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Clinical Trial Design for Triglyceride-Rich Lipoprotein-Lowering Therapies

JACC Focus Seminar 3/3

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ABSTRACT

Triglyceride-rich lipoproteins (TRLs) are a source of residual risk in patients with atherosclerotic cardiovascular disease, and are indirectly correlated with triglyceride (TG) levels. Previous clinical trials studying TG-lowering therapies have either failed to reduce major adverse cardiovascular events or shown no linkage of TG reduction with event reduction, particularly when these agents were tested on a background of statin therapy. Limitations in trial design may explain this lack of efficacy. With the advent of new RNA-silencing therapies in the TG metabolism pathway, there is renewed focus on reducing TRLs for major adverse cardiovascular event reduction. In this context, the pathophysiology of TRLs, pharmacological effects of TRL-lowering therapies, and optimal design of cardiovascular outcomes trials are major considerations. (J Am Coll Cardiol 2023;81:1646–1658) © 2023 by the American College of Cardiology Foundation.

Elevations in triglyceride-rich lipoproteins (TRLs) are associated with atherosclerotic cardiovascular disease (ASCVD) events.[1-3] Although reduction of low-density lipoprotein cholesterol (LDL-C) remains the mainstay of primary and secondary ASCVD prevention, TRLs have emerged as a target of interest in patients with residual cardiovascular risk.[4-7]

Randomized controlled trials of TG-lowering therapies have yielded conflicting results. In early trials, the fibrates demonstrated ASCVD risk reduction when compared with placebo,[8,9] but later trials failed to substantiate this benefit when peroxisome proliferator-activated receptor-alpha (PPAR-α) agonists were added to statin therapy[10-12]; trials assessing the effect of omega-3 fatty acids have shown variable results.[13-16]

The discordance between epidemiological and genetic data with efficacy of current clinical therapies in reducing the risk of ASCVD associated with hypertriglyceridemia (HTG) has prompted a re-evaluation of TRL-lowering study design and a focus on novel

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HIGHLIGHTS

- Remnant cholesterol, a marker of apolipoprotein B-containing lipoproteins, is associated with increased risk of ASCVD.
- New therapies targeting proteins in the triglyceride metabolism pathway, particularly apolipoprotein C-III and ANGPTL3, can reduce blood levels of triglycerides and remnant cholesterol.
- Important considerations in trial design include clinical and laboratory risk assessment tools, and the pharmacological effects of inhibiting various pathways.

targets in the TG metabolic pathway. In this review, we address challenges in TRL-lowering clinical trial design and identify methods to demonstrate ASCVD risk reduction in future clinical trials.

CHALLENGE 1: THE SPECTRUM OF TRIGLYCERIDE-RICH LIPOPROTEINS AND ASSOCIATION WITH CARDIOVASCULAR RISK

Plasma total TG concentration encompasses the TG content of all lipoprotein particles, including chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and to a far lesser extent, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). As intestinally derived chylomicrons and liver-derived VLDL undergo lipolysis, these lipoprotein particles become depleted of TGs and enriched in cholesterol while maintaining apolipoprotein (apo) B-48 in chylomicron remnants and apoB-100 in VLDL remnants.[17] ApoB-containing lipoproteins drive atherogenesis; therefore, atherosclerosis is reflective of the cumulative exposure to apoB-containing lipoproteins.[18] After accounting for LDL and Lp(a), the remainder of these lipoproteins are reflected in VLDL, IDL, and chylomicron remnants, which are relatively TG-rich compared with LDL and Lp(a), which are relatively TG poor. Therefore, measured plasma TG serves as an indirect, albeit imprecise, reflection of circulating TRLs.[19]

The range of TGs at which ASCVD risk is increased remains controversial. An expert consensus from the American College of Cardiology/American Heart Association recommend a threshold of 150 mg/dL (fasting) and 175 mg/dL (nonfasting) to reflect increased ASCVD risk, whereas TGs ≥500 mg/dL are defined as severe HTG and reflect increased risk of acute pancreatitis.[20] The European Society of Cardiology guidelines define the cutpoint of TGs <150 mg/dL for low risk of ASCVD and ≥880 mg/dL for pancreatitis.[19]

To more accurately capture the ASCVD risk associated with TGs, it may be more appropriate to estimate the remnant cholesterol (RC) content carried on TRLs (chylomicron remnants, VLDL, and IDL particles). RC is estimated using the formula: RC = total cholesterol − HDL-C − LDL-C.[17] Multiple epidemiological studies have reported significant associations between RC (whether directly measured or estimated) and ASCVD risk.[21-23]

CHALLENGE 2: DOES LOWERING TRIGLYCERIDES REDUCE ASCVD RISK?

