patient-reported outcome (PRO)
	3. FCS Diet Questions for Daily Diary ☒ Patient-reported outcome (PRO)
	4. Patient-Reported Outcome Measurement Information System (PROMIS) Adult Short Form v1.1 – Pain Interference 8a ☒ Patient-reported outcome (PRO)
	5. PROMIS 29+2 Profile v2.1 (PROPr) ☒ Patient-reported outcome (PRO)
	6. PROMIS Short Form v2.0 – Cognitive Function 4a ☒ Patient-reported outcome (PRO)

This Clinical Outcome Assessment (COA) consult review is provided as a response to a request for consultation by the Division of Diabetes, Lipid Disorders and Obesity (DDLO) regarding NDA 218614 (DARRTS Reference ID: 5383083).

In this submission, the Applicant is seeking approval of olezarsen[1] for the treatment of adult patients with familial chylomicronemia syndrome (FCS)[2] based on a single multi-center, randomized, double-blind, placebo-controlled phase 3 Study (ISIS 678354-CS3; here on

[1] Olezarsen is a GalNAc3 conjugated antisense oligonucleotide inhibitor of apolipoprotein C-III (ApoC-III) production indicated as an adjunct to diet to reduce triglycerides in adults with FCS. ApoC-III is a major regulator of lipoprotein metabolism and plays a pivotal role in regulating plasma TG levels.

[2] FCS is a rare genetic disorder. Individuals have very high concentrations of triglycerides (TG) and are at increased risk for pancreatitis, a potentially life-threatening consequence of this condition.

referred to as Study CS3). The Applicant also conducted an exit interview substudy to provide further support for the content validity of the FCS Symptoms and Impacts Scale (FCS-SIS) and to facilitate the interpretation of the olezarsen clinical trial results.

The Applicant is not seeking COA-related labeling claim(s). The primary objective of this review is to evaluate from a COA perspective if the submitted information supports regulatory decision-making (e.g., benefit-risk assessment).

The exploratory COA-based efficacy endpoints in Study CS3 were to evaluate the effect of olezarsen as compared to placebo on:

- Patient-reported abdominal pain, other FCS-related symptoms (including physical fatigue, difficulty thinking and diarrhea)[3], diet[4], and impacts[5], health-related quality of life (HRQoL) [6], pain interference[7], and cognitive function[8].

The data from Study CS3 demonstrated that for olezarsen (80mg and 50mg) there were no change from baseline differences in any of the patient-reported outcome (PRO) scores compared with placebo. The data from the exit interview sub-study indicated that a total of 14 of the 18 patients (77.8%) reported improvement in at least 1 of their pre-trial symptoms and impacts and most improvements were considered meaningful.

From a COA perspective, in the absence of evidence, the Applicant has not demonstrated that any of the COAs are fit-for-purpose in the specified context of use. Further, the findings from Study CS3 and exit interview substudy were not conclusive with improvements in FCS symptoms and impacts (median change was generally 0 in the FCS-SIS measure). As such, it is difficult to determine whether any treatment benefits were observed.

Review Conclusions

The Applicant did not submit an evidence dossier for any of the COAs and/or evidence to assess the fitness-for-purpose of the measures utilized in Study CS3. Therefore, these instruments could not be reviewed for content validity, other measurement properties, and clinically meaningful change. In the absence of evidence, we cannot make a definitive conclusion as to whether the COAs can support regulatory decision-making. While the Applicant generated qualitative data to interpret olezarsen clinical trial results, the data are difficult to interpret due to limitations of the substudy.

Key Issues Identified

Issue #1: Content Validity

- Lack of qualitative evidence to support that the PRO instruments measure important aspects of FCS to patients, as well as whether the instruments are well-understood.

[3] Assessed by the FCS-SIS.
[4] Assessed by the FCS Diet Questions for Daily Diary.
[5] Assessed by the FCS-SIS.
[6] Assessed by the Patient-Reported Outcome Measurement Information System (PROMIS) 29+2 Profile v2.1 (PROPr).
[7] Assessed by the PROMIS Adult Short Form v1.1 – Pain Interference 8a.
[8] Assessed by PROMIS Short Form v2.0 – Cognitive Function 4a.

Issue 2: Data Interpretability

- The content validity of the PRO instruments is questionable (see Issue 1).
- The meaningful change threshold(s) are unknown for the PRO instruments. While the Applicant administered anchor scales, the sample size is insufficient to conduct anchor-based analyses.
- While the Applicant used qualitative methods to help interpret the results of Study CS3, the exit interview substudy was conducted in the open-label extension (OLE) phase of Study CS3 where all patients were given olezarsen. It is unknown whether patients' knowledge of treatment assignment influenced their responses within the substudy.

  Reviewer's comment(s): *The submission includes an exit interview final report dated January 11, 2024*[9]*. Of note, the FDA did not previously review or comment on the qualitative study protocol/interview guide for the exit interview substudy.*

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.
- Qualitative studies should be designed to provide a complete understanding of the disease and/or treatment including presentation of symptoms and associated impacts in the target population, as well as establish the appropriateness (content relevance) and comprehensiveness of the assessment (e.g., conceptual coverage).
- It will be important for the Agency to review and comment on Sponsor's plans for qualitative research (e.g., concept elicitation interviews, cognitive interviews, exit interviews) early in the development program to the extent possible to provide input on the study design to help generate meaningful information.

Regulatory Background and Materials Reviewed

- There have been previous communications in the IND phase with the Applicant regarding their COA measurement strategy, which included the following (starting with the most recent advice):

  - Type B Meeting Preliminary Comments IND 136692 dated December 6, 2023:
    - Agreed with the Applicant's (b) (4)

      Reviewer's comment(s): *In their response to the FDA preliminary comments, the Applicant indicated that they* (b) (4)

[9] Patient Experiences with CSF Exit Interview Sub-Study Final Report Study ISIS 678354-CS13 [Section 1.13 Additional reports, SDN 1]

[redacted] (b) (4)

- Type C Meeting IND Information Request/Advice letter dated March 10, 2021:
  - [redacted] (b) (4)
  - Identified limitations of an 11-point numeric rating scale to measure some core symptoms (e.g., diarrhea, difficulty thinking). Recommended exploring this in exit interviews to help interpret the results.
  - Indicated that the regulatory utility of the impacts items is unclear and would be a review issue.
  - Recommended collecting and separately analyzing the most important disease-related symptoms, if any, and functional impacts that are responsive to treatment to the extent possible.
  - Commented on the limitations of the proposed anchor scales.
  - Indicated that exit interviews may be considered to obtain patient input on the most important disease-related symptoms/functional impacts and meaningful score changes in the FCS-SIS.

The materials reviewed in this submission are shown in Table 1.

Table 1. Materials reviewed

Document	**SDN**	**eCTD#**	**Date Received**
• NDA submission which includes: • Study ISIS 678354-CS3 Report Body • Protocol ISIS 678354-CS3 Amendment 9 dated August 2, 2023 • Exit Interview Sub-Study Final Report dated January 11, 2024	1	0001	19 April 2024
Communications and Reviews	**DARRTS Ref ID**		**Date**
Type B Meeting Preliminary Comments	5289258		06 Dec 2023
Type Correspondence Advice Information Request letter	4760243		10 March 2021
Previous COA Review: C2020538_IND 136692_Patel	4757300		05 March 2021
Previous COA Review: C2023402_IND 136692_Chung	5316317		30 Jan 2024

Trial Design and Study Endpoints

Study CS3[10] is a multi-center, randomized, double-blind, placebo-controlled phase 3 trial to evaluate the efficacy and safety of olezarsen (50mg and 80mg subcutaneously) in adults (n=60; ≥18 years) with a diagnosis of familial chylomicronemia syndrome (type 1 hyperlipoproteinemia). Eligible participants must have had a fasting triglyceride (TG) ≥ 880 mg/dL, a history of pancreatitis[11], and be willing to follow a diet comprising ≤ 20 g fat per day during the study.

This study consisted of the following:

- Screening Period (4-week but no more than 8-weeks). This period included an at least 2-week diet stabilization[12]/run-in period for patients not already on a stable diet, and an approximately 2-week qualification period.

- Treatment Period (53 weeks). Following qualification, approximately 60 eligible patients were randomized 1:1 to Cohort A[13] (n=30) or Cohort B[14] (n=30); each cohort was further randomized 2:1 to groups receiving either the olezarsen dose for the cohort or a matching volume of placebo, respectively.

- OLE. Following the Week 53 visit, eligible patients could elect to enroll in an OLE study

- Post-Treatment Evaluation Period (13 weeks). Patients not participating in the OLE study entered this period.

The study endpoints were:

Primary efficacy endpoint

- Percent change in fasting TG from Baseline at end of Month 6 (defined as the average of Weeks 23, 25, and 27) compared to placebo.

Exploratory efficacy COA endpoints

[10] Amendment 9 dated 2 Aug 2023.
[11] Defined as a recorded diagnosis of acute pancreatitis or hospitalization or emergency room (ER) visit for severe abdominal pain consistent with acute pancreatitis and for which no alternate diagnosis was made
[12] Dietary counseling commenced at the start of the diet stabilization period and was reinforced at intervals throughout the Treatment and Follow-up Period.
[13] Patients in Cohort A received 50 mg of ISIS 678354 once every 4 weeks or matching volume of placebo (0.5 mL) Weeks 1-49 of the Treatment Period.
[14] Patients in Cohort B will receive 80 mg ISIS 678354 once every 4 weeks or matching volume of placebo (0.8 mL) Weeks 1-49 of the Treatment Period.

- Patient-reported abdominal pain, other FCS-related symptoms (including physical fatigue, difficulty thinking and diarrhea)[15], diet[16], and impacts[17], health-related quality of life (HRQoL) [18], pain interference[19], and cognitive function[20].

COA Description(s)

Familial Chylomicronemia Syndrome Symptoms and Impacts Scale (FCS-SIS)
The FCS-SIS is a 17-item PRO instrument designed to assess FCS symptoms and associated impacts. It consists of two domains: Symptom Domain (4 items) and Impact domain (13 items). Items in the Symptom domain are rated on an 11-point numeric rating scale (NRS) ranging from 0 ("No [symptom]) to 10 ("worst possible [symptom]). Items in the Impact domain are rated on a verbal rating scale (VRS) ranging from "Never" to "Always." The recall period for the Symptom domain is the previous two weeks[21]. The recall period for the Impact domain is momentary ("currently"). The FCS Symptom domain (2-week recall) was administered at baseline, thereafter the 24-hour recall version was administered daily (minimum of 4 daily assessments per week) at baseline to Week 17. The FCS Impacts scale was administered at screening, baseline, and then every 4 weeks thereafter. Refer to Appendix A for a copy of the instrument.

Reviewer's comment(s): *The Applicant did not provide any information regarding the scoring of this instrument.*

FCS Diet Questions for Daily Diary
The FCS Diet Questions for Daily Diary is a patient-reported diary that consists of two items designed to assess fasting status and dietary fat intake. Both items are rated on a VRS with varying descriptors. The recall period is the previous 24 hours ("past 24 hours"). The FCS Diet Questions for Daily Diary was administered daily. Refer to Appendix B for a copy of the instrument.

Reviewer's comment(s): *The Applicant did not provide any information regarding the scoring of this instrument.*

Patient-Reported Outcome Measurement Information System (PROMIS) Adult Short Form v1.1 – Pain Interference 8a
The PROMIS Adult Short Form- Pain Interference 8a is an 8-item PRO instrument designed to assess pain interference on different aspects of a person's life. Each item is rated on a 5-point VRS ranging from 1 ("Not at all") to 5 ("Very much"). The recall period is the previous 7 days

[15] Assessed by the FCS-SIS.
[16] Assessed by the FCS Diet Questions for Daily Diary.
[17] Assessed by the FCS-SIS.
[18] Assessed by the Patient-Reported Outcome Measurement Information System (PROMIS) 29+2 Profile v2.1 (PROPr).
[19] Assessed by the PROMIS Adult Short Form v1.1 – Pain Interference 8a.
[20] Assessed by PROMIS Short Form v2.0 – Cognitive Function 4a.
[21] There are two versions of the Symptoms Domain, one with a recall period of "past 24 hours" (referred to as FCS Symptom) and another with a 2-Week recall period (referred to as FCS Symptoms Two Week Recall). Note the two week recall version was administered at screening and the 24-hour recall version was administered at baseline and post-baseline visits.

("past 7 days"). The PROMIS Adult Short Form- Pain Interference 8a was administered at baseline and then every 4 weeks thereafter. Refer to Appendix C for a copy of the instrument.

The PROMIS Adult Short Form v1.1 – Pain Interference 8a generates a total raw score (sum the values of the response to each question). The total summed raw score can be converted into a T-score. The T-score rescales the raw score into a standardized score with a mean of 50 (average for the United States general population) and a standard deviation (SD) of 10. A higher T-score indicates more pain interference.

PROMIS 29+2 Profile v2.1 (PROPr)

The PROPr[22] is a 14-item PRO instrument designed to assess eight dimensions of health status – physical function, anxiety, depression, fatigue, sleep disturbance, social functioning, pain interference and intensity, as well as cognitive abilities. Each item is rated on a 5-point VRS with varying descriptors. The recall period is the previous 7 days ("past 7 days"). The PROPr was administered at baseline, Months 3, 6, and12(end of study). Refer to Appendix D for a copy of the instrument.

The PROMIS-29+2 Profile v2.1 (PROPr) is used to calculate a preference score (PROMIS Preference, PROPr). Preference-based scores provide an overall summary of health-related quality of life on a common metric. Preference-based scores summarize multiple domains on a metric ranging from 0 (as bad as dead) to 1 (perfect or ideal health). Scores can be used in comparisons across groups and for cost-utility analyses. The profile includes all items in the PROMIS-29 Profile v2.1 plus two Cognitive Function Abilities items. T-scores from the measure can be used to calculate a preference-based score.

PROMIS Short Form v2.0 – Cognitive Function 4a

The PROMIS Short Form v2.0– Cognitive Function 4a is a 4-item PRO instrument designed to assess patient-perceived cognitive deficits. Each item is rated on a 5-point VRS ranging from 1 ("Very often/several times a day") to 5 ("Never"). The recall period is the previous 7 days ("past 7 days"). The PROMIS – Cognitive Function was administered at baseline, and Months 3, 6, and 12. Refer to Appendix E for a copy of the PROMIS Cognitive Function 4a.

The PROMIS Short Form v2.0– Cognitive Function 4a generates a total raw score (sum the values of the response to each question). The total summed raw score can be converted into a T-score. The T-score rescales the raw score into a standardized score with a mean of 50 (average for the United States general population) and a standard deviation (SD) of 10. Higher scores indicate better perceived cognitive functioning.

Appendices

Appendix A: FCS Symptoms and Impacts Scale
Appendix B: FCS Diet Questions for Daily Diary

[22] The PROPr is comprised of the PROMIS-29 content, plus two Cognitive Function—Abilities items.

Appendix C: Patient-Reported Outcome Measurement Information System (PROMIS) Adult Short Form v1.1 – Pain Interference 8a
Appendix D: PROMIS 29+2 Profile v2.1 (PROPr)
Appendix E: PROMIS Short Form v2.0 – Cognitive Function 4a
Appendix F: Summary of the Exit Interview Report

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Appendix F: Summary of the Exit Interview Report

The Applicant conducted an exit interview substudy within the open-label extension (OLE) study of Study CS13 in patients with familial FCS.[23] The interview completion was planned after patients completed their week 13 study visit of the OLE study.

The objectives of the qualitative patient interviews were, to describe:

- Patients' experiences prior to starting Study CS3
- Effect of study treatment on the symptoms and impacts related to FCS (either during Study CS3 or the OLE study)
- Meaningfulness of any changes experienced with treatment
- Patients' experiences specific to the FCS-SIS, including how well the combination of the symptom items captured their FCS symptom experiences and whether any important symptoms are missing.

Methodology of the Exit interviews:
Qualitative interviews (1 hour each; via telephone using an interview guide) were conducted with a subset of OLE study participants in the United States (US), Canada, Italy, and Spain after they had completed their week 13 study visit (i.e., allowing uptake for full dose efficacy for any patients having been randomized to placebo in Balance study). All patients completing week 13 of the OLE study were eligible for the exit interview sub-study. Information regarding the interviews was included in the OLE study protocol and informed consent form (Protocol ISIS 678354-CS13, Amendment 5; 29 Nov 2023). The clinical trial study staff were trained on the interview procedures via training slides.

The objectives of the qualitative patient interviews were:

- Patients' experiences prior to starting the Balance study (index study)
- Effect of study treatment on the symptoms and impacts related to FCS (either during the Balance or OLE study)
- Meaningfulness of any changes experienced with treatment
- Patients' experiences specific to the FCS Symptoms and Impacts Scale including how well the combination of the symptom items captured their FCS symptom experiences and whether any important symptoms are missing.

At the time of the interview all interview participants had finished the Balance study, had enrolled in the OLE study, had completed their week 13 OLE study visit, and continued to receive olezarsen. Each interview began with a few general, open-ended questions (followed by probing questions). The interviews were recorded and transcribed.

The majority of the interview data was analyzed for the following two time periods:

- Pre-trial: The time prior to patients' enrollment in the Balance clinical trial

[23] The global phase 3 study (Balance study, 678354-CS3) for olezarsen in patients with FCS was followed by an open-label extension (OLE) study (678354-CS13); the FCS-SIS was administered in the Balance study. As part of the OLE study (see 678354-CS13 Protocol, dated 29 November 2023), Ionis collaborated with (b) (4) to conduct an exit interview sub-study to provide further support for the content validity of the FCS-SIS and to facilitate the interpretation of the olezarsen clinical trial results.