Previous landmark clinical trials of TG-lowering therapy have been reviewed.[17,24] Clinical trials assessing the efficacy of fibrates and PPAR-α agonists have failed to establish a consistent association between TG lowering and reduction in major adverse cardiovascular events (MACE) (Table 1).[8-12,25] However, a significant benefit with respect to vascular events in patients with dyslipidemia was observed, as suggested by subgroup analyses of participants with TGs ≥200 mg/dL and HDL-C ≤40 mg/dL.[26] The PROMINENT (Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes) trial investigated this hypothesis with pemafibrate.[12] Pemafibrate resulted in a 26.2% reduction in TGs and 25.6% reduction in RC, but a 12.3% increase in LDL-C and 4.8% increase in apoB. When evaluating the absolute mass changes, during follow-up this translated into an ~10 mg/dL reduction in remnant cholesterol vs an ~10 mg/dL increase in LDL-C, underscoring the fact that pemafibrate did not reduce the absolute mass of proatherogenic apoB-cholesterol particles. This may account for the observation that there was no significant impact of pemafibrate vs placebo on incidence of ASCVD, despite a marked reduction (40-50 mg/dL) in TG levels.

Evidence supporting the use of n-3 polyunsaturated fatty acids (PUFA) for the purpose of TG lowering and MACE reduction has been mostly neutral (Table 2).[13,15,16,27-29] In the REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl Intervention Trial), an unanticipatedly high 25% relative risk reduction in ASCVD was observed, despite a modest (39 mg/dL) absolute change in TG; interestingly, the benefit was similar regardless of

ABBREVIATIONS AND ACRONYMS

apoB = apolipoprotein B
ACS = acute coronary syndrome
ANGPTL3 = angiopoietin-like protein 3
ASCVD = atherosclerotic cardiovascular disease
ASO = antisense oligonucleotide
HDL = high-density lipoprotein
HTG = hypertriglyceridemia
LDL-C = low-density lipoprotein
MACE = major adverse cardiovascular event(s)
MR = Mendelian randomization
siRNA = small-interfering RNA
TG = triglyceride
TRL = triglyceride-rich lipoproteins
VLDL = very low-density lipoprotein

TABLE 1 Clinical Trials of Fibrates for Reduction of TG

Study, Ref. #	Year	Population	Sample Size (n)	Fibrate and Dose	TG Lowering Effect	Primary Outcome	Primary Outcome HR (95% CI)
Helsinki Heart Study[8]	1987	Asymptomatic middle-aged men (ages 40-55 y) with non-HDL ≥200 mg/dL	4,081	Gemfibrozil 600 mg twice daily	−43% at 2 y	Fatal and nonfatal MI and cardiac death	0.66 (0.474-0.918)
VA-HIT[9]	1999	Men with known coronary heart disease, HDL-C ≤40 mg/dL, LDL-C ≤140 mg/dL	2,531	Gemfibrozil 1,200 mg daily	−31% at 1 y	Nonfatal MI or death from coronary causes	0.78 (0.65-0.93)
BIP[25]	2000	Patients with previous MI or stable angina, TC 180-250 mg/dL, HDL-C ≤45 mg/dL, TG ≤300 mg/dL, LDL-C ≤180 mg/dL	3,090	400 mg bezafibrate daily	−21% at 1 y	Fatal or nonfatal MI or sudden death	0.91 ($P = 0.26$)
FIELD[10]	2005	Participants age 50-75 y with type 2 diabetes and not on statin therapy	9,795	Fenofibrate 200 mg daily	−29% at 4 mo	Coronary events (coronary heart disease death or nonfatal MI)	0.89 (0.75-1.05)
ACCORD-Lipid[11]	2010	Participants with type 2 diabetes being treated with simvastatin	5,518	Fenofibrate 160 mg daily	−25.6% at 5 y	First occurrence of nonfatal MI, nonfatal stroke, or death from CV causes	0.92 (0.79-1.08)
PROMINENT[12]	2022	Participants with type 2 diabetes, TG 200-499 mg/dL, HDL-C ≤40 mg/dL on guideline statin therapy	10,497	Pemafibrate 0.2 mg BID	−26.2% at 3.4 y	Composite of nonfatal MI, stroke, hospitalization for UA requiring revascularization, and CV death	1.03 (0.91-1.15)

ACCORD = Action to Control Cardiovascular Risk in Diabetes; CV = cardiovascular; FIELD = Fenofibrate Intervention and Event Lowering in Diabetes; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MI = myocardial infarction; PROMINENT = Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes; TG = triglycerides; UA = unstable angina; VA-HIT = Veterans Affairs HDL Intervention Trial.

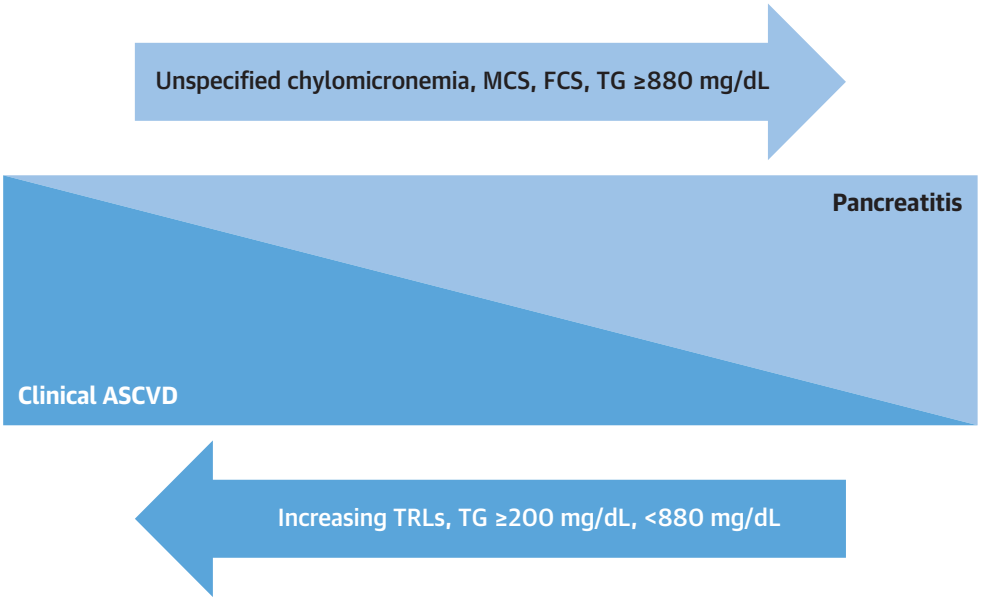
baseline TG concentration.[13] The large effect size may have been in part caused by the use of mineral oil as placebo, because mineral oil can increase inflammation and oxidized phospholipids.[30] However, recent results from the open-label RESPECT-EPA (Randomized Trial for Evaluation in Secondary Prevention Efficacy of Combination Therapy-Statin and Eicosapentanoic Acid) lend further support for the potential benefits of EPA for the treatment of HTG.[16] Although the trial failed to meet its primary endpoint, this may have been caused by being underpowered rather than the lack of efficacy of EPA. In patients with low EPA levels and HTG, supplementation with EPA may provide significant cardiovascular benefit, likely independent of the change in TG levels. Conversely, docosahexanoic acid supplementation does not show significant benefit, despite comparable changes in TG levels.[15] The discrepancies

TABLE 2 Clinical Trials of Omega-3 Fatty Acids

Study, Ref. #	Year	Population	Sample Size (n)	Omega-3 PUFA and Dose (g)	TG-Lowering Effect Compared With Placebo	Primary Outcome HR (95% CI)
EPA clinical trials						
JELIS[29]	2007	Hypercholesterolemia patients with baseline LDL-C ≥182 mg/dL	18,645	EPA EE 1.8 g	−5.0%	0.81 (0.69-0.95)
REDUCE-IT[13]	2019	Diabetic patients and patients with ASCVD	8,179	EPA EE 4 g	−20.5%	0.75 (0.68-0.83)
RESPECT-EPA[16]	2022	Patients with chronic coronary artery disease with EPA/AA ratio <0.4	2,506	EPA 1.8 g	—	0.785 (0.62-1.00)
EPA/DHA clinical trials						
VITAL[27]	2018	Healthy volunteers (men >50 y or women >55 y)	25,871	EPA EE 0.46 g DHA EE 0.38 g	Unknown	0.92 (0.8-1.06)
ASCEND[73]	2018	Diabetic patients without ASCVD	15,480	EPA EE 0.46 g DHA EE 0.38 g	Unknown	0.97 (0.87-1.08)
STRENGTH[15]	2020	Diabetic patients, patients with ASCVD, and high-risk patients	13,078	EPA 2.2 g DHA 0.8 g	−18.1%	0.99 (0.9-1.09)

ASCEND = A Study of Cardiovascular Events in Diabetes; DHA EE = docosahexaenoic acid ethyl ester; EPA EE = eicosapentaenoic acid ethyl ester; JELIS = Japan EPA Lipid Intervention Study; PUFA = n-3 polyunsaturated fatty acids; REDUCE-IT = Reduction of Cardiovascular Events with Icosapent Ethyl Intervention Trial; RESPECT-EPA = Randomized Trial for Evaluation in Secondary Prevention Efficacy of Combination Therapy-Statin and Eicosapentanoic Acid; STRENGTH = Long-Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia; VITAL = Vitamin D and Omega-3 Trial; other abbreviations as in Table 1.

FIGURE 1 The Spectrum of TGs and Associated Risk for ASCVD vs Pancreatitis



As triglyceride (TG) concentration increases, it reflects an increase in chylomicrons, which increasingly predisposes to pancreatitis rather than atherosclerotic cardiovascular disease (ASCVD). FCS = familial chylomicronemia syndrome; MCS = multifactorial chylomicronemia syndrome; TRL = triglyceride-rich lipoproteins.