- Post-treatment: The time after which any given patient either (1) believed they received olezarsen in the Balance study or (2) began receiving olezarsen as part of the OLE study.

Results of the Exit Interviews:

Demographics:

A total of 18 participants (US n=5, Canada n=6, Italy n=4, Spain n=3) completed the interviews; of these,

- Eight (44%) participants were males and n=10 (55%) were females; the mean age of the participants was 43 (range, 23-73) years. Note that the ethnicity/race and the level of education of the participants was not included in the submission.

- The mean age of the participants at FCS diagnosis was 15.6 years (range, 0-53), including 6 patients reporting a diagnosis at birth or prior to 1 year of age. On average, time since diagnosis was 27 years, ranging from 3 to 50 years. The mean age when FCS symptoms were first experienced was 10.8 years (range, 0-48.5). Seven of the 18 interview participants experienced FCS symptoms for more than a year prior to their FCS diagnosis (for a duration of 2 to 42 years), 8 were diagnosed within 1 year after experiencing their first symptoms (for a duration of 0 to 1 year[s]), and 3 were diagnosed prior to their experience of FCS symptoms.

- Ninety four percent (n=17/18) participants reported experiencing an acute pancreatitis event at some point in their life, prior to the Balance study; the mean age at first acute pancreatitis event was 16 years (range, 0-62 years); 14/17 (82%) participants had an acute pancreatitis event in the 10 years prior to the Balance study, including 13 patients with associated hospitalizations. Three individuals spontaneously reported having had some type of surgery on their pancreas.

- In the Balance study, 6/18 patients were in each of the two treatment groups and 6 patients were in the placebo group. A total of 6 patients had previously received volanesorsen and 5 were identified as having no history of acute pancreatitis that resulted in a hospital visit within the 10 years prior to starting the Balance study.

The majority of the interview data was analyzed for the following two time periods:

- Pre-trial: Defined as the time prior to patients' enrollment in the Balance clinical trial.
- Post-treatment: Defined as the time after which any given patient either (1) believed they received olezarsen in the Balance study or (2) began receiving olezarsen as part of the OLE study.

The sponsor's results are summarized below:

Pre-trial Experiences

FCS-related symptoms

All 18 patients reported experiencing at least one FCS-related symptom prior to starting the Balance study.

- The most commonly reported symptoms were:

- Abdominal pain reported by n = 17 (94%) of the participants.
  - Abdominal pain was also reported by n=11(64%) participants as their most bothersome symptom. Participants described this pain as debilitating with interference with their daily activities and the worry associated with whether the pain would progress into an acute pancreatitis event.

- Physical fatigue reported by n = 12 (66%) of the participants.
  - Physical fatigue was reported as most bothersome by n=3 (17%) participants as it interfered with daily activities and responsibilities (often severe during an acute pancreatitis event).
  - Physical fatigue severity ranged from mild to severe, and reported frequency ranged from occasional to daily fatigue.
  - Participants described physical fatigue using the terms: "being so tired even if I get a good night's sleep" "weak legs" "no motivation," "a shutdown of vital energy" and "heavy tiredness" all of which ultimately had an impact on their everyday lives. For instance, patients reported experiencing difficulty with daily tasks, such as getting mail or climbing stairs.
  - Physical fatigue was often severe during an acute pancreatitis event; participants reported feeling fatigued before an event and recognizing fatigue as a warning sign for a potential acute pancreatitis event. Additionally, patients reported continuing to feel fatigued as they recovered from an event.

- Diarrhea (that occurred outside an acute pancreatitis event) was reported by n=10 (56%) of the participants.
  - Patients' diarrhea severity ranged from mild to severe and the frequency ranged from several times daily to monthly ("not often"). For the 4 patients with acute diarrhea during acute pancreatitis events, both the severity and frequency of their diarrhea increased during this time.

- Vomiting (experienced only during acute pancreatitis events) was reported by n=9 (50%) of the participants.

- Nausea was spontaneously reported by 6 patients (33%), primarily those experiencing this symptom only during acute pancreatitis events (n = 5). Nausea was usually mild at the beginning of an event and could escalate to severe during an event.

- Difficulty thinking was reported by 5 (27%) participants.
  - n=3 participants said that these problems were ongoing, occurring during and between acute pancreatitis events.
  - Participants described difficulty thinking as "brain fog", "being unable to think clearly", "problems remembering things, concentrating, and completing tasks as quickly as they would like to".

        - The severity of difficulties with thinking varied for all 5 patients, with the worst experiences usually occurring during and immediately following an acute pancreatitis event.

- Acute pancreatitis events: Of the 18 participants,
    - Seventeen (94%) noted at least 1 acute pancreatitis event had occurred in their lifetime (1 patient reported no history of acute pancreatitis), including 14 patients who had an acute pancreatitis event within the past 10 years (77%). The frequency of these events also varied, from 1 patient being routinely hospitalized for events every 15 days to another patient with only 1 event in the past 10 years.
    - For most patients, acute pancreatitis events were described as extremely painful, "paralyzing," and "unbearable." All participants described events as severe and very impactful to daily life, with many noting the total inability to function in daily activities. Acute pancreatitis events usually prevented patients from caring for their family and home or participating in work/school and social activities.
    - Participants reported that the severe debilitating nature of events led to a constant fear of experiencing such attacks in the future, as well as worries related to medical bills; missed work or school and decreased productivity; and potential hospitalization.
    - Patients also mentioned how the acute pancreatitis events often resulted in additional dietary restrictions, including taking nothing by mouth (including food and liquids) at home and during hospitalizations.

Chronic[24] FCS-related symptoms

- Chronic FCS-related symptoms were reported by n=15 (83%) participants. Of these, abdominal pain was reported by n=12 (80%), fatigue was reported by n=8 (53%), diarrhea was reported by n=6 (40%), difficulty thinking was reported by n=3 (20%) participants.
    - Notable differences in pre-trial symptom experiences or trends are not easily identifiable among the 6 patients who previously took volanesorsen, compared with the 12 participants who had not taken volanesorsen.
        - All 6 participants who had taken volanesorsen noted severe abdominal pain during acute pancreatitis events; of these, n=5 (83%) reported chronic abdominal pain; and n=2 (33%) participants had not experienced events in the 10 years prior to the Balance study.

***Reviewer's comments**: The finding above indicates that participants experienced abdominal pain outside of the pancreatitis events.*

FCS-related impacts

- The FCS-related impacts reported by the majority (>60%) of the participants were related to dietary restrictions, mood/emotions, social activities, hospitalizations, daily

[24] For each symptom discussed, patients were asked whether they experienced the symptom only during acute pancreatitis events (acute) or also at times outside an acute pancreatitis event (chronic).

and physical activities, work, career, or schooling, and relationships with family and friends. See Table 1.

Table 1. Patient Report of Pre-trial Impacts

Impact	Reported, n (%) (N = 18)[a]	Most bothersome, n (%) (N = 16)[b]
Dietary restrictions	18 (100.0)	4 (25.0)
Mood and emotions (e.g., anxiety, fear of pancreatitis, social stigma, worry about ability to become pregnant, worry about future health)	17 (94.4)	5 (31.3)
Social activities (e.g., those involving food, planning events)	14 (77.8)	4 (25.0)
Hospitalizations	14 (77.8)	4 (25.0)
Daily/physical activities (e.g., walking, childcare, leaving the home)	12 (66.7)	0 (0)
Work or school	11 (61.1)	2 (12.5)
Relationships (e.g., intimate, family, friends)	7 (38.9)	3 (18.8)
Finances	4 (22.2)	0 (0)

FCS = familial chylomicronemia syndrome.

Note: Other impacts of FCS spontaneously reported by < 3 participants include limited contraceptive options (n = 2), health during pregnancies (n = 2), weight loss (n = 1), hunger (n = 1), and scarring from surgeries related to FCS signs/symptoms (n = 1).

[a] Patients were asked to spontaneously comment on the impact of FCS on their lives. General probes were used to expound upon the patient report. Given the conversational nature of the interview, the absence of report does not equate with an absence of impact.

[b] Two patients were not asked to identify their most bothersome pre-trial impact of FCS. Seven of the 16 patients who identified most bothersome impacts identified more than 1 impact as most bothersome.

Source: Table 5 under Section 3.3.3, page23/128 Exit Interview Report.

Post-treatment Experiences and Meaningful Improvement

Reviewer's comments: *Per the interview guide, the participants were asked the following questions:*

- (b) (4)

- [redacted] (b) (4)

Reviewer's comment(s): *Overall, this reviewer finds the questions reasonable with the exception of questions on the meaningful change. The participants were not queried on the different categories of the anchor scales to determine the meaningfulness of change.*

Symptoms and Acute Pancreatitis Events

- Participants (n=14/18; 77%) reported improvement in at least one of their pre-trial symptoms and most improvements were considered meaningful[25] (See Table 2).

Table 2. Patient Report of Post-treatment Symptom Improvement

Symptom	Reported pre-trial, n	Improved n/base of reported as most bothersome symptom pre-trial	Improved n/base of responses (%)[a]	Meaningful improvement, n/base of responses (%)[b]
Abdominal pain	17	11/11 (100.0)	14/17 (82.4)[c]	13/14 (92.9)
Physical fatigue	12	3/3 (100.0)	7/12 (58.3)	6/7 (85.7)
Diarrhea	10	0/1 (0)[d]	6/10 (60.0)	6/6 (100)
Vomiting	9	—	7/8 (87.5)	7/7 (100)
Nausea	6	—	3/5 (60.0)	3/3 (100)
Difficulty thinking	5	1/1 (100.0)	3/5 (60.0)	3/3 (100)
Bloating	4	—	1/1 (100.0)	1/1 (100.0)
Constipation	3	—	2/2 (100.0)	0/0 (—)

IDI = in-depth interview; OLE = open-label extension.

Note: Other signs and symptoms spontaneously reported pre-trial by < 3 participants include headaches or migraines (n = 2), gas (n = 1), fever (n = 1), blood pimples (n = 1), and xanthomas (n = 1). One patient reported gas as their most bothersome symptom. All of these patients reported symptom improvement, except for the 2 patients who reported experiencing headaches and migraines pre-trial, who described no improvement (n = 1) or worsening (n = 1) of these symptoms post-treatment.

[a] Based on those reporting pre-trial symptoms responding to question on improvement.

[b] Based on those reporting pre-trial symptom improvement and responding to question on meaningfulness.

[c] One patient ((b) (6) profiled in Table 7) reported the worsening of abdominal pain associated with liver function during the OLE study.

[d] One patient reporting pre-trial diarrhea as most bothersome did not report improvement; they had not recently experienced diarrhea and attributed earlier improvement to volanesorsen.

Source: Table 6 under Section 3.4, page 29/128 of the Exit Interview Report.

  - Post-treatment improvement was seen in abdominal pain (n=14/17, 82%) and physical fatigue (n=7/12, 58%) of the participants; note that these symptoms

[25] Meaningful symptom improvements often related to patients being able to increase their participation in daily and social activities due to a noticeable difference in the symptoms and number of acute pancreatitis events they experienced.

were also considered most bothersome as well by the participants who were asked about bothersomeness.
    - For many (n=not known) patients, less abdominal pain also meant fewer acute pancreatitis events.
    - Of the patients who did not experience improvements in physical fatigue, two attributed their chronic mild fatigue to health conditions other than FCS (i.e., previous cancer treatment, cardiac issues), and one experienced a significant improvement in fatigue following pancreas surgery before treatment.
    - All but 1 patient (n = 6 of 7; 85%) who reported an improvement in physical fatigue described these improvements as significant, such that they were able to have more energy to accomplish their goals, including tasks at work and school and spending more time with family and friends.
  - While diarrhea also showed improvement (n=6/10, 60%), the participants did not consider this symptom as most bothersome.
  - For difficulty thinking, improvement was seen in 3/5 participants who reported this symptom pre-trial. This symptom was not reported by the majority of the participants pre-trial.

- Four patients experienced no symptom improvement post-treatment (See Table 7 under Section 1.41 of the exit interview report). These 4 patients reported that they continued to experience generally mild symptoms from before the trial to after treatment.
  - Two of these 4 patients were pancreatitis naïve ((b) (6)), including 1 patient ((b) (6)) who previously received volanesorsen.
  - (b) (6) shared that they did not have knowledge of their TG levels to confirm if anything had improved.
  - The other 2 patients ((b) (6)) had experienced previous acute pancreatitis events and received placebo during the Balance study.

- Acute pancreatitis events

  Reviewer's comments: *It is difficult to compare the rate of acute pancreatitis events pre- and post-treatment given that the pre-trial patient acute pancreatitis events appear to be sparse spread over a few years in time. For example, "n=17 (94.4%) noted at least 1 acute pancreatitis event had occurred in their lifetime (1 patient reported no history of acute pancreatitis), including 14 patients who had an acute pancreatitis event within the past 10 years (77%). The frequency of these events also varied, from 1 patient being routinely hospitalized for events every 15 days to another patient with only 1 event in the past 10 years.*

- Dietary Restrictions: Less than half (8/17; 47%) of participants reported improvements in dietary restrictions, many who did not report improvements noted that they were instructed to continue following their usual dietary restrictions throughout the Balance study and OLE or were not comfortable trying foods outside their usual diets. Overall,

those who did report improvements in the impact of dietary restrictions reported that these improvements were meaningful.

- Hospitalizations: Most patients who reported pre-trial hospitalizations reported post-treatment improvements (n 11/14; 78%). These improvements included less frequent visits to the hospital, which improved their quality of life.

- Daily and Physical Activities: An improvement in daily and physical activities was reported by n 8/12; 66% of the participants who reported this impact before the trial. All these improvements were meaningful to patients, including being able to participate in activities they "wanted to do" and being able to "live normally."

Reviewer's comment(s):
In their Exit Interviews, the Applicant has demonstrated improvement in some symptoms (i.e., abdominal pain, physical fatigue and diarrhea), frequency of acute pancreatitis events and impacts (on dietary restrictions, hospitalizations, and daily activities). It should be noted that these results do not mirror the results of the exploratory analyses of Study CS3 (see Section 11.4.1.4. of the CS3 Body Report, SDN 1).

However, this reviewer believes that the results should be viewed with caution for the following reasons:

Exit Interview Report:

1. *The patient transcripts were not included in the NDA submission therefore, we are unable to confirm the Applicant's findings. Further, DCOA did not review the exit interview study protocol and the interview guide prior to the conduct of this study.*
    a. *The interview guide was deficient in certain areas particularly the querying of the participants on meaningful change. The participants were not queried on meaningful change on each category of the anchor scales.*

2. *Whether symptoms/impacts of FCS were reported spontaneously by the participants or probed by the interviewer(s) is not stated with all symptoms/impacts in the exit interview report. The frequency counts of the patients who understood each item in the instrument, along with descriptive statistics regarding item relevancy (i.e., number of patients who found each item relevant) is also not included in the exit interview report.*

3. *The following concerns regarding the interpretability of the PRO items (identified during the IND phase) still exist as the Applicant did not address them.*
    a. *The response scale for the FCS Symptoms is a numeric rating scale (NRS); a verbal rating scale may be more appropriate and interpretable for some of the item concepts (e.g., diarrhea, difficulty thinking). It is unclear what attribute of diarrhea is being rated and what constitutes an "8" for diarrhea; and what is the difference between an 8 and 9 related to diarrhea.*

b. *The anchor scales (i.e., Patient Global Impression of Severity and Patient Global Impression of Change) have limitations in that they assess the severity of FCS instead of severity of "FCS symptoms". This concern was also raised in the IND phase and the Applicant did not address. However, per the interview guide when the participants were queried on these anchors they were told to keep their symptoms in mind.*

4. *While the exit interview report states that there was improvement in the impacts (dietary restrictions, number of hospitalizations, number of acute pancreatitis), it is difficult for us to determine whether these patient-reported numbers are correct. We defer to the Division/Biostats to confirm these numbers from the Study CS3 report.*

5. *Measurement properties for the FCS SIS were not included in the NDA submission.*

Design of the substudy:

6. *The interviews were conducted in the OLE phase of Study 678354-CS3 where all patients were given olezarsen. As such there is concern that open-label study design may limit interpretability of COA data. Respondents' knowledge of treatment assignment may lead to systematic overestimation or underestimation of the treatment effect, the magnitude of which is currently unknown.*

    a. *Of the 18 participants,* n 10/12 (83%) *were accurate in their self-assessment of having received either treatment or placebo, often due to the change (or lack of change) in their pre-trial symptoms and acute pancreatitis event experiences and 6 participants were unsure.*

    b. *Additionally, some patients were provided their triglyceride (TG) levels by their personal (non-trial doctors) while they were participating in the Balance study or shortly thereafter. This knowledge, as well as the appearance of their blood (i.e., pink blood), also led a few participants to believe they either did or did not receive olezarsen during the Balance study.*

    c. *Furthermore, per OLE study protocol, patients may have been unblinded at the principal investigators' discretion following the week 13 study visit, which may account for some patients' awareness of their TG levels at the time of interview participation.*

    d. *The interviews were conducted 3 to 48 weeks after completion of the study. This raises the concern of recall error.*

    e. *Due to time considerations, not all interview questions were asked of all participants.*

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

YASMIN A CHOUDHRY
11/21/2024 12:07:38 PM

SELENA R DANIELS
11/21/2024 12:12:52 PM

Date: November 7, 2024

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

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

To: Eileen Navarro Almario, MD (Office of New Drugs, Office of Immunology and Inflammation, Division of Hepatology and Nutrition [OND/OII/DHN]); Paul Hayashi, MD (OND/OII/DHN); Edwige ChiogoVouffo, MD (OND/OII/DHN); Hobart Rogers, PharmD, PhD (OCP, Division of Translational and Precision Medicine)

Subject: Hepatobiliary toxicity of liver targeting ASO/SiRNA products intended for dyslipidemia indications (NDA 218614, olezarsen)

EXECUTIVE SUMMARY

DHN OII/OND/CDER consulted DARS regarding the potential mechanism of hepatobiliary events described for olezarsen based on the ASO and/or its linker.