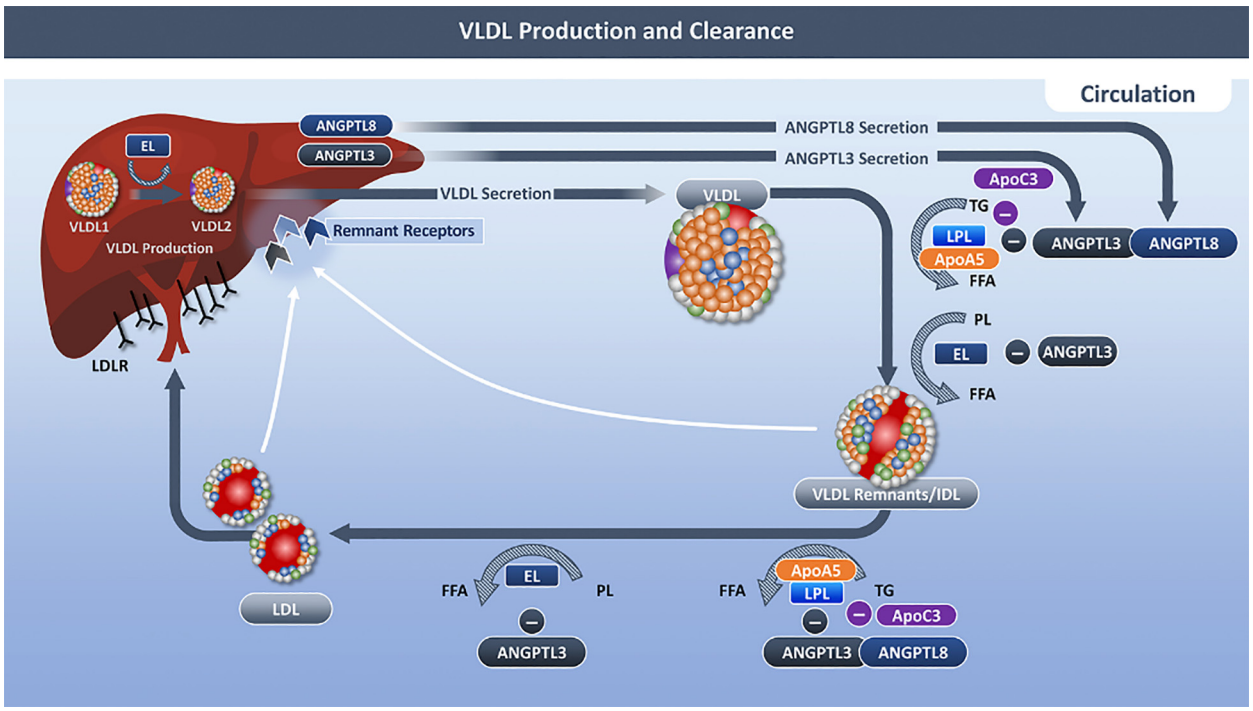
between the impact of EPA and docosahexanoic acid have been reviewed previously[31] and are outside the scope of this review. Regardless, the heterogeneous results of n-3 PUFA trials have resulted in overall uncertainty regarding the magnitude of the ASCVD benefit of n-3 PUFA therapies.

These findings are consistent with several Mendelian randomization (MR) analyses; lipoprotein lipase (LPL) variants resulting in reduced TG were associated with a similarly lower ASCVD risk as lowering LDL-C and apoB; however, the risk associated with lower TG was proportional to the net absolute difference in total apoB.[32] Additionally, single nucleotide polymorphisms exclusively associated with increased TGs and non-HDL-C are associated with increased CAD risk.[33,34] Collectively, this implies that the cholesterol carried by either LDL or TRLs drives atherogenicity, whereas the TG load carried by these particles inaccurately mirrors this atherogenic load.

Cumulatively, these findings imply that therapies that merely enhance TG metabolism are insufficient to convey cardiovascular benefit. Only when this is combined with increased clearance of the cholesterol-enriched TRLs can one expect to achieve significant cardiovascular benefit.

OPTIMAL THRESHOLDS FOR TRL-LOWERING THERAPY TRIALS. In the design of placebo-controlled TRL-lowering trials for the purpose of reducing clinical ASCVD, it is important to avoid recruiting patients with multifactorial chylomicronemia syndrome (MCS) and familial chylomicronemia syndrome (FCS) caused by the increased risk of acute pancreatitis. In one study of 7.1 million patients, the annualized incidence rate for a first event acute pancreatitis increased from 0.08% with TG <200 mg/dL to 1.21% with TG >1,000 mg/dL, whereas the risk for a second and third episode in the year after the first event was 9.26% with TG <200 mg/dL to 23.62% with TG >1,000 mg/dL.[35] Mild to moderate HTG is also associated with increasing risk for acute pancreatitis as TGs increase. In the Copenhagen City Heart Study, a 1.17 increase in HR for acute pancreatitis was seen for every 89-mg/dL increase in TG.[36] The wide range of TGs makes determining cutoffs for recruitment for TRL-lowering therapy trials difficult. At one end of the spectrum, there is higher risk of ASCVD with low risk of pancreatitis, whereas at the other end, severe HTG with chylomicronemia increases the risk of pancreatitis while the risk of clinical ASCVD is a lesser near-term concern (**Figure 1**). Using a threshold that focuses on ASCVD

FIGURE 2 Triglyceride-Rich Lipoprotein Metabolic Pathways



Schematic depicting the regulation of very low-density lipoprotein (VLDL) production and clearance by apolipoprotein (apo) C-III, ANGPTL3, and ANGPTL8. Inhibition of apoC-III and ANGPTL3 leads to increased lipoprotein lipase (LPL) activity, and ANGPTL3 also leads to increased endothelial lipase (EL) activity, thereby increasing the breakdown of triglyceride-rich lipoproteins (TRL) into low-density lipoprotein cholesterol, which can be more effectively cleared by the low-density lipoprotein receptor (LDLR). Additionally, increased EL activity leads to increased production of mature VLDL which is secreted into the circulation. Reprinted with permission from Rosenson et al.[17] IDL = intermediate-density lipoprotein.

risk while monitoring for pancreatitis as a safety outcome will be necessary.