Olezarsen is an antisense oligonucleotide (ASO)-GalNAc conjugate designed to inhibit *apolipoprotein C-III (APOC3)* mRNA expression and thus APOC3 protein synthesis and is under review as a potential adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS). Olezarsen contains a GalNAc complex at the 5′ position for specific targeting to the liver where unconjugated ASO binds to its target *APOC3* mRNA for degradation. Olezarsen has identical sequence, chemical composition, and mechanism of action as volanesorsen. The difference is that olezarsen is (b) (4) GalNAc3-conjugated while volanesorsen is not. This suggests that any liver enzyme elevations and/or potential for adverse events or toxicity is a function of the cellular/tissue concentration differences of olezarsen and/or its metabolites and thus on-target or off-target effects.

Our review of the literature and findings in FDA submissions do not suggest potential olezarsen on-target or non-specific off-target sequence effects on mRNAs in the liver that may be related to hepatic/hepatobiliary dysfunction and toxicity. Metabolites produced across species were low in liver tissue and no unique or disproportionate metabolites were identified in humans compared to the animal species. Comparative evaluation of other GalNAc-conjugated-ASOs with (b) (4) linkers submitted to the FDA characterized an inconsistent signal related to liver adverse events across ASOs, suggesting that the (b) (4) linker is unlikely to be associated with hepatobiliary injury. The relationship between APOC3 and liver steatosis or metabolic dysfunction-associated steatotic liver disease (MASLD) is conflicting across different studies, however, clinical data has shown that APOC3 inhibition by volanesorsen resulted in reduced hepatic fat fraction (HFF) in patients with diverse etiologies of severe hypertriglyceridemia including familial chylomicronemia syndrome (FCS). Lastly, information on the impact of chronic administration of olezarsen in subjects with impaired liver function was not available and has not been assessed for volanesorsen (which is an approved FCS therapy in Europe). However, it is noted that ASO oligonucleotides are not metabolized by the cytochrome P450 enzyme system in the liver. Further, we found no evidence linking high uptake of GalNAc-conjugated-ASOs and slow/delayed release from hepatic endosomes with changes in liver function/injury or whether ASO on-target effects on the lipodome can influence endosomal function or endosomal-lysozyme systems in hepatic cells.

Because olezarsen and volanesorsen differ only in the (b) (4) GalNAc3-conjugate, further exploration, and comparison of these ASOs in a suitable *in vitro* cell system could be an option to provide further information on the relationship between potential liver changes and endosomal trafficking and release, and reduced APOC3-mediated changes in the transcriptome and lipodome following ASO degradation.

BACKGROUND

Familial chylomicronemia syndrome (FCS) is an inherited lipid disorder marked by hypertriglyceridemia affecting 3000-5000 patients globally. Lipoprotein lipase, the enzyme responsible for TG hydrolysis, is inhibited by apoC-III. By degrading *APOC3* mRNA and subsequent protein production, an ASO volanesorsen restored LPL activity, reduced TG levels and pancreatitis events in FCS. (b) (4) and the sponsor moved forward with a second-generation ligand conjugated ASO (olezarsen, the active product in this NDA) that can be delivered hepatically at lower doses (80 mg intended dose) and less frequently.

The development program consists of 2 main studies. CS3 enrolled 44 olezarsen exposed FCS subjects in a placebo-controlled study of 66 subjects followed for a year; 54 patients were treated in an open label extension phase 3 study. Safety is buttressed by study CS8, that enrolled 115 patients with moderate hypertriglyceridemia with or at risk for cardiovascular disease or patients with severe hypertriglyceridemia.

There are 3 to 5 thousand subjects with FCS globally, whereas severe hypertriglyceridemia (~3.4 million Americans in 2006 with triglyceride >= 500 mg/dL) and moderate hypertriglyceridemia (<26.8 million in the US with TG >150-499 mg/dL) with or at risk of CVD are highly prevalent. Approximately 68% of patients with moderate to severe hypertriglyceridemia have MASLD.

Olezarsen is not a substrate or inhibitor of major transporters including BSEP. It has very low potential to involve CYP and transporter mediated DDI. Hence, drug interaction experiments with CYP and transporters were not necessary per sponsor. Fragmented oligonucleotide metabolites were eliminated in urine and full length olezarsen eliminated in urine was <1%. This suggests that olezarsen has low evidence to impact the bile or to form gallstones.

Olezarsen is a 2'-MOE chimeric antisense oligonucleotide (ASO) conjugated to N-acetylgalactosamine 3 (GalNac3) via a (b) (4) linker to enhance liver delivery. Increases in alanine aminotransferase (ALT) over baseline were observed in two clinical studies, as well as adverse events related to liver and biliary signals. DHN OII/OND/CDER thus consulted DARS regarding the potential mechanism of hepatobiliary events described for Olezarsen based on the ASO and/or its linker. ASOs bind to mRNA to modify protein expression via complementary base-pairing hybridization. All ASOs can exhibit off-target effects by binding to non-target mRNAs via partial base-pairing – a mechanism that is dependent on the ASO sequence. One possibility for olezarsen's liver and biliary signals is thus related to both GalNAc-targeting: GalNAc-targeting delivers this oligonucleotide in mass quantities to the liver. If off-target mechanisms for this sequence can cause cell injury, then liver and biliary events can happen.

Assistance was requested for:

1) Assessing the potential mechanism of the liver and biliary events described with olezarsen based on the ASO or its linker.
2) Assessing whether disrupted lipid metabolism in the hepatocyte could result in lipid dysregulation (e.g. altered feedback loops) and hepatobiliary injury.
   Any insight on the impact of chronic administration in subjects with impaired liver function would also be useful to consider in risk mitigation.

EVALUATION

Olezarsen is an antisense oligonucleotide (ASO)-GalNAc conjugate designed to inhibit *apolipoprotein C-III (APOC3)* mRNA expression and thus APOC3 protein synthesis and activity as a potential adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS). It characterizes a 5′-(b) (4) GalNAc3 (GalNAc) conjugated 2′-O-(2-methoxyethyl) (MOE) ASO containing a phosphorothioate (PS) backbone with sequence: (b) (4)AG^{Me}C^{Me}U^{Me}U^{Me}CTTGT^{Me}C^{Me}CAG^{Me}C^{Me}U^{Me}U^{Me}UA^{Me}U-3′. The underlined residues are 2′-O-(2-methoxyethyl) nucleosides; all other residues are 2′-deoxynucleosides. (b) (4) . The 2' -O-(2-methoxyethyl) methyluridine (2'-MOE MeU) nucleosides are sometimes referred to as 2' -O-(2-methoxyethyl) ribothymidine (2'-MOE T) (**Figure 1**). The 5' nucleotides at each end are 2′O-(2-methoxyethyl)-(b) (4) . In addition, the compound contains a GalNAc complex at the 5′ position (b) (4). In the liver, the GalNAc3 conjugated uptake in kidney and other organs is like the PS-backbone driven uptake seen for unconjugated ASOs-conjugated ASO binds to the asialoglycoprotein receptor (ASGPR) and is taken up into endosomes. It then dissociates and the ASO enters the cytoplasm and nucleus where it binds to its complementary sequence within the *APOC3* transcript. Specifically, olezarsen binds to *APOC3* mRNA to form a DNA: RNA hybrids that recruits RNase H1 enzyme. RNase H1 then cleaves the RNA in the duplex structure, causing mRNA degradation [1,2] after which the ASO is released and can bind another *APOC3* transcript.

Figure 1: Olezarsen drug sequence with base labeled for each nucleotide shown below to correlate with schematic presentation using colored squares

1. Assessing the potential mechanism of the liver and biliary events described with olezarsen based on the ASO or its linker.

ASO and (b) (4) Linker

Olezarsen has identical sequence, chemical composition, and mechanism of action as volanesorsen. Therefore, the difference in toxicity or potential for elevated liver enzyme levels is unlikely to be mediated through sequence or chemical composition differences. The difference is that olezarsen is (b) (4) GalNAc3-conjugated while volanesorsen is not. The advantage of ASO conjugation to GalNAc

is that delivery to hepatocytes is markedly increased, which reduces the dose and administration frequency required to inhibit *APOC3* which is expressed primarily in hepatocytes. High systemic concentrations of volanesorsen in plasma is likely related to the thrombocytopenia events observed. This suggests that liver enzyme elevations and/or potential for toxicity is a function of the hepatic cellular/tissue concentration differences of olezarsen and/or its metabolites and on-target or off-target effects.

Chemistry-wise, olezarsen is a 2nd generation PS-ASO. (b) (4) eplontersen (file://cdsesub1/EVSPROD/NDA218614/0001/m2/27-clin-sum/summary-clin-safety.pdf) clinical summary reports conclude no increased liver adverse events for these treatments.

(b) (4) For eplontersen, the integrated review included an assessment of drug-induced liver injury. The review did not observe any Hy's law cases and concluded that there did not appear to be a significant increased risk of hepatic adverse events with eplontersen. However, the review did note cases of ALT elevations (from >ULN to 3.5xULN) from 4 health participants with dosing of 90 mg eplontersen every 4 weeks, which is twice the approved dosing. It also described six cases of abnormal laboratory findings from patients, with 3 classified as possibly related to eplontersen. The product labeling for eplontersen does not include any information regarding liver enzyme elevations.

As the clinical data from (b) (4) eplontersen do not suggest an association with liver adverse events and as these products also have (b) (4), while limited, this data would suggest that the (b) (4) linker is unlikely to be directly associated with hepatobiliary injury.

Table 1. GalNAc-conjugated 2nd generation PS-ASOs that have (b) (4) -linkers.

NDA 218614	Olezarsen	2nd Gen PS-ASO (2'-MOE)
		(b) (4)
NDA 217388	Eplontersen/ION-682884	2nd Gen PS-ASO (2'-MOE)
		(b) (4)

RNA oligonucleotides (including ASOs and siRNAs) are too charged, too large and/or too hydrophilic to diffuse across the cell membrane and instead are taken up by endocytosis[3,4]. As described above, GalNAc-conjugated ASOs bind to ASGPR in the liver and are taken up in endosomes where the GalNac-conjugated ASO dissociates from the receptor. Olezarsen is cleared rapidly from plasma primarily because of distribution to tissues. GalNAc3 conjugated uptake in kidney and other organs is like the PS-backbone driven uptake seen for unconjugated ASOs. However, endosomes are also composed of a lipid

bilayer barrier that results in endosomal capture and retention of ~ 99% of the conjugated ASO (**Figure 2**) with only 1-2% of GalNAc ASO conjugates or ASOs escaping from endosomes in hepatocytes *in vivo* in what remains a poorly understood mechanism[4]. Similarly, only 0.3% of endocytosed GalNAc–siRNA conjugates have been identified in the cytoplasm *in vivo* at any given time. Although endosomal trapped RNA oligonucleotides serve as a depot (enabling long single dose duration of response as observed), this also results in ~99% of endocytosed RNA ASOs failing to enter the cytoplasm[4]. Consequently, escape/release from endosomes remains an important rate-limiting delivery problem while maintaining a partial depot effect for long duration of responses [3]. The rate of endosomal escape (or maximal response in humans) for olezarsen is not defined.

Figure 2: Endosomal uptake and escape

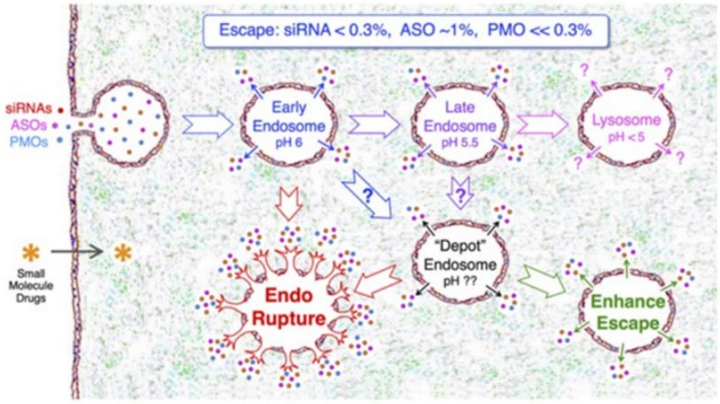


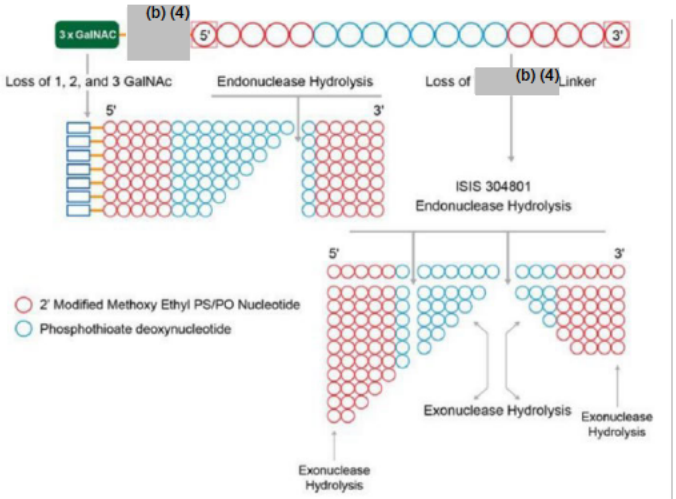
Image from Dowdy et al., PMID: 36669888

Independent of the entry route, the initial sorting compartment along the intracellular endocytic trafficking pathway is the early endosome. In general, endosome contents are recycled back to the plasma membrane or targeted for degradation. For degradation, contents are trafficked to the late endosome or multivesicular body (MVB) and subsequently to the lysosomes and/or Golgi. Whether increased tissue concentrations of conjugated-ASO and non-conjugated ASOs in endosomes may have implications for malfunction of the endosomal-lysosome systems is not known, but malfunction of this system(s) by other mechanisms is associated with liver disease such as non-alcoholic fatty liver disease[5,6].

Further, metabolism and/or degradation of olezarsen may occur within cells/tissues (both in the cytoplasm following release from the endosome as well as inside the endosome) and in circulation. While different metabolites[7] of olezarsen (e.g., chain-shortened oligonucleotide metabolites of unconjugated olezarsen, **Figure 3**) were identified in plasma, liver, kidney, and urine across species 48 hours after dosing (\\CDSESUB1\EVSPROD\nda218614\0001\m2\25-clin-over\clinical-overview.pdf
and 2.6.4 Pharmacokinetics Written Summary; \\CDSESUB1\EVSPROD\nda218614\0001\m2\26-nonclin-sum\pharmkin-written-summary.pdf), levels accounted for < 8% of total relative peak area in liver and can be considered low or rare and therefore unlikely to contribute to toxicity via a RNase H1 sequence-dependent or -independent mechanism. Concentrations (µM) of -1, -2 and -3 GalNAc metabolites were either below the limit of quantitation or low in concentration in monkey liver (0.681 ± 0.124) 48 hours after administration of 30 mg/kg. Similarly, a total of 11 (b) (4) linker-related metabolites[7] of olezarsen were detected in human urine samples. However, metabolite levels were also low (< 10% of administered dose). The most abundant human linker metabolites were M5 and M8. (b) (4) linker-related metabolites were minimally released to circulation and subsequently rapidly

excreted in urine (or via biliary excretion in monkeys) within 24 hours similar to other conjugated ASOs of the same chemical class (*2.6.4 Pharmacokinetics Written Summary;* \\CDSESUB1\EVSPROD\nda218614\0001\m2\26-nonclin-sum\pharmkin-written-summary.pdf)). Based on current data no unique or disproportionate olezarsen metabolites were identified in humans compared to animal species that suggest potential for involvement in hepatic or hepatobiliary toxicity of injury.

Figure 3. Oligonucleotide-related metabolism of oleszasen



ASO species (i.e., fully conjugated, partially conjugated [with 1, 2, and/or 3 GalNAc sugar deletions], and unconjugated olezarsen) and oligonucleotide metabolites associated with nuclease-mediated metabolism of ASO species (chain-shortened metabolites)

ASO On-target effects of olezarsen

The olezarsen ASO was designed to target the 3'untranslated region (UTR) of *APOC3* mRNA that is primarily conserved across species including humans and rhesus and cynomolgus monkeys (*Mammals Multiz Alignment & Conservation*, UCSC bowser). Early phase 1/2a clinical studies confirmed effective dose-dependent pharmacodynamic impact on the target, *APOC3,* with a clear difference in the TG-lowering effect (NCT02900027). The sponsor further characterized on-target effects or degradation of the *APOC3* by olezarsen *in vitro*, in human primary hepatocyte cells and hepatocytes from human *Apoc3* transgenic mice using reverse transcriptase-quantitative PCR (RT-qPCR). Following either an overnight or 48-hour incubation (with recovery), olezarsen produced a concentration dependent reduction of *APOC3* mRNA up to 82% (IC50 of ~0.18 µM) in human primary hepatocytes and up to 89% (IC50 of ~0.012 µM) in transgenic mouse hepatocytes. Using literature and database information from Ingenuity Pathway Analysis (IPA, Qiagen), we identified only a single proteomics study suggesting that reduced levels of APOC3 protein in serum was associated with hepatocellular carcinoma (HCC)[8]. We observed no *in vivo* or *in vitro* evidence to suggest that the deficiency of *APOC3* in the liver and thus on target effects by olezarsen in humans might be associated with liver dysfunction or toxicity. Further, data from genome-wide association study in the broader population and/or natural experiments involving loss-of-function mutations in the *APOC3* gene (and thus the impact of reduced APOC3 levels by ~50% in closed founder populations) do not provide evidence of a potential link between reduced endogenous APOC3 synthesis with liver dysfunction or toxicity. Rather, the results suggest increased efficiency of conversion of VLDL to LDL, thereby reducing remnant concentrations, and thus potential to reduce the risk of coronary heart disease. In contrast, APOC3 deficiency is associated with diet-induced steatosis, obesity, and insulin resistance in APOC3 knock-out mice[9].

ASO sequence-dependent off-target effects of olezarsen

RNase H1 the enzyme that cleaves the mRNA transcripts in the duplex can tolerate some base sequence divergence resulting in unintended RNA cleavage if partial complementarity occurs. Off-target ASO dependent RNase H1 recruitment depends on several factors. The nature of the heteroduplex formed between the ASO and its non-intended target including the number, position and type of mismatches can influence RNase H1 activity. The length of the ASO is also important. Shorter ASOs matching off-target regions can either perfectly or nearly perfectly match [10], while longer ASOs provide opportunities for partial hybridization throughout the transcriptome. In both cases this represents a loss of specificity. Olezarsen is a 20 nt chimeric oligonucleotide. This is in keeping with other RNase H1 dependent ASO sequences which are between 16 and 20 nucleotides in length [11-15]. Several studies have observed that off-target effects (or the binding affinity of the ASO for a target) are more often seen when mismatches in the flank regions of ASOs than when mismatches are in the central gap DNA region [16]. Olezarsen (and thus volanesorsen) characterizes a 2'-O-methoxyethyl (MOE) ASOs with lower binding affinity compared to ASOs with high-affinity ribose modifications, such as locked nucleic acid (LNA) or constrained ethyl (cEt). The latter ASOs are also shorter thereby increasing the likelihood of aligning to off-targets and producing liver toxicity[11,17,18].

In NDA 218614, STUDY ID 23-133f, off-target study the sponsor identified six gene transcripts or mRNAs (*RAC1, STIM2, CADM1, FOXP2, RAC1P2*, and *RAC1P4*) as potential off-target transcripts of olezarsen based on *in silico* predictions. Of the six, four transcripts (*CADM1, FOXP2, RAC1P2,* and *RAC1P4*), were reported not to be expressed in liver and were excluded from analysis. This result based on a median transcripts per million (TMP) < 1 was confirmed for liver tissue and single primary hepatocyte cells using expression data from GTEX (https://gtexportal.org/home/) and Protein Atlas (https://www.proteinatlas.org/humanproteome/single+cell/tissue+cell+type/liver). Exploratory analyses of the remaining two predicted off-target transcripts together with *APOC3* using Ingenuity Pathway Analysis knowledge base (Qiagen LLC) did not show any association of the gene products (*RAC1*, and *STIM2*) with liver toxicity endpoints, responses (e.g., fibrosis) or causal phenotypes (e.g., liver cancer, cirrhosis, and degeneration) in humans. The effect of olezarsen on the mRNA levels of *RAC1*, and S*TIM2* was assessed in cultured primary hepatocytes using reverse transcriptase-quantitative PCR (RT-qPCR) and was shown to be > 10-fold less potent than the on-target APOC3 mRNA reduction. Olezarsen showed selectivity for human *APOC3* mRNA as described above. The *in silico* predicted off-targets *RAC1*, and *STIM2* were not reduced significantly in human cells *in vitro.* For olezarsen, searching for putative *in silico* sequence-dependent off-targets in mature- and pre-mRNA should be considered. Additionally, non-coding RNAs should be considered as potential off targets because olezarsen is an ASO. Many computational *in silico* tools and databases are now available publicly (including *blastn*; https://blast.ncbi.nlm.nih.gov/Blast.cgi) that may have utility in evaluating sequence-of-target effects. We evaluated the sequence for volanesorsen which is available publicly using *blastn* and the USCS browser. Searching a reference mRNA database using *blastn*, we identified 22 mRNA targets with ≥ 70% homology to the 20 nt ASO, but only one target (*APOC3* mRNA) had 100% homology and an *E p-value* < 0.01 indicating a good hit and match. Six of the nineteen off-target mRNAs known to be expressed in liver (including *RAC1* previously identified by the sponsor) showed a match to the central DNA gapmer or 10 bases but had E *p-values* ≥ 0.08 indicating poor/weak match and potential for false positives (data not shown).

ASO Other mechanisms:

ASOs can interact with cellular proteins.

ASOs are designed to hybridize or bind to target ribonucleic acid (RNA) sequences to silence a gene through induced cleavage of the targeted sequence by RNase H1. However, while PS-ASOs are known to enhance nuclease resistance and cellular uptake both *in vitro* and *in vivo,* they can also interact and bind to intracellular and extracellular proteins[19] . Using the UCSC browser and empirical data to build a

genetic and epigenetic track including we showed that the nucleotide sequence of volanesorsen (and therefore olezarsen) located to a region containing multiple DNA binding motifs important for interaction with several intracellular proteins including POLRG2, AGO2, AGO1, and RBM22. The target sequence of volanesorsen/olezarsen in the 3'UTR of *APOC3*, also mapped to a liver-specific promoter and regulatory region in primary liver hepatocytes and HepG2 cells. Whether the volanesorsen /olezarsen ASO can interfere with protein binding and dysregulate gene expression and thus liver function via protein interaction as an off-target effect is not known. However, in the absence of potential differences in cellular cytoplasmic concentrations between the two ASOs (after endosome release), this observation is noteworthy of the potential to highjack cellular machinery but would not help explain any hepatic event(s) observed for olezarsen *vs* volanesorsen as both ASOs have identical sequences and MOA and would therefore be expected to dysregulate similarly.

2. Assessing whether disrupted lipid metabolism in the hepatocyte could result in lipid dysregulation (e.g. altered feedback loops) and hepatobiliary injury.

Predominantly synthesized in the liver, APOC3 is a key regulator of plasma triglyceride-rich lipoprotein (TRL) metabolism. APOC3 circulates on very low-density lipoprotein (VLDL), LDL, Lp(a), and HDL particles and can be present in multiple copies per particle. APOC3 play a key role in determining triglyceride levels by two main mechanisms, by inhibiting LPL activity (or lipolysis of TRLs) as well as by directly inhibiting hepatic uptake of TRL, thus leading to increased levels of chylomicrons and TRLs[20,21].

Previously known as nonalcoholic fatty liver disease (NAFLD), metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized in part by steatosis, excess lipid deposition as lipid droplets within hepatocytes[22]. These lipid droplets consist largely of triglycerides and are the result of an imbalance of hepatic lipid handling. Steatosis can occur when one or more of the following conditions is present; 1) excess delivery of free fatty acids (FFA) to the liver from adipose tissue, 2) increased de novo lipogenesis (DNL) within the liver, 3) decreased oxidation of fatty acids within hepatocytes and 4) impaired export of triglycerides from the liver in the form of very-low density lipoproteins (VLDL)[22,23].

The relationship between APOC3 and liver steatosis remains controversial. Petersen et al.[24] reported that human subjects with *APOC3* genetic variants (rs2854117 and rs2854116) are at increased risk of developing MASLD. In contrast, Kozlitina et al.[25] reported that neither of the two *APOC3* variants (rs2854117 and rs2854116) were associated with MASLD, although both were associated with hypertriglyceridemia. This latter observation was supported by clinical studies, in which humans with *APOC3* loss-of-function alleles are associated with decreased plasma TG levels but not with increased susceptibility to developing steatosis[26-28]. Lee et al.[29] demonstrated that *Apoc3* overexpressing transgenic mice develop mild steatosis in response to high fat feeding. A subsequent study by Cheng et al. reported that Apoc3 expression neither exacerbated diet-induced adiposity nor aggravated the degree of steatosis in high fructose or high fat-fed Apoc3-transgenic mice[21]. In contrast, ApoC3 deficiency in knock-out mice is associated with diet-induced obesity, insulin resistance, and steatosis as a result of free fatty acids being delivered to the liver from adipose tissue, and the accumulation of fat in the liver and due to impaired lipid clearance and increased lipolysis[9]. In humans, APOC3 inhibition (with/without LPL deficiency) reduced HFF (ranging from -1.0 to -8.34 placebo-corrected absolute percentage points) in patients with hypertriglyceridemia (SHTG), familial partial lipodystrophy (FPL), and FCS. Decreases in HFF were lowest in FCS, modest in SHTG and greatest in FPL. Further, a strong inverse correlation was observed between baseline HFF and change in HFF in the drug groups, but not in the placebo groups[30]. For example, in the BROADEN trial[30], volanesorsen, significantly reduced HFF by -8.34 percentage points ($p=0.001$) from placebo-adjusted baseline after 12 months of treatment[30,31]. In contrast, vupanorsen, a GalNAc-conjugated ASO and inhibitor of ANGPTL3 protein which also inhibits LPL, was shown to

cause dose-dependent increases in HFF and higher doses were associated with elevations in ALT and aspartate aminotransferase (AST)[32,33]. However, increases in HFF were only moderately correlated with elevations in ALT (and AST) suggesting that liver enzymes are an imperfect indicator to detect increases in hepatic fat.

Although APOC3 is being targeted for the development of anti-hypertriglyceridemia therapy for reducing the cardiovascular risk Cheng et al.[21] indicate caution in using APOC3 therapy in patients with established MASLD. Given its intracellular role in facilitating VLDL-TG assembly and secretion, selective APOC3 inhibition in subjects with steatosis may impair hepatic VLDL-TG secretion, resulting in further fat accumulation in the liver. Further research is be needed to determine whether APOC3 inhibition for lowering plasma TG levels is inadvertently coupled with a risk of developing MASLD.

Lastly, ASO oligonucleotides are not metabolized by cytochrome P450 enzyme system in the liver. We are not able to provide insight into the impact of chronic administration of olezarsen in subjects with impaired liver function as such information was not available from studies provided by the sponsor or in the literature. Moreover, the impact of volanesorsen (which is an approved FCS therapy in Europe) in subjects with hepatic impairment was not studied (Waylivra: EPAR- Product Information).

CONCLUSIONS

Olezarsen has identical sequence, chemical composition, and mechanism of action as volanesorsen. Therefore, the difference in toxicity or potential for elevated liver enzyme levels is unlikely to be mediated through sequence or chemical composition differences. The difference is that olezarsen is (b) (4) GalNAc3-conjugated while volanesorsen is not. This suggests that any liver enzyme elevations and/or potential for adverse events or toxicity is a function of the cellular/tissue concentration differences of olezarsen and/or its metabolites and thus on-target or off-target effects.

Present findings do not suggest potential olezarsen/ASO on-target or non-specific off-target sequence effects on mRNAs in the liver that may be related to hepatic/hepatobiliary dysfunction and toxicity. However, total RNA sequencing (compared to RT-qPCR) would allow a more comprehensive assessment of the whole transcriptome including mRNA transcripts and non-coding RNAs and would complement *in silico* predictions. Total transcriptomic data could also indirectly inform on the potential for non-specific sequence effects involving binding of the ASO with intracellular proteins involved in transcriptional regulation, which was observed for olezarsen (and thus volanesorsen) here. Further, olezarsen metabolite levels were low in liver tissue and no unique or disproportionate metabolites were identified in humans compared to animal species that might suggest involvement in hepatic or hepatobiliary toxicity of injury. Comparative evaluation of GalNAc-conjugated-ASOs with (b) (4) linkers submitted to the FDA characterized a differential or inconsistent signal related to liver AEs across the ASOs, suggesting that the (b) (4) linker is unlikely to be associated with hepatobiliary injury.

Lastly, while the relationship between APOC3 and liver steatosis when evaluated across species and across different data types appears conflicting, clinical data has shown that inhibition of human APOC3 by volanesorsen (which has the same sequence as olezarsen), with/without germline LPL deficiency resulted in reduced hepatic fat fraction (HFF) in patients with hypertriglyceridemia, familial partial lipodystrophy, and familial chylomicronemia syndrome. Further, while GalNac facilitates high liver uptake of conjugated-ASO or administered dose (e.g., 50% - 70%) by hepatic endosomes it does not directly impact the rate of endosomal escape of the ASOs. Recycling endosomes deliver receptors back to the plasma membrane, whereas degradation occurs within multivesicular bodies and late endosomes that secrete their contents directly into the bile canaliculus for export. Presently, there is no insight into whether high uptake and slow/delayed release of unconjugated ASOs/siRNA from hepatic endosomes

may be associated with changes in liver function/injury. Or whether lipid changes within the cells because of the ASO intended target impacts endosome function or endosomal-lysozyme systems in hepatic cells.

Because olezarsen and volanesorsen differ only in the (b) (4) GalNAc3-conjugate, an exploratory comparison of these ASOs in a suitable *in vitro* cell system (e.g., hepatic microphysiological systems, 3D-spheroids, and/or organ-on-chip approaches) might provide further information on the relationship between potential liver changes or injury and endosomal trafficking and release, and reduced APOC3-mediated changes in the transcriptome and lipodome following ASO degradation.

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M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: October 30, 2024

TO: John Sharretts, M.D.
Director
Division of Diabetes, Lipid Disorders and Obesity (DDLO)
Office of Cardiology, Hematology, Endocrinology and Nephrology (OCHEN)
Office of New Drugs (OND)

FROM: Xikui Chen, Ph.D.
Pharmacologist
Division of Generic Drug Study Integrity (DGDSI)
Office of Study Integrity and Surveillance (OSIS)

THROUGH: Seongeun Cho, Ph.D.
Director
Division of Generic Drug Study Integrity (DGDSI)
Office of Study Integrity and Surveillance (OSIS)

SUBJECT: Review of Clinical Inspection at Medpace, Inc., Cincinnati, OH

1. Inspection Summary

The Office of Study Integrity and Surveillance (OSIS) arranged a clinical inspection of study ISIS 678354-CS20 (NDA 218614, olezarsen injection) conducted at Medpace, Inc., Cincinnati, OH

Form FDA 483 was not issued at the inspection close-out. There was one discussion item regarding the original paper print outs from the pharmacy balances without dates. Based on the inspection finding and my review, I conclude this discussion item unlikely has an impact on reliability of the data and human subject protection for inspected study ISIS 678354-CS20.

2. Inspected Study

NDA 218614

Study Number: ISIS 678354-CS20
Study Title: "A Single-Dose, Randomized, Open-Label, Two-Period Crossover Bioequivalence Study Comparing Two Subcutaneous Formulations: Vial and Autoinjector (AI) with Olezarsen, at Two Dose Levels, in Healthy Adult Participants"

Dates of study conduct: 10/5/2022 to 6/1/2023

Clinical site: Medpace, Inc., Clinical Pharmacology Unit
5355 Medpace Way, Cincinnati, OH 45227, USA

Principal Investigator: Leela H. Vrishabhendra, M.D.

3. Inspectional Findings

3.1 Medpace, Inc., Cincinnati, OH

ORA investigator D'Arbra R Blankenship inspected Medpace, Inc., Cincinnati, OH from September 16 to 20, 2024.

3.1.1 Previous Inspection

A previous clinical inspection was performed in January 2017 and final OSIS classification was NAI.

3.1.2 Current Inspection

The current inspection included auditing the following items:
- Case report forms (CRFs)
- Informed consent process
- Protocol deviations
- Institutional Review Board approvals
- Randomization
- Test article accountability, and storage
- Adverse events

3.1.3 Inspection findings

At the conclusion of the inspection, investigator D'Arbra R Blankenship, did not issue Form FDA 483. One discussion item was communicated with management as the following:

Discussion item 1:
The original paper print outs from the pharmacy balances do not automatically add dates. The original print outs are attached to the Pharmacy Worksheet and used to document the weights of the autoinjector and syringe prior to and after dosing.

OSIS Evaluation:
According to the EIR, all the information on the Pharmacy Worksheet is verifiable and trackable through multiple other documented study source records. Even though the date of weights of the autoinjector and syringe prior to and after dosing was not recorded on the Pharmacy Worksheet, other information such

as study number, subject number, subject initials, randomization number, period 1, period 2, pre-dose weight print out, post-dose weight print out, enter pre-dose weight and enter post dose weight were recorded on the pharmacy worksheet and OII investigator did not identify any discrepancies in EIR. Thus, the discussion item unlikely has an impact on reliability of the data or human subject protection for the inspected study ISIS 678354-CS20.

Draft: XC 10/29/2024, 10/30/2024
Edit: MO 10/29/2024; JC 10/30/2024

OSIS File: BE 10271

eNSpect assignment ID: 246229
eNSpect operation ID: 288504

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MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING

Division of Medication Error Prevention and Analysis 1 (DMEPA 1)
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:	October 22, 2024
Requesting Office or Division:	Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number:	NDA 218614
Product Name, Dosage Form, and Strength:	Tryngolza (olezarsen) injection, 80 mg/0.8 mL
Applicant Name:	Ionis Pharmaceuticals, Inc.
FDA Received Date:	October 18, 2024
TTT ID #:	2024-9221-2
DMEPA 1 Safety Evaluator:	Vraj Patel, PharmD
DMEPA 1 Team Leader:	Damon Birkemeier, PharmD, FISMP, NREMT

1 PURPOSE OF MEMORANDUM

Ionis Pharmaceuticals, Inc. submitted a revised container label received on October 18, 2024 for Tryngolza. The Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container label for Tryngolza (Appendix A) to determine if it is 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

Ionis Pharmaceuticals, Inc. implemented all of our recommendations and we have no additional recommendations at this time.