CHALLENGE 3: CAN INHIBITION OF NOVEL TARGETS IN TRL METABOLISM PATHWAYS RESULT IN ASCVD RISK REDUCTION?

The pathways under consideration for new pharmacotherapeutic options for lowering TRLs have been reviewed extensively.[17,24] Emerging targets include apoC-III, angiopoietin-like protein 3 (ANGPTL3), ANGPTL3/ANGPTL8, and ANGPTL4 (Figure 2).

TARGET: apoC-III. Pathophysiology and mechanism of action. ApoC-III is an endogenous inhibitor of LPL and hepatic lipase (HL), enzymes involved in hydrolysis and clearance of TGs and TRLs from the circulation.[17,37,38] Increasing apoC-III levels correlate with reduced TRL lipolysis, potentially because of displacement of LPL from TRL particles,[39] as well as the reduced hepatic uptake of TRL remnants.[40] The latter may contribute to the observed LPL-independent effects of apoCIII inhibition observed in patients with FCS with complete LPL deficiency.[41]

Genetic evidence for APOC3 inhibition. *APOC3* was first established as a potential target when heterozygous carriers of a null mutation in the Amish population were found to have significantly lower TGs and coronary calcification.[42] In the Exome Sequencing Project, carrying an *APOC3* loss of function variant (LOFV) resulted in a 39% reduction in plasma TGs and 40% reduction in incident ASCVD events when compared with noncarriers.[43] In another study, a 44% reduction in nonfasting TGs and 36% reduction in ischemic heart disease was observed in carriers of *APOC3* LOFVs compared with noncarriers.[44] A meta-analysis of 137,895 individuals showed *APOC3* LOFV carriers had a 43% reduction in RC compared with noncarriers. Conversely, only a 4% reduction in LDL-C was observed when compared with noncarriers, suggesting that the cardioprotective effects of *APOC3* LOFVs are independent of LDL-C

TABLE 3 Lipid-Lowering Efficacy of ApoC-III Inhibitors

Therapy	Trial Phase, Ref. #	Study Population	Sample Size (n)	Dose	Apo-C-III	TG	LDL-C	HDL-C	Non-HDL-C
Volanesorsen (ASO)	2[74]	Severe HTG (between 350 mg/dL and 2,000 mg/dL)	57	100 mg SC QW	−40%	−38%	+48%	+27%	−7%
				200 mg SC QW	−64%	−70%	+79%	+36%	−7%
				300 mg SC QW	−80%	−72%	+118%	+46%	−11%
	3[46]	FCS (TG >750 mg/dL)	66	300 mg SC QW	−84%	−77%	+136%	+46%	−46%
	3[47]	Severe HTG/MCS (TG ≥500 mg/dL)	113	300 mg SC QW	—	−72%	—	—	—
Olezarsen (ASO)	1/2a[48]	Healthy volunteers; single ascending dose	40	10 mg SC	0%	−12%	—	—	—
				30 mg SC	−42%	−7%	—	—	—
				60 mg SC	−73%	−42%	—	—	—
				90 mg SC	−81%	−73%	—	—	—
				120 mg SC	−92%	−77%	—	—	—
		Healthy volunteers; multiple ascending dose	17	15 mg SC QW	−65%	−61%	−3%	+50%	−22%
				30 mg SC QW	−84%	−71%	−17%	+56%	−30%
				60 mg SC Q4W	−80%	−61%	−10%	+64%	−27%
ARO-APOC3 (siRNA)	1/2a[49]	FCS (confirmed 2 biallelic variants)	4	50 mg	−98%	−91%	—	+152%	−58%
		MCS (severely elevated TG ≥880 mg/dL)	26	10 mg, 25 mg, 50 mg, 100 mg	−96%	−90%	—	+111%	−49%
	2b[63]	Severe HTG (TG ≥500 mg/dL)	177	10 mg	−73%	−78%	—	+76%	−37%
				25 mg	−82%	−86%	—	+99%	−45%
				50 mg	−87%	−86%	—	+83%	−34%

ASO = antisense oligonucleotide; FCS = familial chylomicronemia syndrome; HTG = hypertriglyceridemia; MCS = multifactorial chylomicronemia syndrome; QW = every week; SC = subcutaneously; siRNA = small-interfering RNA; other abbreviations as in Tables 1 and 2.

lowering.[45] Unfortunately, apoB levels were not available.

Effects of APOC3 inhibition on TGs. Multiple pharmacotherapeutics have been developed targeting *APOC3* mRNA. Volanesorsen (second generation) and Olezarsen (AKCEA-APOCIII-L_{RX}) (third generation) are 2 antisense oligonucleotides (ASOs), whereas ARO-APOC3 is a small-interfering RNA (siRNA) directed at APOC3 mRNA (Table 3). Volanesorsen was studied in patients with FCS with TG ≥750 mg/dL; volanesorsen administration resulted in a 77% reduction in mean TGs at 3 months.[46] In patients with MCS, volanesorsen administration at a dose of 300 mg weekly for 26 weeks resulted in a mean 71.2% decrease in TGs at 3 months, which coincided with a numerical decrease in reported pancreatitis episodes during follow-up.[47]

In a phase 1/2a trial of Olezarsen, single doses of 60, 90, and 120 mg resulted in median reductions of 42%, 73%, and 77% in TGs and 73%, 81%, and 92% in apoC-III 2 weeks after dosing, respectively. In multiple-dose cohorts of 15 and 30 mg weekly, and 60 mg every 4 weeks, reductions of 59%, 73%, and 66% in TGs and 65%, 84%, and 80% in apoC-III were seen, respectively.[48] ApoB was reduced by up to 26%. Two phase 3 trials, CORE (in patients with MCS) (A Study of Olezarsen Administered to Patients with Severe Hypertriglyceridemia; NCT05079919) and BALANCE (in patients with FCS) (A Study of Olezarsen Administered to Patients with Familial Chylomicronemia Syndrome; NCT04568434) are currently ongoing.