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[a] Patel, V. Label and Labeling Review for Tryngolza (NDA 218614). Silver Spring (MD): FDA, CDER, OSE, DMEPA 1 (US); 2024 Oct 09. TTT ID: 2024-9221-1.

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VRAJ K PATEL
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DAMON A BIRKEMEIER
10/22/2024 09:23:23 AM

Clinical Inspection Summary

Date	October 18, 2024
From	Ling Yang, M.D., Ph.D., FAAFP Min Lu, M.D., M.P.H., Team Leader Jenn Sellers, M.D., Ph.D., Branch Chief Good Clinical Practice Assessment Branch (GCPAB) Division of Clinical Compliance Evaluation (DCCE) Office of Scientific Investigations (OSI)
To	Mary Roberts, M.D., Clinical Reviewer Eileen Craig, M.D., Clinical Team Leader Ronald Picking III, Consumer Safety Officer, RPM Division of Diabetes, Lipid Disorders and Obesity (DDLO)
NDA #	218614
Applicant	Ionis Pharmaceuticals, Inc.
Drug	Olezarsen
NME (Yes/No)	Yes
Review Priority	Priority
Proposed Indication(s)	As an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS)
Consultation Request Date	May 21, 2024
Summary Goal Date	November 19, 2024
Action Goal Date	December 19, 2024
PDUFA Date	December 19, 2024

I. OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

Clinical data from studies **ISIS 678354-CS3** entitled "A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Study of AKCEA-APOCIII-LRX Administered Subcutaneously to Patients with Familial Chylomicronemia Syndrome (FCS)" and **ISIS 678354-CS8** entitled "A Randomized, Double-blind, Placebo-controlled, Phase 2b Study of ISIS 678354 in Patients with Hypertriglyceridemia and Atherosclerotic Cardiovascular Disease (Established or at Increased Risk for), and/or with Severe Hypertriglyceridemia" were submitted in this original new drug application (NDA) for Olezarsen, a GalNAc3 conjugated antisense oligonucleotide inhibitor of apolipoprotein C-III production, for the proposed indication of as an adjunct to diet to reduce triglycerides (TG) in adults with FCS. Two domestic clinical investigators (CIs): Drs. Seth Baum (Site #2138 for both studies) and Alejandro De La Cruz (Site #3195 for Study ISIS 678354-CS8) and Contract Research Organization (CRO) Medpace were inspected for the submitted studies.

The inspection of Dr. Cruz found 2 unreported AEs (redness at the injection site and nausea) in 2 subjects, both in olezarsen 80 mg group. OSI recommend the review division include these 2 AEs in the safety analysis. Dr. Cruz also enrolled 3 ineligible subjects in Study ISIS 678354-CS8, which were reported to the FDA as protocol deviations.

Based on the overall inspection results of these CIs, CRO and the regulatory assessments, the data generated by the CIs; managed by the CRO, and submitted by the sponsor are verifiable. Studies

ISIS 678354-CS3 and ISIS 678354-CS8 appear to have been conducted adequately and the clinical data submitted by the sponsor appear acceptable in support of the respective indication.

II. BACKGROUND

Ionis Pharmaceuticals, Inc. (Ionis) submitted an original NDA 218614 on 04/19/2024 for Olezarsen for the proposed indications of as an adjunct to diet to reduce TG in adults with FCS. Data from a Phase 3 pivotal Study **ISIS 678354-CS3** and a Phase 2b confirmatory efficacy Study **ISIS 678354-CS8** were submitted to support the approval.

Study ISIS 678354-CS3

Study ISIS 678354-CS3 was a Phase 3, multicenter, randomized, double-blind, placebo-controlled, 53-week study of olezarsen 50 mg or 80 mg or matching placebo administered once every 4 weeks in patients with FCS.

The primary study objective was to evaluate the efficacy of olezarsen as compared to placebo on the percent change in fasting TG from baseline.

The primary efficacy endpoint was the mean percent change in fasting TG from baseline at Month 6 (the average of Weeks 23, 25, and 27) compared with placebo.

Study Design:

The study consisted of three study periods:

- **Screening Period**: 4-8 weeks: included an at least 2-week Diet Stabilization/Run-In Period for subjects not already on a stable diet; and a 2-week Qualification Period.
- **Treatment Period**: 53 weeks. Eligible subjects were randomized at a 1:1 ratio to Cohort A (olezarsen 50 mg) or Cohort B (olezarsen 80 mg). Subjects in each cohort was further randomized at a 2:1 ratio to receive SC injection of olezarsen (50 mg or 80 mg) or matching placebo once every 4 weeks.
- **Post-Treatment Evaluation Period**: 13 weeks.

The study screened 144 subjects, randomized 66 (22 in olezarsen 50 mg group, 21 in olezarsen 80 mg group and 23 in placebo group) subjects at 29 study sites in the US (9); Canada (3); European Union (16) and United Kingdom (1). The first subject was screened on 11/18/2020 and the study was going on with the data cutoff date on 07/14/2023. The study data base was locked on 08/30/2023. A total of 60 subjects completed the Treatment Period of the study (19 in olezarsen 50 mg group, 19 in olezarsen 80 mg group and 22 in placebo group).

Study ISIS 678354-CS8

Study ISIS 678354-CS8 was a multicenter, randomized, double-blind, placebo-controlled, 53-week study of 50 mg or 80 mg olezarsen, or matching placebo, administered once every 4 weeks in patients with hypertriglyceridemia and ASCVD and/or severe hypertriglyceridemia.

The primary study objective was to evaluate the effect of olezarsen compared to placebo on the percent change in fasting TG levels from baseline.

The primary efficacy endpoint was the percent change in fasting TG from baseline at Month 6 (the average of Week 25 and Week 27) compared with the placebo group.

Study Design:
The study consisted of three study periods:

- **Screening Period**: 4-8 weeks: included an at least 2-week Diet Stabilization/Run-In Period for patients not already on a stable diet; and a 2-week Qualification Period.
- **Treatment Period**: 53 weeks. Eligible subjects were randomized at a 1:1 ratio to Cohort A (olezarsen 50 mg) or Cohort B (olezarsen 80 mg). Subjects in each cohort was further randomized at a 3:1 ratio to receive SC injection of olezarsen (50 mg or 80 mg) or matching placebo once every 4 weeks. Randomization was stratified by fasting TG levels of < 500 mg/dL vs. ≥ 500 mg/dL.
- **Post-Treatment Evaluation Period**: 13 weeks.

The study screened 304 subjects, randomized 154 (58 in olezarsen 50 mg group, 57 in olezarsen 80 mg group and 39 in placebo group) subjects at 28 study sites in the US (21) and Canada (7). The first subject was randomized on 06/01/2022 and the last subject's last visit was on 01/17/2023. The study data base was locked on 10/20/2023. A total of 130 subjects completed the study (44 in olezarsen 50 mg group, 50 in olezarsen 80 mg group and 36 in placebo group).

III. RESULTS

1. **Seth Baum, M.D. (Site # 2138)**
   7900 Glades Road, Suite 400
   Boca Raton, FL 33434-4104

This CI was inspected on 08/05-07/2024 as a data audit for Studies ISIS 678354-CS3 and ISIS 678354-CS8. This was the first FDA inspection of Dr. Baum.

For Study ISIS 678354-CS3, the site screened 9 subjects and enrolled 5 subjects. All 5 subjects completed the study. The first subject consented on 11/18/2020 and the last subject's last visit was on 03/07/2023. Source records for all 9 screened subjects were reviewed.

For Study ISIS 678354-CS8, the site screened 14 subjects and enrolled 7 subjects. Five (5) subjects completed the study, and two subjects discontinued the study: 1 due to AE of gastrointestinal (GI) disorder (Subject (b) (6); olezarsen 50 mg group) and 1 due to GI cancer diagnosis (Subject (b) (6); olezarsen 80 mg group). The first subject consented on 05/10/2022 and the last subject's last visit was on 11/14/2023. Source records for all 14 screened subjects were reviewed.

The inspection reviewed the studies' protocols and amendments, Informed Consent Forms (ICFs) and versions, documentation of eligibility criteria and enrollment logs, medical records [including visit data, laboratory tests, physical exam results, concomitant medications, adverse events (AEs) and serious AEs (SAEs) reports], investigational product (IP) accountability records, both paper and electronic Case Report Forms (eCRFs) and electronic data capture (EDC) system, protocol deviations and related regulatory documents [e.g., Institutional Review

Board (IRB) approvals and communications, staff trainings, monitoring log, records retention, financial disclosures and delegation of authority].

The submitted data were verifiable with source records at the study site. The primary efficacy endpoint data of the mean percent change in fasting TG from baseline at Month 6 were centrally adjudicated and were initially blinded to the site. The inspection verified the results with no discrepancies noted. There were no underreporting of AEs or SAEs.

In general, the inspection verified adequate source data for the inspected study subjects, with no deficiencies reported.

2. Alejandro De La Cruz, M.D. (Site # 3195)
13218 SW 8th Street
Miami, FL 33184-1176

This CI was inspected on 07/08-16/2024 as a data audit for Study ISIS 678354-CS8. This was the first FDA inspection of Dr. De La Cruz.

For the inspected study, the site screened 64 subjects and enrolled 32 subjects. Except one subject ((b) (6); olezarsen 50 mg group) withdrew due to AE of ecchymosis at the injection site, 31 subjects completed the study. The first subject consented on 06/20/2022 and the last subject's last visit was on 11/29/2023. ICFs for 12/64 screened subjects, all source records for 11/32 of the enrolled subjects, and primary efficacy endpoint and AEs for all 32 randomized subjects were reviewed.

The inspection reviewed the protocol and amendments, English and Spanish language ICFs and versions, documentation of eligibility criteria and enrollment logs, medical records (including visit data, laboratory tests, AEs and SAEs reports), IP accountability records, paper CRFs with EDC entries and audit trails, protocol deviations and related regulatory documents (e.g., IRB approvals and communications, staff trainings, monitoring procedure and logs, records retention, financial disclosures and delegation of authority).

The submitted data were verifiable with source records at the study site. The primary efficacy endpoint data of the mean percent change in fasting TG from baseline at Month 6 were verified with no discrepancies noted. There was no underreporting of SAEs.

The inspection noted the following protocol deviations:

- Three (3) subjects did not meet all inclusion or exclusion criteria:
  a. Subject (b) (6) (olezarsen 50 mg group) was diagnosed with diabetes mellitus type 2 at Screening and met exclusion criterion #1a "newly diagnosed with diabetes within 12 weeks of Screening".
  b. Subject (b) (6) (olezarsen 80 mg group) did not have the follicle-stimulating hormone (FSH) levels checked at Screening, per inclusion criteria #6a.
  c. Subject (b) (6) (olezarsen 80 mg group) fasting TG was 148 mg/dL that did not meet inclusion criteria #3 "a fasting triglyceride value of 150 mg/dL or greater".

- Two (2) AEs were recorded in source documentation but were not entered into the EDC.
  a. Subject (b) (6) (olezarsen 80 mg group) "redness at the injection site" documented on (b) (6) was not submitted.
  b. Subject (b) (6) (olezarsen 80 mg group) moderate severity of "nausea" reported during (b) (6) was not submitted.

Reviewer's Comments:

- *Three ineligible subjects should not have been enrolled in the study. These cases were all reported and submitted in the list of protocol deviations in the NDA submission.*
- *The above two AEs should have been reported. They appear to be the isolated events that may not significantly impact the safety profile of the product. Nevertheless, these AEs should be included in the safety analysis because the IP is NME.*
- *The CI responded to the inspection observation on 08/02/2024, acknowledged the inspection findings, and submitted plans with corrective actions to improve the quality of future clinical studies. The CI's responses are deemed acceptable.*

In general, the inspection verified adequate source data for the inspected subjects, with no significant deficiencies noted.

3. Medpace Inc. (CRO)
5400 Medpace Way
Cincinnati, OH 45227-1530

This CRO was inspected on 07/8-11/2024 as a data audit for Studies ISIS 678354-CS3 and ISIS 678354-CS8. This was the second FDA inspection of the CRO Medpace. Previous inspection was in 02/2024 with no GCP issues identified.

For both studies, Medpace was responsible for clinical trial management, study startup and maintenance, subject recruitment and retention, clinical monitoring, Data and Safety Monitoring Board (DSMB) meeting organization and attendance; and biostatistics analysis for Study ISIS 678354-CS8.

The inspection reviewed the CRO's standard operating procedures (SOPs), training records, monitoring records, site investigator documents, contracts with the sponsor and 3rd party vendors, meeting minutes, non-compliance issues management, DSMB meeting documents, and biostatistics data transfer verification.

Financial disclosure forms and monitoring files were reviewed for four sites (#1943, 1956, 2673 and 2674) for Study ISIS 678354-CS3 and 5 sites (#3193, 3195, 3211, 2138 and 3192) for Study ISIS 678354-CS8 with no GCP issues identified.

In general, the CRO's management and monitoring of Studies ISIS 678354-CS3 and ISIS 678354-CS8 appear adequate, with no significant deficiencies noted.

{See appended electronic signature page}

Ling Yang, M.D., Ph.D.
Good Clinical Practice Assessment Branch
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Jenn Sellers, M.D., Ph.D.
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DDLO\Reviewer\Mary Roberts
DDLO\Project Manager\Ronald Picking
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OSI\DCCE\GCPAB\Reviewer\Ling Yang
OSI\DCCE\Program Analysts\Yolanda Patague

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MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING

Division of Medication Error Prevention and Analysis 1 (DMEPA 1)
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:	October 09, 2024
Requesting Office or Division:	Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number:	NDA 218614
Product Name, Dosage Form, and Strength:	Tryngolza (olezarsen) injection, 80 mg/0.8 mL
Applicant Name:	Ionis Pharmaceuticals, Inc.
FDA Received Date:	September 20, 2024
TTT ID #:	2024-9221-1
DMEPA 1 Safety Evaluator:	Vraj Patel, PharmD
DMEPA 1 Team Leader:	Damon Birkemeier, PharmD, FISMP, NREMT

1 PURPOSE OF MEMORANDUM

Ionis Pharmaceuticals, Inc. submitted revised container label and carton labeling received on September 20, 2024 for Tryngolza. The Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container label and carton labeling for Tryngolza (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

We evaluated the proposed Tryngolza carton labeling and determined that it is acceptable from a medication error perspective.

However, the container label is unacceptable from a medication error perspective. We provide recommendations in section 3 for Ionis Pharmaceuticals, Inc.

3 RECOMMENDATIONS FOR IONIS PHARMACEUTICALS, INC.

A. Container Label

1. We acknowledge you accepted our recommendation for deleting (b) (4) from next to the established name. However, you stated you "*deleted 'injection' due to space constraints*." Not including the dosage form next to the active ingredient may lead to wrong route medication errors. We recommend adding the dosage form, "injection", either on the same line as the active ingredient, "olezarsen" or directly below it. For example,

   Tryngolza

   (olezarsen) injection

   OR

   Tryngolza

   (olezarsen)

   Injection

   See *Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors* for additional information.[b]

2. As currently presented, the container label states, "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original (b) (4)". However, the carton labeling states "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton." We

[a] Patel, V. Label and Labeling Review for Tryngolza (NDA 218614). Silver Spring (MD): FDA, CDER, OSE, DMEPA 1 (US); 2024 Aug 13. TTT ID: 2024-9221.

[b] *Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors*. May 2022. Available from: https://www.fda.gov/media/158522/download.

recommend revising this sentence to read, “Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton.” on the container label for consistency with the carton labeling.

3. As currently presented, the “(b) (4)” statement competes in prominence with other critical information on the principal display panel (PDP). Additionally, we note that the statement “(b) (4)” is not required on small labels; thus, in this case, since the “(b) (4)” statement is present on the carton labeling, it could be removed from the container labeling to decrease clutter. We recommend removing the “(b) (4)” statement from the container label or relocating the statement to a side panel on the container label.

4. As currently presented, the container label has an NDC number on the side panel of the container label. The human-readable NDC is often used for product verification and identification prior to dispensing or administering a drug. It is an important safety feature that is recommended to be prominently displayed on the PDP of the container label. We recommend that you move the NDC number to the PDP on the container label.

5. As currently presented, the statements “for subcutaneous use” and “single-dose (b) (4)” compete in size and prominence with other critical information on the PDP. Critical product information should appear as the most prominent information on the PDP in accordance with 21 CFR 201.15. We recommend decreasing the size and prominence of these statements so they do not compete in size or prominence with critical information on the PDP.

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10/09/2024 09:29:15 AM

LABEL, LABELING AND HUMAN FACTORS REVIEW

Division of Medication Error Prevention and Analysis 1 (DMEPA 1)
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:	August 13, 2024
Requesting Office or Division:	Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number:	NDA 218614
Product Name, Dosage Form, and Strength:	Tryngolza (olezarsen) injection, 80 mg/0.8 mL
Product Type:	Combination Product (Drug-Device)
Rx or OTC:	Prescription (Rx)
Applicant Name:	Ionis Pharmaceuticals, Inc.
FDA Received Date:	April 19, 2024, July 24, 2024
TTT ID #:	2024-9221; 2024-9223
DMEPA 1 Safety Evaluator:	Vraj Patel, PharmD
DMEPA 1 Human Factors Evaluator:	Avinash Konkani, PhD, MS, BE
DMEPA 1 Team Leader:	Damon Birkemeier, PharmD, FISMP, NREMT
DMEPA 1 Human Factors Team Leader:	Murewa Oguntimein, PhD, MHS, CPH, MCHES

1 INTRODUCTION

As part of the approval process for Tryngolza (olezarsen) injection, the Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the proposed Tryngolza Prescribing Information (PI), Patient Package Insert (PPI), Instructions for Use (IFU), container labels, and carton labeling for areas of vulnerability that may lead to medication errors. This review also discusses the human factor (HF) data requirements for this product based on our previous HF review.[a]

2 MATERIALS REVIEWED

This section lists the materials considered for our review of NDA 218614.

Table 1. Materials Considered for this Label, Labeling and Human Factors Review	
Materials Reviewed	Appendix Section
Relevant Product Information	A
Labels and Labeling	B
Previous DMEPA Reviews	C
Information Requests Issued During the Review	D

3 HUMAN FACTORS (HF) DISCUSSION

On October 04, 2022, under IND 136692, the Applicant submitted the use-related risk analysis (URRA) and comparative analyses (CA) as a justification for not submitting human factors (HF) validation study results to support their future marketing application for Tryngolza (olezarsen) injection, (b) (4) 80 mg/0.8 mL autoinjector (AI).

On April 09, 2024, the DMEPA I HF Team reviewed the URRA and CA. The HF team determined the Applicant did not need to submit HF validation results to support their future marketing application for Tryngolza (olezarsen) injection, (b) (4) 80 mg/0.8 mL AI. The HF team also stated that if Ionis modified the product user interface, additional human factors considerations may apply.[b]

The Applicant confirmed that the product user interface, URRA, and CA submitted in this application is identical to the version we reviewed previously under their IND 136692.[c] As such, we maintain that the Applicant does not need to submit human factors (HF) validation results

[a] Konkani. A. Use-Related Risk Analysis and Comparative Analyses review for ISIS 678354 (olezarsen) injection, (b) (4) (b) (4) 80 mg/0.8 mL AI. (IND 136692). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 09 April 2024. TTT No.: 2022-2622.

[b] Konkani. A. Use-Related Risk Analysis and Comparative Analyses review for ISIS 678354 (olezarsen) injection, (b) (4) (b) (4) 80 mg/0.8 mL AI. (IND 136692). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 09 April 2024. TTT No.: 2022-2622.

[c] NDA 218614 Response to Information Request. Carlbab (CA): Ionis Pharmaceuticals, Inc. 2024 JUL 24. Available from: \\CDSESUB1\EVSPROD\nda218614\0014\m5\53-clin-stud-rep\535-rep-effic-safety-stud\fcs\5354-other-stud-rep\hfe-ue\human-factor-autoinjector-report.pdf

with their marketing application for the proposed Tryngolza (olezarsen) injection, 80 mg/0.8 mL AI.

4 CONCLUSION

We evaluated the proposed Tryngolza PI, PPI, and IFU and determined that they are acceptable from a medication error perspective.

From an HF perspective, we maintain that the Applicant does not need to submit human factors (HF) validation results with their marketing application for the proposed Tryngolza (olezarsen) injection, 80 mg/0.8 mL AI.

However, the proposed Tryngolza container labels and carton labeling may be improved to promote the safe use of this product from a medication error perspective. We provide the identified medication error issues, our rationale for concern, and our proposed recommendations to minimize the risk for medication error for Ionis Pharmaceuticals, Inc. in Section 4.

5 RECOMMENDATIONS FOR IONIS PHARMACEUTICALS, INC.

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)

	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
Container Label and Carton Labeling			
1.	As currently presented, the format for the expiration date is not defined.	We are unable to assess the proposed expiration date format from a medication safety perspective.	To minimize confusion and reduce the risk for deteriorated drug medication errors, identify the expiration date format you intend to use. FDA recommends that the human-readable expiration date on the drug package label include a year, month, and non-zero day. FDA recommends that the expiration date appear in YYYY-MM-DD format if only numerical characters are used or in YYYY-MMM-DD if alphabetical characters are used to represent the month. If there are space limitations on the drug package, the human-readable text may include only a year and month, to be expressed as: YYYY-MM if only

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	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
			numerical characters are used or YYYY-MMM if alphabetical characters are used to represent the month. FDA recommends that a hyphen or forward slash to separate the portions of the expiration date. See *Guidance for Industry: Product Identifiers under the Drug Supply Chain Security Act - Questions and Answers (June 2021).*
2.	As currently presented, the proprietary name and established name lack prominence on the principal display panel.	The proprietary name and established name along with the product strength, route of administration, and warnings or cautionary statements should be the most prominent information on the principal display panel (PDP).	We recommend increasing the prominence of the proprietary name and established name. Consider the use of different font type or size, bolding, color, or other means to achieve increased prominence. See Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (May 2022).
3.	As currently presented, it is difficult to tell if the established name is at least half the size of the proprietary name.	We refer you to 21 CFR 201.10(g)(2) which states that the established name shall be printed in letters that are at least half as large as the letters comprising the proprietary name or designation with which it is joined, and the established name shall have a prominence commensurate with the prominence with which such proprietary name or designation appears, taking	Confirm the established name is at least half the size of the proprietary name. If it is not, revise the established name to be in accordance with 21 CFR 201.10(g)(2).

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
		into account all pertinent factors, including typography, layout, contrast, and other printing features.	
4.	As currently presented, the manufacturer name, "Ionis", is too prominent compared to other information.	The proprietary name and established name along with the product strength, route of administration, and warnings or cautionary statements should be the most prominent information on the principal display panel (PDP).	We recommend decreasing the prominence of the manufacturer name, "Ionis", relative to the prominence of the critical information on the PDP needed for proper identification and use of your proposed product in accordance with 21 CFR 201.15(a)(6). See Guidance for Industry: Safety Considerations for Container Labels and Carton Labeling Design to Minimize Medication Errors (May 2022).
5.	As currently presented, the (b) (4) to the right of the established name, olezarsen, is not readable. (b) (4)	Not being able to read the (b) (4) next to the established name can lead to medication errors.	We recommend deleting the (b) (4) next to the established name and putting the dosage form, "injection", either on the same line as the active ingredient or directly below the active ingredient. Make sure the dosage form is readable by increasing its prominence.
Container Label			
1.	The statement, "(b) (4)" is too prominent compared to the proprietary name, established name, and dosage form.	The proprietary name and established name along with the product strength, route of administration, and warnings or cautionary statements should be the	Decrease the prominence of the statement, "(b) (4)" compared to the proprietary name, established name, and dosage form.

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
		most prominent information.	
2.	The statement, "for subcutaneous use" is not prominent enough compared to other information.	The proprietary name and established name along with the product strength, route of administration, and warnings or cautionary statements should be the most prominent information.	Move the statement, "for subcutaneous use" above the statement, "single-dose (b) (4)". Decrease the prominence of the statement, "single-dose (b) (4)" by decreasing the font size and/or de-bolding.
Carton Labeling			
1.	(b) (4) "FCS" (b) (4) not spelled out on the principal display panel.	Abbreviations that are not spelled out may lead to wrong indication medication errors.	We recommend spelling out (b) (4) on the carton labeling or removing the indication from the principal display panel.
2.	As currently presented, the carton labeling does not include the assigned NDC number on the principal display panel.	The human-readable NDC is often used for product verification and identification prior to dispensing or administering a drug. It is an important safety feature that is recommended to be prominently displayed on the principal display panel of the carton labeling.	We request that you include the NDC number on the principal display panel of the carton labeling per 21 CFR 201.2.
3.	The route of administration on the Principal Display Panel and Side Panel are different ("for subcutaneous use" *versus* "(b) (4)").	To ensure consistency with the terminology across labeling.	Revise the statement on the side panel to read, "for subcutaneous use."
4.	The "1" used for the net quantity statement on	Using the number instead of spelling out the intended	We recommend spelling out the number "1" on the

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
	the Principal Display Panel is not spelled out.	meaning may lead to medication errors.	principal display panel when discussing the net quantity so that it reads “One single-dose autoinjector”.
5.	The "Rx" only" statement is missing on the Principal Display Panel on the carton labeling.	The "Rx" only" statement is required on the drug label in accordance with Section 353(b)(4)(A) of the Federal Food, Drug, and Cosmetic Act.	Add the “Rx only” statement to the Principal Display Panel on the carton labeling. Ensure the "Rx only" statement does not compete in size or prominence with critical information on the principal display panel.
6.	The terminology within the statement of dosage statement (i.e., “(b) (4)”) is inconsistent with the terminology in the Prescribing Information.	To ensure consistency with the terminology in the Prescribing Information.	We recommend revising the statement of dosage statement to read, “Recommended Dosage: see Prescribing Information.”
7.	The placeholder for the lot number is missing.	Lot number statement is required on the immediate container AND carton labeling when there is sufficient space per 21 CFR 201.10(i)(1).	Add the placeholder for the lot number in accordance with 21 CFR 201.10(i)(1).
8.	The placeholder for the expiration date is missing.	The label of an official drug product shall bear an expiration date per USP General Chapter <7>.	Add the placeholder for the expiration date in accordance with USP General Chapter <7>. The USP Chapter <7>Labeling requires the expiration date to appear on the immediate container and all other packaging. When all-numeric dates are used, they must be formatted using the year, the month, and, if applicable, the day, separated by hyphens or forward slashes in one of the following formats: YYYY-MM-

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
			DD or YYYY-MM. When alphanumeric dates are used, months must be displayed using at least three letters in one of the following formats: YYYY-MMM-DD or YYYY-MMM. We recommend you ensure that there are no other numbers located in close proximity to the expiration date. To minimize confusion and reduce the risk for deteriorated drug medication errors, identify the expiration date format you intend to use.
9.	As currently presented, there is no space for end-users to write the beyond-use date which is the [date or time] the opened product must be discarded after taking the product out of the refrigerator.	Since the product has a different expiration date after taking out of the refrigerator, 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 ___/____/____
10.	We note that the words "(b) (4)" and "carton" interchangeably within the storage information.	Carton and (b) (4) are different terms and should not be used interchangeably.	To increase consistency, we recommend replacing the word "(b) (4)" with the word "carton" in the storage statement so the storage statement reads, "Store refrigerated at 2°C to 8°C (36°F to 46°F) in the original carton and protect from direct light."
11.	We note the storage information is located on	This information is repetitive.	We recommend deleting the storage information from the (b) (4).

Table 2. Identified Issues and Recommendations for Ionis Pharmaceuticals, Inc. (entire table to be conveyed to Applicant)			
	IDENTIFIED ISSUE	RATIONALE FOR CONCERN	RECOMMENDATION
	the (b) (4) and the Side Panel.		
12.	We note on the Principal Display Panel, you use the words "(b) (4) for subcutaneous use"	The words, "(b) (4) " is repetitive.	We recommend revising the route of administration statement, so it reads, "For subcutaneous use".

APPENDICES: METHODS & RESULTS FOR EACH MATERIALS REVIEWED

APPENDIX A. RELEVANT PRODUCT INFORMATION

Table 3 presents relevant product information for Tryngolza received on April 19, 2024 from Ionis Pharmaceuticals, Inc.

Table 3. Relevant Product Information for Tryngolza	
Initial Approval Date	N/A
Active Ingredient	olezarsen
Indication	As an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS).
Dosage Form	injection
Strength	80 mg/0.8 mL
Route of Administration	Subcutaneous
Dose and Frequency	80 mg subcutaneously once monthly
How Supplied	Tryngolza injection is a sterile, preservative-free, clear, colorless to yellow solution supplied in a single-dose autoinjector. Each autoinjector of Tryngolza is filled to deliver 0.8 mL of solution containing 80 mg of olezarsen.
Storage	Store the Tryngolza autoinjector in the refrigerator between 36°F to 46°F (2°C to 8°C) in the original carton. The TRYNGOLZA autoinjector can be stored at room temperature (b) (4) in the original carton for up to 6 weeks. If not used within the 6 weeks stored at room temperature, discard TRYNGOLZA. Do not freeze. Do not expose to heat. Protect from light.
Container Closure	Autoinjector

APPENDIX B. LABELS AND LABELING

B.1 List of Labels and Labeling Reviewed

Using the principles of human factors and Failure Mode and Effects Analysis,[d] along with postmarket medication error data, we reviewed the following Tryngolza labels and labeling submitted by Ionis Pharmaceuticals, Inc.

- Prescribing Information received on April 19, 2024, available from \\CDSESUB1\EVSPROD\nda218614\0001\m1\us\114-labeling\draft\labeling\draft-labeling-text.pdf.
- Patient Package Insert received on April 19, 2024, available from \\CDSESUB1\EVSPROD\nda218614\0001\m1\us\114-labeling\draft\labeling\draft-patient-info.pdf.
- Instructions for Use received on April 19, 2024, available from \\CDSESUB1\EVSPROD\nda218614\0001\m1\us\114-labeling\draft\labeling\draft-ifu-text.pdf.
- Container label received on April 19, 2024.
- Carton labeling received on April 19, 2024.

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

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

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IMMUNOGENICITY ASSESSMENT

Application Type	NDA
Application Number	218614
Submit Date	04/19/2024
Received Date	06/12/2024
Division/Office	DDLO
Review Completion Date	07/22/2024
Proposed Proper Name[1]	olezarsen
Proposed Proprietary Name[1]	Tryngolza
Pharmacologic Class	2'-MOE chimeric antisense oligonucleotide (ASO) covalently bound to $GalNA_{C3}$
Applicant	Ionis Pharmaceuticals, Inc
Applicant Proposed Indication(s)	An adjunct to diet to reduce triglycerides (TG) in adults with familial chylomicronemia syndrome

Immunogenicity Assessors

Primary Assessor(s)	Ha Na Lee PhD
Secondary Assessor (s)	Daniela Verthelyi, MD PhD

Immunogenicity Consult requests:

OPQR was requested to

1) Review ADA assay method and validation reports for anti-drug antibody (ADA, Validation report # 678354-MV09).
2) Comment on the lack of neutralizing antibody assay despite high ADA incidence observed.
3) Provide the comments to the question: Do the nonclinical alterations noted in cytokine and immunoglobulin levels, response to KLH challenge, and associated clinical pathology and organ/tissue effects observed in chronic rat and/or monkey studies indicate a need for additional immunologic assessments for the indicated clinical population?

Assessor Recommendation to each question (highlighted in blue):

1) Review ADA assay method and validation reports for anti-drug antibody (ADA, Validation report # 678354-MV09): The assay detects total ADA that bind to the product so incidence and ADA titer can be correlated with PD, PK, efficacy and safety. However, the specificity of ADA assay the sponsor used is unclear, as the assay does not differentiate between ADA to the ASO sequence or the GalNAc moieties. Since there is an evolving landscape in the treatments for this disease, this may have bearing on future alternative treatments.

2) Comment on the lack of neutralizing antibody assay despite high ADA incidence observed: A Nab assay was not developed. It is generally acceptable for this type of oligonucleotide therapeutics as their target is intracellular.

3) Provide the comments to the question: Do the nonclinical alterations noted in cytokine and immunoglobulin levels, response to KLH challenge, and associated clinical pathology and organ/tissue effects observed in chronic rat and/or monkey studies indicate a need for additional immunologic assessments for the indicated clinical population? Given the non-clinical findings, we would have recommended monitoring of the coagulation cascade, complement activation and liver function during the clinical trials. The increase in C_{trough}, could potentially increase these concerns; however, whether to request additional clinical assessments via PMR will be a clinical decision.

   The reasoning behind the recommendation is that, given the non-clinical evidence, close monitoring of complement activation and coagulation cascade during the clinical studies is a reasonable request. At this point, the clinical observations supersede the non-clinical ones. Of note, 1. Thrombocypenia: Due to the affinity of ASOs with PS backbone linkages for plasma and cellular surface proteins and their known interactions with platelet receptors and PF4, it would be reasonable to monitor platelet numbers and activation states in the clinical study. Monitoring GPVI and/or PF4 levels may help identify patients at higher risk of ASO-induced thrombocytopenia. 2. Complement activation: Although complement activation by 2'-MOE ASO is not evident in humans, the non-clinical data certainly raises concerns. We would have suggested close monitoring of complement activation and coagulation cascade during the clinical studies. At this point, if there have been no clinical signals, then there is no clinical argument to request complement activation assessment through a PMR. 3. Liver enzyme elevation: The observed increases in ALT and AST levels following Olezarsen treatment could be related to GalNAc conjugation and/or ADA-induced increases in C_{trough}. It might be informative to determine whether these increases are linked to ADA from an immunogenicity perspective.

Review

Document Reviewed	**Link to Document**
Validation of an ELISA Method for the Detection of Anti-ISIS678354 Antibodies in Human Plasma	\\CDSESUB1\EVSPROD\nda218614\0001\m5\53-clin-stud-rep\531-rep-biopharm-stud\5314-bioanalyt-analyt-met\678354-mv09\678354-mv09-body.pdf

Validation of Anti-Drug Antibody Assays

The sponsor has provided validation exercises for the detection of ADA against Olezarsen but no a Nab assays.

Method Principle

A brief description of each assay is given below and tables which detail the key assay parameters are given below for each assay.

Validation Exercises

Critical assay parameters are summarized below for each assay.