ARO-APOC3 is a siRNA targeted at hepatic *APOC3* mRNA. Results from a phase 1/2a trial demonstrated that 4 patients with FCS and 26 patients with MCS who received ARO-APOC3 showed similar maximum median reductions in TG of 91% and 90% and in apoC-III of 98% and 96%, respectively.[49] The phase 2b trial interim results for patients with severe HTG (fasting TGs >500 mg/dL) were recently presented.[50] At week 16, ARO-APOC3 resulted in an up to 87% decrease in apoC-III, 86% decrease in TGs, 45% decrease in non-HDL-C, 77% decrease in RC, and a 99% increase in HDL-C. The phase 2b trial is estimated to be completed in October 2023 (NCT04720534).

TARGET: ANGPTL3. Pathophysiology and mechanism of action. ANGPTL3 is a key regulator of lipoprotein metabolism, and when complexed with ANGPTL8, ANPGPTL3 has an enhanced ability to repress LPL and endothelial lipase (EL) activity (Figure 2), thereby increasing the level of TRLs and decreasing the efficiency of clearance.[51,52]

Genetic evidence for ANGPTL3 inhibition. Whole exome sequencing and genome-wide association studies confirmed LOFVs in *ANGPTL3* lead to a significant reduction in TGs and risk for CAD.[53-55] In 1 study of 58,335 participants, participants with LOFVs in *ANGPTL3* had 27% lower TGs than noncarriers, 9%

TABLE 4 Lipid-Lowering Efficacy of ANGPTL3 Inhibitors

Therapy	Trial Phase, Ref. #	Study Population and Study Design	Sample Size (n)	Dose	TG	Non-HDL-C	LDL-C	apoB
Evinacumab (mAb)	1[60]	HTG (TG ≥150 mg/dL and ≤450 mg/dL) single dose study	83	5 mg/kg IV	−80.3%	−30.6%	−18.2%	−24.6%
				10 mg/kg IV	−88.0%	−31.2%	−22.8%	−21.4%
				20 mg/kg IV	−83.9%	−35.4%	−14.8%	−26.8%
				75 mg SC	−20.8%	−6.9%	−12.4%	−3.4%
				150 mg SC	−40.8%	−12.9%	−15.9%	−8.3%
				250 mg SC	−55.5%	−23.7%	−20.6%	−13.6%
	1[61]	HTG (≥450 and <1,500 mg/dL) single dose	16	10 mg/kg IV	−81.8%	−32.0%	+54.4%	—
	[61]	Severe HTG (>1,000 mg/dL) multiple ascending dose	56	20 mg/kg IV	−0.9% to −93.2%	−45.8%	+74.4%	—
				250 mg SC	−37.8%	—	+79.6%	—
	2[75]	HeFH	252	15 mg/kg IV Q4W	−45.9%	−50.9%	−50.5%	−39.4%
				300 mg SC QW	−55.8%	−53.8%	−52.9%	−42.0%
				450 mg SC QW	−61.5%	−58.5%	−56.0%	−45.5%
	3[59]	HoFH	64	15 mg/kg IV QW	−50.4%	−51.7%	−49.0%	−36.9%
Vupanorsen (ASO)	2[64]	TG >150 mg/dL, T2DM, and hepatic steatosis	105	80 mg SC Q4W	−53%	−18%	−7%	—
	3[65]	Adults with non-HDL-C ≥100 mg/dL, TG 150-500 mg/dL, on statin therapy	286	80 mg SC Q4W	−44.0%	−22.4%	−10.0%	−15.1%
				120 mg SC Q4W	−41.3%	−24.1%	−11.4%	−11.5%
				160 mg SC Q4W	−45.9%	−26.6%	−14.5%	−12.6%
				60 mg SC Q2W	−43.8%	−22.0%	−7.9%	−10.6%
				80 mg SC Q2W	−50.5%	−27.7%	−16.0%	−12.5%
				120 mg SC Q2W	−50.7%	−24.7%	−7.9%	−6.0%
				160 mg SC Q2W	−56.8%	−26.5%	−9.0%	−8.5%
ARO-ANG3 (siRNA)	1/2a[66]	Healthy volunteers with TG >1.13 mmol/L and LDL-C >1.81 mmol/L, not on statins or other lipid-lowering medications	12	100 mg SC	−62%	−49%	−45%	−33%
				200 mg SC	−72%	−48%	−37%	−29%
				300 mg SC	−67%	−54%	−50%	−42%
	2[67]	Adults with mixed dyslipidemia, fasting TG ≥150 mg/dL and <500 mg/dL and either LDL-C ≥70 mg/dL or non-HDL-C ≥100 mg/dL on optimal statin therapy	152	50 mg SC	−53%	−37%	−23%	−13.2%
				100 mg SC	−56%	−45%	−22%	
				200 mg SC	−59%	−34%	−32%	−21.8%