Validation Parameter	Validation of an ELISA Method for the Detection of Anti-ISIS678354 Antibodies in Human Plasma	Assessor Comment
Contract Research Org	(b) (4)	
Assay principle	Multi-Tiered Testing Approach An indirect ELISA using TMB substrate	
Sample Pretreatment	N/A	
Positive control (PC)	Rabbit polyclonal antibody raised against Olezarsen Antibodies diluted into neat pooled normal human plasma containing K_2EDTA	
Detection Ab	HRP-conjugated Protein A/G	Acceptable. This will detect PC and all human IgG subclasses, IgA, IgE, IgM and to a lesser extent IgD in human serum samples.
PC Dose Curve and Hook Effect	No hook effect was observed up to 100 ug/mL	Acceptable since Olezarsen plasma concentrations over time do not exceed 2 µg/mL
LPC	50.0 ng/ml	
MPC	1500 ng/ml	
HPC	10000 ng/ml	
Matrix and NC	human serum matrix pool	
MRD	1:50 with blocking buffer	
Screening cut- point (SCP) (non-parametric, multiplicative, 95% upper limit)	Thirty normal human plasma (15 male and 15 female) and 30 disease human plasma (15 male and 15 female) were analyzed n=1 in duplicate on 3 runs performed by 2 analysts. Evaluation was based on the log-transformed S/N values, where each subject's sample result	*Use of 5% FPR for SCP determination is acceptable.* *Out of 360 samples, the statistical outliers including analytical (25 samples) and biological outliers (5 subjects;*

	(S) on a plate is divided by the median NC instrument response (N) of that plate. Outliers identified by a mixed effects model were excluded. A non-parametric approach was used to determine SCP. A one-sided 90% lower confidence limit for the 95th percentile to detect a 5% false positive rate with 90% confidence. The SCP factor was determined to be 1.1345. NC was used to adjust the SCP on each plate to determine the PSCP as below: PSCP = 1.1345 x Median NC	*23 samples) were identified and excluded from the further analyses. Acceptable*
Confirmatory cut-point (CCP) (% inhibition, Fixed, 99% upper limit)	Fixed cut-point 20.34% - % inhibition data of the same subject plasma used in the SCP factor evaluation after spiking the samples with 50 ug/ml of drug - A one-sided 80% lower confidence limit for the 99th percentile, and thus assures at least a 1% false positive rate with 80% confidence - Robust parametric approach was used for evaluating the CCP	*Use of 1% FPR for CCP determination is acceptable.*
Titer Cut Point (TCP)	TCP factor of 1.412 was determined using the same data and non-parametric approach used to establish the SCP factor but with a more stringent FPR (0.1% FPR) NC was used to adjust the TCP on each plate to determine the PSTCP as below: PSTCP = 1.412 x Median NC	*Use of 0.1% FPR for TCP determination is acceptable.*
Assay Drug tolerance	PC were prepared at 2x LLPC2, 2x LPC, 200 ng/ml and 2 x HPC levels and were spiked with an equal volume of ISIS678354 prepared in NC at 9 selected concentrations (final conc of 4000 ng/ml to 10 ng/ml). After 1 hour incubation, samples were stored at -80°C for a minimum of 12 hours before assessment. Samples were analyzed one occasion n=1 in duplicate. The impact of drug interference on titers was not evaluated. At the LLPC2, LPC, 100 ng/ml and HPC levels, the assay tolerated to ISIS678354 concentrations ≤ 4000 ng/ml.	The assay could detect as low as 15 ng/ml of anti-olezarsen antibodies in the presence of up to 4000 ng/ml of olezarsen, based on the mean $A_{450nm} \geq$ PSCP. C_{max} levels are 344-558 ng/ml in 50 mg of Olexarsen-treated group and 479-764 ng/ml in 80 mg-treated group. C_{trough} levels were <1% of C_{max} (below 10 ng/ml) after multiple sc injections of 50 or 80 mg once every 4 weeks. Screening assay tolerance for on board drug is acceptable

Sensitivity (95% CI)	HPC sample was diluted using fourteen 2-fold serial dilutions in NC. Sensitivity samples were subsequently diluted 1/50 in blocking buffer in the absence or presence of excess ISIS678354 and tested n=1 in 8 reportable runs performed by two analysts. The screening assay sensitivity was calculated from 7 runs as below (one plate was out of range): Mean PC concentration at PSCP + ($t_{0.05, df}$ x SD) The confirmatory assay sensitivity was the lowest PC concentration tested that consistently confirmed positive in all runs (i.e. spiked PC concentration with % signal inhibition ≥ CCP in all runs) Screening 9.65ng/ml Confirmatory 39.1 ng/ml	Acceptable
Repeatability/Intra-assay variability	PC samples were assays n=6, each one in duplicate on a total of two occasions performed by two analysts. Three duplicate sets (6 wells) of NC samples were tested in the confirmatory assays. <u>Screening</u> *Acceptance criteria*: %CV ≤ 20% *Results:* NC not tested LLPC2 (15 ng/ml) 5.8, 13.9 %CV LLPC1 (40 ng/ml) 5.8, 8.1 %CV LPC (50 ng/ml) 1.3, 3.8 %CV MPC (1500 ng/ml) 4.7, 5.3%CV HPC (10000 ng/ml) 1.5, 0.7 %CV <u>Confirmatory</u> *Acceptance criteria*: %CV ≤ 20% % of inhibition of NC (at least 1 out of 2) < CCP *Results:* LLPC2 (15 ng/ml) 26.2, 13.9 %CV LLPC1 (40 ng/ml) 13.7, 13.6 %CV LPC (50 ng/ml) 4.0, 6.5%CV MPC (1500 ng/ml) 0.3, 0.4%CV HPC (10000 ng/ml) 0.1, 0.2 %CV	Screening LLPC (LLPC2) is 13.36 ng/ml (adjusted to 15 ng/ml) and Confirmatory LLPC (LLPC1) is 39.1 ng/ml (adjusted to 40 ng/ml). The intra-assay precision results all met the acceptance criteria for screening assay. In confirmatory assay, one of the LLPC2 replicates showed 26.2 %CV, however, above confirmatory LLPC (LLPC1), the intra-assay precision results met the acceptance criteria. So acceptable
Intermediate Precision (IP)/inter-assay variability	All screening and confirmatory assays in which PC samples were analyzed n=2 in duplicate (total of at least 4 wells).	Intermediate precision results did not meet the acceptance criteria of %CV ≤ 20%.

	PC samples with %CV between duplicate values >20% were included all calculations <u>Screening</u> *Acceptance criteria:* %CV ≤ 20% *Results:* NC 11.5 %CV LLPC2 16.3 %CV LLPC1 19.5 % CV LPC 113.3% CV MPC 26.6% CV HPC 16.5% CV Without rejected runs NC 10.4 %CV LLPC2 13.8 %CV LLPC1 14.0 % CV LPC 20.6 % CV MPC 22.5 % CV HPC 11.8 % CV <u>Confirmatory</u> *Acceptance criteria*: %CV ≤ 20% % of inhibition of NC (at least 2 out of 3) < CCP *Results:* LLPC2 34.8 %CV LLPC1 23.8 %CV LPC 30.5 %CV MPC 1.6 %CV HPC 0.5% CV Without rejected runs LLPC2 28.7 %CV LLPC1 12.5 % CV LPC 29.8 % CV MPC 1.6 % CV HPC 0.5 % CV	Sponsor provided the justification to explain why higher %CV should be acceptable: 1) Screening assay The variability at the LLPC1 and LLPC2, which was tested at a lower conc than the LPC is passing the inter-assay variability assessment **Not acceptable** 2) Confirmatory assay High variability of LLPC2 due to technical issue in Run-116, which was not reproducible. No justification for LPC **Not acceptable** Sponsor should consider the need to refine the assay parameters to optimize the assay precision to the extent possible.
Selectivity	For matrix interference, LLPC2, LLPC1, LPC and HPC concentration were spiked into at least of 10 individual lots of normal human plasma (5 males and 5 females) or lipemic plasma (at least 5 males and at least 5 females) <u>Screening assay</u> *Acceptance criteria:* - At least 80% of the unspiked samples < PSCP - At least 80% of the individual samples spiked at LLPC2, LLPC1 and LPC levels	Samples spiked at LLPC2 concentration did not meet the acceptance criteria for screening and confirmatory assay. Also, non-spiked lipemic samples did not meet the acceptance criteria for confirmatory assay.

	should have OD ≥ PSCP, and at least 80% of the individual samples spiked at HPC level should have ODs > global mean MPC value of plate controls prepared in PNHP *Results:* 1) Normal plasma - 8 out of 10 unspiked normal plasma lots were < PSCP - 5 out of 10 (50%) individual lots of plasma spiked at LLPC2 were ≥ PSCP - 10 out of 10 individual lots of plasma spiked at LLPC1 and LPC were ≥ PSCP - 10 out of 10 individual lots of plasma spiked at HPC were > MPC 2) Lipemic plasma - 13 out of 15 unspiked lipemic plasma lots were < PSCP - 6 out of 8 (75%) lipemic individual lots spiked at LLPC2 were ≥ PSCP - 8 out of 8 lipemic individual lots spiked at LLPC1 and LPC were ≥ PSCP - 8 out of 8 lipemic individual lots of plasma spiked at HPC were > MPC <u>Confirmatory assay</u> *Acceptance criteria:* - At least 80% of normal plasma lots spiked with ISIS678354 should have % signal inhibition < CCP - At least 80% of the individual samples spiked at LLPC2, LLPC1, LPC and HPC and with ISIS678354 should have % signal inhibition ≥ CCP *Results:* 1) Normal plasma - 9 out of 10 unspiked normal plasma lots were < CCP - 6 out of 10 (60%) individual lots of plasma spiked at LLPC2 were ≥ CCP - 10 out of 10 individual lots of plasma spiked at LLPC1, LPC and HPC 2) Lipemic plasma - 11 out of 15 (73.3%) unspiked lipemic plasma lots were < CCP - 8 out of 8 individual lots of plasma spiked at LLPC2, LLPC1, LPC and HPC ≥ CCP	
Specificity	The ADA assay developed for anti-olezarsen antibodies can detect anti-ISIS304801 antibodies with sensitivity of 34 ng/ml in screening assay. Clinical samples with known status for anti-ISIS304801 antibodies were evaluated for comparison:	ISIS304801 (volanesorsen): same sequence and chemistry as olezarsen but without (b) (4) GalNAC cluster and gapmer design (i.e. mixed backbone with 2'MOE modification)

	Out of 60 clinical samples, 22 samples screened negative (2 out 22: positive in reference lab results) and 38 screened positive (8 out of 38: negative in reference). Out of 38 positive samples, 7 samples confirmed negative (all negative in reference) and 31 confirmed positive (2 out of 31: negative in reference) Out of 29 titrated samples, 26 out of 29 were within 2-fold dilution between labs.	Difference between Olezarsen and ISIS304801: $GalNAC_3$
Stability	The stability assessment was performed with the PC spiked into PNHP at LLPC2, LPC and HPC levels Short-term stability The short-term stability was evaluated samples kept at 4°C for 24 hours and 10 min Freeze-thaw stability 8 cycles of freeze (at least 12 hours) and thaw at RT (at least 4 hours) Bench-top stability Samples were stored at RT for 30 hours and 48 min All results met the acceptance criteria	
Lipemia	Impact of lipemia was evaluated under selectivity assessment	Unspiked lipemic plasma samples did not meet the acceptance criteria in the confirmatory assay. Lipemic plasma samples spiked at LLPC2 did not meet the acceptance criteria in the screening assay
Hemolysis	Impact of hemolysis was evaluated under selectivity assessment. LLPC2, LLPC1, LPC and HPC were prepared in PNHP spiked with hemolyzed whole blood at a final target level of 2% v/v *Acceptance criteria:* - Unspiked hemolyzed PNHP < PSCP - LLPC2, LLPC1 and LPC spiked hemolyzed PNHP ≥ PSC - HPC spiked hemolyzed PNHP > MPC *Results:* - All met the acceptance criteria within 73.9-125% recovery - At least 80% of the individual samples spiked at LLPC2, LLPC1 and LPC levels	Data show that hemolysis does not impact ADA detection

	should have OD ≥ PSCP, and at least 80% of the individual samples spiked at HPC level should have ODs > global mean MPC value of plate controls prepared in PNHP	
ADA Assay Assessment		*The specificity of the assay is unclear*

Assessor's Comments:

While immunogenicity has not been observed with GalNAc siRNA, it has been reported with GalNAc ASO (Eplotersen; ADA rate: 37%). The assay developed by the sponsor can detect antibodies that bind to both olezarsen and ISIS304801 (volanesorsen), however, the specificity of the assay remains unclear. It is uncertain whether the assay detects anti-olezarsen antibodies based on sequence, structure, or the GalNAc moiety.

A Nab assay was not developed, however, it is generally acceptable for this type of oligonucleotide therapeutics since their target is intracellular.

Another limitation of the assay the sponsor used for validation is high inter-assay variability and selectivity. Regarding these, we sent the following comments to the applicant during the review process and received sponsor's answers as below:

Information Request #1:

The information provided on the reproducibility of your assay, particularly inter-assay variability, raises concerns regarding the interpretability of the immunogenicity assessment reports. Consider whether there is a way to analyze the data that takes into account this deficiency and provides better information on the ADA incidence and sample titers or explain why this is not needed.

The sponsor's response to question 1:

The Positive control (PC) samples were prepared at five different concentrations: 15.0 ng/mL (Screening Lowest PC, LLPC2), 40.0 ng/mL (Confirmatory Lowest PC, LLPC1), 50.0 ng/mL (Low PC, LPC), 1500 ng/mL (Medium PC, MPC) and 10000 ng/mL (High PC, HPC) (678354-MV09 Amendment 2, Section 4.1). We acknowledge the higher variability seen at the LPC and MPC level for the screening assay, and at the LLPC2 and LPC level for the confirmatory assay during the method validation. Of note, the assay sensitivity was 9.65 and 39.1 ng/mL for the screening and confirmatory assay, respectively (678354-MV09 Amendment 2, Tables 6 and 7). In the screening assay, the slightly higher inter-assay variability at the LPC (20.6%) and MPC (22.5%) was not a concern as the inter-assay variability at the LLPC2 and LLPC1 was 13.8% and 14.9%, respectively, which were tested at lower concentrations than the LPC and MPC (15 and 40 vs 50 and 1500 ng/mL), met inter-assay variability acceptance criteria of 20% (678354-MV09 Amendment 2, Section 5.5).

In the confirmatory assay, higher inter-assay variability was observed for the LLPC2 (28.7%) and LPC (29.8%). The high variability seen at the LPC level in confirmatory assay is acceptable since the inter-assay variability at the LLPC1 was 12.5%, which was tested at a lower concentration than the LPC, met the acceptance criteria of 20% (678354-MV09 Amendment 2, Section 5.5). Since the confirmatory assay had an assay sensitivity of 39.1 ng/mL, the LLPC2 level (15 ng/mL) is below confirmation cut point level, thus the 20% precision acceptance criteria would not be applicable. Nonetheless, the higher %CV at the LLPC2 was mainly caused by one value (most likely due to a spiking error), in Run-116 (678354-MV09 Amendment 2, Table 4). An outlier test was

performed, and the result confirmed that the observed % signal inhibition value of -11.4% was an outlier in the data set. The %CV after removal of the outlier was decreased to 22.6% (Appendix 1).
Furthermore, in-study assay performance during clinical sample analyses across clinical studies showed that the inter-assay variability for both the screening assay and confirmatory assay at all the positive controls evaluated were well below 20% (Table 1 and Table 2).
Taken together, the sponsor concluded that the assay for olezarsen ADA assessment was deemed robust and reproducible, and no additional analysis is needed. The higher inter-assay variability observed during method validation would not impact the interpretability of the immunogenicity results.

Table 1: Summary of the In-Study Screening Assay Precision for the Detection of Anti-ISIS678354 Antibodies in Human Plasma Across Olezarsen Clinical Studies

Study	LLPC2 (15 ng/mL)		LPC (50 ng/mL)		HPC (10000 ng/mL)	
	N	%CV	N	%CV	N	%CV
AKCEA-CS1	30	11.5	30	14.0	30	9.0
ISIS 678354-CS2	120	9.6	120	9.6	124	7.3
ISIS 678354-CS3	185	7.6	186	10.9	185	10.5
ISIS 678354-CS7	49*	8.3	52*	12.7	51*	9.9
ISIS 678354-CS8	330*	9.0	330*	10.4	336*	5.8
ISIS 678354-CS13	147*	9.2	147*	12.0	148*	4.9

Source: Report AKCEA-CS01BA06, Report 678354_CS02BA02, Table 2; Report 678354-CS03BA02, Table 2; Report 678354-CS07BA02, Table 2; Report 678354-CS08BA02, Table 2; Report 678354-CS13BA02, Table 2.
*: N is counted manually and is not presented in the report tables, PCs that did not meet acceptance %CV were excluded from total number.

Table 2: Summary of the In-Study Confirmatory Assay Precision for the Detection of Anti-ISIS678354 Antibodies in Human Plasma Across Olezarsen Clinical Studies

Study	LLPC2 (15 ng/mL)		LLPC1 (40 ng/mL)		HPC (10000 ng/mL)	
	N	%CV	N	%CV	N	%CV
AKCEA-CS1	8	4.0	NA	NA	8	7.0
ISIS 678354-CS2	28	15.6	NA	NA	28	0.2
ISIS 678354-CS3	11	6.8	62	12.3	74	0.2
ISIS 678354-CS7	NA	NA	16	0.1	16	0.0
ISIS 678354-CS8	NA	NA	126	0.1	127	0.0
ISIS 678354-CS13	NA	NA	42	7.7	42	0.2

Source: Report AKCEA-CS01BA06, Table 3; Report 678354-CS02BA02, Table 3; Report 678354-CS03BA02, Table 3; Report 678354-CS07BA02, Table 3; Report 678354-CS08BA02, Table 3; Report 678354-CS13BA02, Table 3.
NA: Not Applicable

Additional Accessor's Comments:
In-study assay meets the acceptance criteria for inter-assay variability.