HeFH = heterozygous familial hypercholesterolemia; HoFH = homozygous familial hypercholesterolemia; IV = intravenous; T2DM = type 2 diabetes mellitus; other abbreviations as in Tables 1 to 3.

lower LDL-C, and 4% lower HDL-C. Additionally, the presence of an LOFV resulted in a 41% lower risk of CAD.[54] In another study, the CAD burden was reduced in patients with LOFVs in *ANGPTL3* compared with patients without variants. Homozygous patients had no plaque, and carrier status was associated with a 34% odds reduction in odds of CAD.[55]

Effects of ANGPTL3 inhibition on lipoproteins. ANGPTL3 inhibition results in decreased LDL-C, HDL-C, and TGs through enhanced lipolysis of TRLs to LDL particles[56] (Table 4). The decreased inhibition of EL results in more extensive "remodeling" of the TRLs into smaller remnant particles, which are more efficiently cleared by the liver, independent of the LDL receptor.[57] In addition, increased EL activity may also decrease endogenous VLDL production.[58] Evinacumab is a fully human monoclonal antibody directed at ANGPTL3; in a phase 3 trial, an intravenous infusion of evinacumab at a dose of 15 mg/kg every 4 weeks resulted in a 41.4%, 55.0%, and 84.1% reduction in total apoB, TG, and apoC-III at 24 weeks, respectively.[59] Additionally, in patients with severe HTG, intravenous evinacumab resulted in significant TG reduction,[60,61] as long as there was sufficient LPL activity.[62] Additionally, evinacumab administration resulted in a 53.1% to 54.2% reduction in RC, respectively.[63]

Vupanorsen is an ASO targeting *ANGPTL3* mRNA. In a phase 2a trial in patients with HTG, diabetes mellitus, and hepatic steatosis, vupanorsen 80 mg every 4 weeks resulted in a 53% reduction in TGs. A 38% reduction was seen in RC, 18% in non-HDL-C, and 9% in apoB.[64] In the phase 2b TRANSLATE-TIMI 70 (Targeting ANGPTL3 With an Antisense Oligonucleotide in Adults With Dyslipidemia—Thrombolysis In Myocardial Infarction 70) trial, vupanorsen

TABLE 5 Liver Safety With ANGPTL3 Inhibition

Compound	Mechanism	Doses	Effect on ANGPTL3	Hepatic Enzymes	Hepatic Fat Fraction
Evinacumab[62]	mAb	15 mg/kg IV Q4 weeks	—	No change	Decrease up to 23%
Vupanorsen[65]	ASO	160 mg SC, 240 mg SC, 320 mg SC monthly	−80.4% to −95.2%	Dose-dependent increase in AST/ALT >3× ULN in up to 33.3% and 44.4%	Dose-dependent increase in hepatic fraction up to 76%
ARO-ANG3[67]	siRNA	50 mg SC, 100 mg SC, 200 mg SC every 3 mo	−15% to −71%	No change	Dose-dependent decrease in hepatic fat fraction up to 30%

ALT = alanine aminotransferase; AST = aspartate aminotransferase; mAb = monoclonal antibody; ULN = upper limit of normal; other abbreviations as in Tables 1 to 4.

administered at a maximum dose of 320 mg monthly resulted in a 26.5% reduction in non-HDL-C and a 56.8% reduction in TGs.[65] The effects on LDL-C and apoB were more modest, ranging from −7.9% to −16.0% and −6.0% to −15.1%, respectively. However, at higher total monthly doses (240 and 320 mg), injection site reactions and >3× elevations of transaminases were seen in 33.3% and 44.4% of the patients, respectively, which coincided with a dose-dependent increase in hepatic fat fraction up to 76%. These effects were not observed with lower doses of vupanorsen.[64] Given the modest reductions in TGs, LDL-C, and apoB, and the subpar safety results at higher doses, future development of this ASO was terminated.

ARO-ANG3 is an siRNA directed at hepatic *ANGPTL3* mRNA. In a phase 1 trial, a dose of 100, 200, and 300 mg resulted in a 62%, 72%, and 67% reduction in TGs, respectively. Additionally, the 200-mg dose resulted in a 50% decrease in LDL-C and 42% reduction in total apoB.[66] Interim results of the phase 2 trial of ARO-ANG3 in participants with mixed dyslipidemia, defined as having TG ≥150 mg/dL but <500 mg/dL and LDL-C ≥70 mg/dL after 4 weeks of stable optimal statin treatment, were recently presented.[67] ARO-ANG3 was found to reduce ANGPTL3 up to 71%, TGs up to 59%, apoB up to 21.8%, and LDL-C up to 32% at week 16. Interestingly, in this case, there was a dose-dependent decrease in liver fat fraction and no adverse events related to liver transaminases. The study is estimated to be completed in December 2024 (NCT04832971).