Information Request #2:
The specificity of the assay you used to detect anti-olezarsen antibodies is unclear although you provide data indicating that your assay would detect antibodies against ISIS304801, as it shares the same sequence and

chemistry as olezarsen but lacks the [redacted] GalNAc cluster and gapmer design. Provide an assessment of the risk for antibodies to each component of the product (the gapmer and the GaNAc moieties) as well as an assessment of what moieties the antibodies in the patient samples are directed to.

Response to Question 2

Olezarsen consists of 20 2´-MOE nucleotides (i.e. ISIS 304801) covalently bonded to a GalNAc3 at the 5´-end. Thus, anti-olezarsen antibodies may include anti-ISIS304801 and/or anti-GalNAc3 antibodies. The immune response against ASOs typically has no clinically meaningful consequences (Bano et al. 2022; Henry et al. 2022). Antibodies against GalNAc3 may occur naturally in a small proportion of the population (New et al. 2016), consistent with the observed baseline ADA-positive rates of 3.47 to 25.0% across olezarsen clinical studies conducted to date (Olezarsen NDA 2.7.2, Section 4.1.5.7). Investigation of pre-existing antibodies in baseline samples against GalNAc3-conjugated ASOs confirmed that the epitope recognized by preexisting antibodies in baseline samples was the GalNAc3-moiety (ADA Exploratory Report, [redacted] Project Code [redacted]). Therefore, patients with treatment-unaffected ADA bare likely having anti-GalNAc3 antibodies.

It is recognized that the anti-olezarsen assay can detect both anti-GalNAc3 and anti-ISIS304801 antibodies but does not distinguish between the two types of antibodies. Nonetheless, antibodies binding to either the ASO-moiety or the GalNAc3-moiety necessitate the evaluations for the potential effects or risk of treatment-unaffected ADAs and treatment-emergent ADAs, which was thoroughly evaluated in terms of pharmacokinetics (PK), pharmacodynamics (PD), clinical efficacy and safety (Module 2.7.2 Section 4.1).

Anti- olezarsen antibodies had no impact on the peak plasma exposures, although higher Ctrough was observed in ADA-positive patients. There was no clinically relevant impact of ADA positivity on measures of PD, efficacy, and safety. These findings indicate that anti-olezarsen antibodies had no neutralizing activity and did not affect pharmacological response or clinical benefit. Therefore, it was concluded that olezarsen has a low-risk immunogenicity profile.

Additional Accessor's Comments:

The specificity of this ADA assay is still unclear, as the assay does not differentiate between ADA to the ASO sequence or the GalNAc moieties.

Non-clinical study summary

Olezarsen had no effects on safety pharmacology parameters including CNS, heart and respiratory, and no blocking of hERG.

In the 9-month chronic monkey study, plasma exposure (Cmax and AUC) in ADA positive monkeys was higher after 13 and 39 weeks of dosing, however, the terminal elimination half-life or tissue exposure was not different between ADA positive and negative animals. Also, ADA showed no impact on C3, PLT counts and hepatic apoC-III mRNA levels.

A 13-/39-week repeat dose toxicity study in cynomolgus monkeys with a 26-week recovery period (study no. 678354-AS02) showed:

1. A marked PLT reduction in one male at 12 mg/kg/week, which is attributed to increased clearance of PLT. This resulted in temporary petechiae and oral mucosal bleeding.
2. Acute transient increases in activated partial thromboplastin time (aPTT) and activation of alternative complement pathway (Bb increase), associated with the plasma C_{max}
3. Slight to moderate increase in IgM at $\geq$ 12 mg/kg/week (1.6 to 1.7-fold over baseline on Day 93) and minimal increase in IgG on day 93 in one male with 2mg/kg/week and in two females dosed with 30 mg/kg/week
4. Minimal to slight decreases in albumin concentrations, indicative of inflammation.
5. Slight increase of IL-6 in one male
6. Liver/spleen weight increase. Liver showed the indication of inflammation.
7. Basophilic granule accumulation primarily in the liver, kidney and lymph nodes

In summary, the production of plasma complement split product Bb was elevated by olezarsen treatment in an acute, transient, and dose-dependent pattern. Elevations in Bb were observed at 12 and 30 mg/kg/week, with increases approximately up to 3- (females) and 21-fold (males and females), respectively, over pre-study values on Day 1. The observed elevations in circulating Bb peaked at 4 hours post-dose and generally returned to pre-dose levels by 24 hours post-dose. Increases in Bb concentration were accompanied by minimal to slight decreases in total C3 levels.

***Assessor's Comment to the Question 'D*o the nonclinical alterations indicate a need for additional immunologic assessments for the indicated clinical population'?**

Due to the polyanionic nature of ASOs with PS backbone linkages, their nonspecific affinity to plasma and cellular surface proteins is enhance. PS ASO is known to bind to platelet collagen receptor GPVI, leading to platelet aggregation, and platelet factor 4 (PF4), inducing a humoral response to the ASO-PF4 complex (similar to heparin-induced thrombocytopenia). Given the non-clinical data and the nature of drug product, platelet numbers and activation state should be assessed in the clinical study. Also, GPVI and/or PF4 levels could be useful as a screening tool to identify patients at higher risk of ASO-induced thrombocytopenia.

Several reports indicate that 2'-MOE ASO induces alternative pathway of complement activation in monkeys (1, 2). Also, marked increases in platelet-bound C3d/C4d were observed in 2'-MOE ASO-induced thrombocytopenic monkeys (3), indicating increased platelet clearance due to complement deposition on the platelets. While this complement activation is not evident in humans, the non-clinical data certainly raises concerns, and we would have suggested close monitoring of complement activation and coagulation cascades during the clinical studies. At this point, if there have been no clinical signals, then there is no clinical argument to request complement activation assessment through a PMR.

Hepatocellular vacuolation, hepatocellular single-cell necrosis and basophilic granule accumulation have been observed in the non-clinical studies for several GalNAc-conjugated oligonucleotides. Among them, ALN-AAT, a GalNAc-conjugated siRNA targeting alpha-1 antitrypsin for the treatment of AAT deficiency-associated liver disease, showed transient, asymptomatic, and dose-dependent increases in liver enzymes in healthy volunteers exposed to single dose. Therefore, its development was discontinued. In this application, it is noted that increases in mean levels of ALT and AST from baseline were observed following treatment with Olezarsen. This could be due to GalNAc and/or ADA-induced increase in Ctrough. From an immunogenicity perspective, it is important to evaluate whether the observed increases in ALT and AST are linked to ADA or not.

1. Crooke ST, Baker BF, Kwoh TJ, Cheng W, Schulz DJ, Xia S, et al. Integrated Safety Assessment of 2'-O-Methoxyethyl Chimeric Antisense Oligonucleotides in NonHuman Primates and Healthy Human Volunteers. Mol Ther. 2016;24(10):1771-82.
2. Shen L, Frazer-Abel A, Reynolds PR, Giclas PC, Chappell A, Pangburn MK, et al. Mechanistic understanding for the greater sensitivity of monkeys to antisense oligonucleotide-mediated complement activation compared with humans. J Pharmacol Exp Ther. 2014;351(3):709-17.
3. Shen L, Wong A, Oneda S, Curtis BR, Schroeder J, Zanardi T, et al. Complement C3d/C4d Deposition on Platelets Correlates with 2'-O-Methoxyethyl Antisense Oligonucleotide-Induced Thrombocytopenia in Monkeys. Nucleic Acid Ther. 2023;33(3):209-25.

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/s/

HA NA LEE
08/05/2024 11:19:11 AM

DANIELA I VERTHELYI
08/05/2024 11:20:20 AM

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: 7/12/2024

TO: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Office of Cardiology, Hematology, Endocrinology and Nephrology (OCHEN)

FROM: Office of Study Integrity and Surveillance (OSIS)

SUBJECT: **Decline to conduct an on-site inspection**

RE: NDA 218614

The Office of Study Integrity and Surveillance (OSIS) determined that an inspection is not needed for the site listed below. The rationale for this decision is noted below.

Rationale

(b) (4)**:** OSIS conducted a Remote Regulatory Assessment (RRA) for the site in (b) (4). The RRA was conducted under the following submission: NON-RESPONSIVE

OSIS concluded that data from the reviewed studies were reliable.

Site

Facility Type	Facility Name	Facility Address
Analytical	(b) (4)	

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/s/

ADANMA S OJI
07/12/2024 01:19:03 PM

Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIOLOGY AND NEPHROLOGY PRODUCTS

Date: June 27, 2024

From: Interdisciplinary Review Team for Cardiac Safety Studies

Through: Christine Garnett, PharmD
Team Lead, Cardiac Safety IRT, DCN

To: Ron Picking
DDLO

Subject: QT Consult to NDA 218614 (SN 0001)

This memo responds to your consult to us dated 6/4/2024 regarding the sponsor's QT related question. We reviewed the following materials:

- Sponsor's label (NDA 218614 / SN 0001; link if applicable);
- Previous IRT review(s) for IND 136692 dated 03/05/2021; 07/05/2023 in DARRTS.

1 Responses for the Division

Question from the Division: We are consulting the IRT for review of language in Section 12.2 of the Prescribing Information submitted with the NDA:

(b) (4)

IRT's response: Yes, we agree with the proposed language.

2 BACKGROUND

As a substitute for a TQT study, the IRT previously communicated to the Applicant that we would consider an integrated risk assessment consisting of nonclinical studies and clinical ECG data from studies ISIS 678354-CS1 and ISIS 678354-CS2 (IRT review dated 3/5/21). The Applicant submitted the integrated risk assessment; IRT concluded that olezarsen did not prolong the QTcF interval ≥ 10 msec and nonclinical studies were negative for QT effects (IRT review dated 7/5/23).

The proposed statement in section 12.2 of labeling is (b) (4) (b) (4) (b) (4) The highest dose evaluated in the clinical QT assessment was a single dose of 120 mg SC, where the ΔΔQTcF (90% CI) was 0.3 msec (-4.9, 5.4). The highest proposed therapeutic dose is 80 mg SC monthly. We agree with the proposed labeling statement, which is consistent with QT labeling guidance (Table 1).

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/s/

ELIFORD N KITABI
06/27/2024 03:52:39 PM

MARIO SAMPSON
06/27/2024 03:56:26 PM

CHRISTINE E GARNETT
06/27/2024 03:57:57 PM

U.S. FOOD & DRUG ADMINISTRATION

ICCR FACILITIES REVIEW MEMO

Date: June 25, 2024

To: Oluwafunmike Ajomale, FDA/OC/CDER/OPQ/OPRO/DRBPMIII/ Oluwafunmike.Ajomale@fda.hhs.gov

Office of Combination Products at combination@fda.gov

RPM: Oluwafunmike Ajomale

Through: Shruti Mistry, Assistant Director, Inj ction Team, OHT3, OPEQ, CDRH

Shruti N. Mistry -S 2024.06.25 16:06:04 -04'00'

From: Farzaneh Akhavannik, THT3C3, OHT3, OPEQ, CDRH

Applicant: Ionis Pharmaceuticals, Inc
2282 Faraday Ave.
Carlsbad, California 92008
United States
FEI# 3000215659

Application # NDA 218614

Consult # ICC# ICC2400423

Product Name: Olezarsen

Combination Product

Intended Use: As an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome (FCS)

Pre-Approval Inspection: Yes

The Office of of Reproductive, Gastro-Renal, Urological, General Hospital Device & Human Factors at CDRH received a consult from CDER requesting the identification of the device manufacturing sites for NDA 218614 which will require a device inspection.

PRODUCT DESCRIPTION

Olezarsen Solution for Injection is a sterile, single-dose, ready-to-use parenteral solution of olezarsen intended for monthly subcutaneous (SC) administration. The formulation contains 100 mg/mL olezarsen free acid (105 mg/mL olezarsen sodium) (b) (4), at a target pH 7.4. Sodium chloride is added to (b) (4). The solution appears clear and colorless to yellow. The drug product is packaged with 0.8 mL deliverable volume in a 1 mL Long (1mL L) staked needle glass prefilled syringe (PFS) closed with a (b) (4) rubber plunger stopper. The PFS is assembled into an autoinjector for the final drug product presentation. The autoinjector delivers a single dose of 80 mg olezarsen.

The following is a description of the container closure systems:

Table 1: Description of Primary Packaging Components

Component	Description	(b) (4) Supplier/ Manufacturer
Syringe[a]	1 mL L (b) (4) glass, (b) (4) clear, compliant with USP <660>, with 27G 1/2" special (b) (4) bevel stainless steel needle and a rigid needle shield (b) (4)	(b) (4)
Rubber Plunger Stopper[a]	(b) (4) rubber, (b) (4) grey, compliant with USP <381> (physicochemical tests) (b) (4)	
	(b) (4) rubber, (b) (4) grey, compliant with USP <381> (physicochemical tests) (b) (4)	

[a] The rubber closure parts (b) (4). In addition, (b) (4) is used in the manufacture of these parts.

REGULATORY HISTORY

The following facilities were identified as being involved in the manufacturing and/or development of the Olezarsen 1 ml prefilled syringe in NDA 218614, SD 1

1. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4). The inspection covered Drug GMP including storage capacities for all temperature, transport of goods between all (b) (4) locations, production activities (optical inspections) and quality control activities (packaging material inspections) as well as import activities and was classified VAI.

Inspection Recommendation:
An inspection is not required because:

- The firm is not responsible for major activities related to the manufacturing and development of the final combination product or the device constituent part;

2. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4). The inspection covered BIMO good laboratory practice for nonclinical labs, specifically the project titled: NON-RESPONSIVE and was classified NAI.

Inspection Recommendation:
An inspection is not required because:

- The firm is not responsible for major activities related to the manufacturing and development of the final combination product or the device constituent part;

3. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4). The inspection covered Inspection of Human Cells, Tissues and

Cellular and Tissue Based Products (HCT/Ps) and CP 7345.848, Inspection of Biological Drug Products (CBER), (PAC 41848F Level 1 CGMP Inspection). System coverage included the Quality, Laboratory Control, and the Facility / Equipment Systems and was classified NAI.

Inspection Recommendation:
An inspection is not required because:

- The firm is not responsible for major activities related to the manufacturing and development of the final combination product or the device constituent part;

4. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4) The inspection covered Drug Process Inspections(DPI) and was classified VAI.

Inspection Recommendation:
An inspection is required because:

- The firm is responsible for major activities related to the manufacturing, assembly and development of the final combination product or the device constituent part.
  The Autoinjector is different from other devices previously covered in the (b) (4) inspection. The assembly processes of the Autoinjector play an important role in the final functionality and specification of the device constituent. Failure to meet the specifications of the device constituent may result is medication errors, such as missed dose or administration of a partial dose, delay or lack of therapy, and injection related risks such as needle stick wounds .Therefore, an inspection of the autoinjector assembly processes is requested.

5. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4). The inspection covered Pre-License Therapeutic Biological Product Inspections and was classified NAI. There have been no device inspection completed at this site.

Inspection Recommendation:
An inspection is not required because:

- The firm is not responsible for major activities related to the manufacturing and development of the final combination product or the device constituent part;

6. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that an inspection was conducted (b) (4) The inspection covered ANDA Pre-Approval Inspection for NON-RESPONSIVE. The inspection was classified OAI.

Inspection Recommendation:
An inspection is not required because:

- The firm is not responsible for major activities related to the manufacturing and development of the final combination product or the device constituent part;

7. (b) (4)

(b) (4)

Inspectional History – An analysis of the firm's inspection history showed that the firm does not have a history of inspections.

Farzaneh S. Akhavannik -S
Digitally signed by Farzaneh S. Akhavannik -S
Date: 2024.06.25 15:57:55 -04'00'

Farzaneh Akhavannik

Prepared: Farzaneh Akhavannik: 05/13/2024
Reviewed: SMistry: 06/25/2024

CTS No.: ICC2400423
NDA 218614

Review Cycle Meeting Attendance:
05/13/2024

Inspectional Assignment

CDRH recommends a preapproval inspection under the applicable Medical Device Regulations (b) (4)
(b) (4)

Firm to be inspected:

1. (b) (4)

A comprehensive baseline Level 2 /limited inspection is recommended focusing on Management Responsibility (21 CFR 820.20), Purchasing Controls (21 CFR 820.50), CAPA (21 CFR 820.100), Final Acceptance Activities (21 CFR 820.80), and Design Controls (21 CFR 820.30)

Additionally, evaluate the manufacturing activities associated with the manufacturing/assembly of the finished combination product, including in process and final acceptance activities. Detailed inspection guidance will be provided upon request.

REGULATORY STRATEGY

The establishment inspection report (EIR) for the firm should be shared with CDRH (The EIR should be assigned to CDER and then sent to CDRH as a consult for review). If the inspection is being classified Official Action Indicated (OAI), the District should consider recommending appropriate regulatory action with consultation from CDER and CDRH and whether the violation is drug or device related.

Questions regarding this consult should be referred to one of the following individuals:

Primary Contact
Farzaneh Akhavannik
CDRH Device Reviewer
General Hospital Team
DHT3C
OHT3: Office of Health Technology 3
Phone: 301-796-7484

Secondary Contacts (if Primary is unavailable and a timely answer is required)
Shruti Mistry
Assistant Director
Injection Team
DHT3C
OHT#: Office of Health Technology 3
Phone: 301-796-6605

THIS ATTACHMENT IS NOT TO BE PROVIDED TO THE FIRM OR SHOWN TO THEM DURING THE INSPECTION. THIS ATTACHMENT CONTAINS PREDECISIONAL INFORMATION

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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