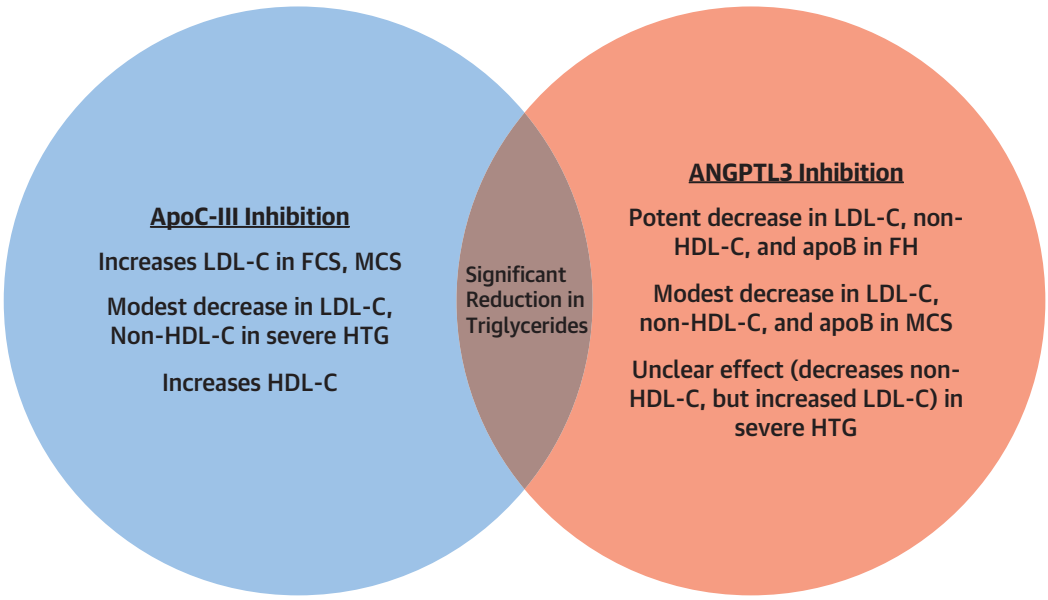
It remains unclear if ANGPTL3 inhibition results in significant hepatotoxicity (Table 5). Due to decreased hepatic VLDL secretion with ANGPTL3 inhibition, there is concern regarding increased hepatic fat caused by reduced VLDL unloading. Evinacumab, a monoclonal antibody that acts in the blood compartment, did not associate with increased liver transaminases in multiple clinical trials, and in a study of patients with severe HTG, there were no changes in the hepatic fat fraction. Vupanorsen did not demonstrate hepatotoxicity, except at higher doses of 240 and 320 mg monthly. ARO-ANG3 also did not demonstrate any hepatotoxicity and, in fact, reduced hepatic fat. The reason for these differences is unclear, but may be compound-specific toxicity or potentially a dose-dependent effect on ANGPTL3 inhibition. The higher doses of vupanorsen resulted in a −80.4% to 95.2% reduction in ANGPTL3, whereas the highest doses of ARO-ANG3 resulted in a 67% to 71% reduction in ANGPTL3. More safety data are needed to determine if maximal ANGPTL3 inhibition may have adverse hepatotoxic effects.

Despite the significant reduction in TGs with both apoC-III and ANGPTL3 inhibitors, it remains to be seen whether these reductions in TG can translate to reductions in MACE. Furthermore, whether clinical benefits also track with differential effects of these agents on LDL-C and apoB also remains unknown. In view of improvement in TG, non-HDL-C, and RC, combined with data from MR analyses, cardiovascular benefit is expected.

CHALLENGE 4: HOW DO WE DESIGN PHASE 3 CLINICAL TRIALS TO DEMONSTRATE EFFICACY OF TRL-LOWERING THERAPIES?

Previous trials focusing on TG lowering did not demonstrate a benefit with respect to MACE. New agents targeting apoC-III and ANGPTL3 result in TRL lowering, which is more likely associated with a reduction in ASCVD. When designing phase 3 trials testing the clinical efficacy of these drugs with respect to reducing MACE, enrollment criteria may differ for APOC3 inhibitors vs ANGPTL3 inhibitors (Figure 3). Through both LPL-dependent and -independent pathways, APOC3 inhibitors lead to potent reductions in TRLs and are particularly effective in FCS and MCS. However, APOC3 inhibitors have a modest effect on LDL-C and apoB in participants with TGs <800 mg/dL, whereas ANGPTL3 inhibition lowers TRLs, total apoB, and LDL-C through LPL- and EL-dependent pathways. In patients with genetically confirmed FCS, ANGPTL3 inhibition is ineffective in lowering TRLs,[62] which contrasts

FIGURE 3 Differential Effects of APOC3 Inhibition vs ANGPTL3 Inhibition



ApoC-III inhibition and ANGPTL3 inhibition both result in reduction in TGs, but apoC-III inhibition may be more useful in patients with severe hypertriglyceridemia (HTG), MCS, and FCS, whereas ANGPTL3 inhibition has a more potent effect on low-density lipoprotein cholesterol (LDL-C) and apoB reduction and may be more useful in residual ASCVD risk reduction. FH = familial hypercholesterolemia; HDL-C = high-density lipoprotein cholesterol; other abbreviations as in Figures 1 and 2.

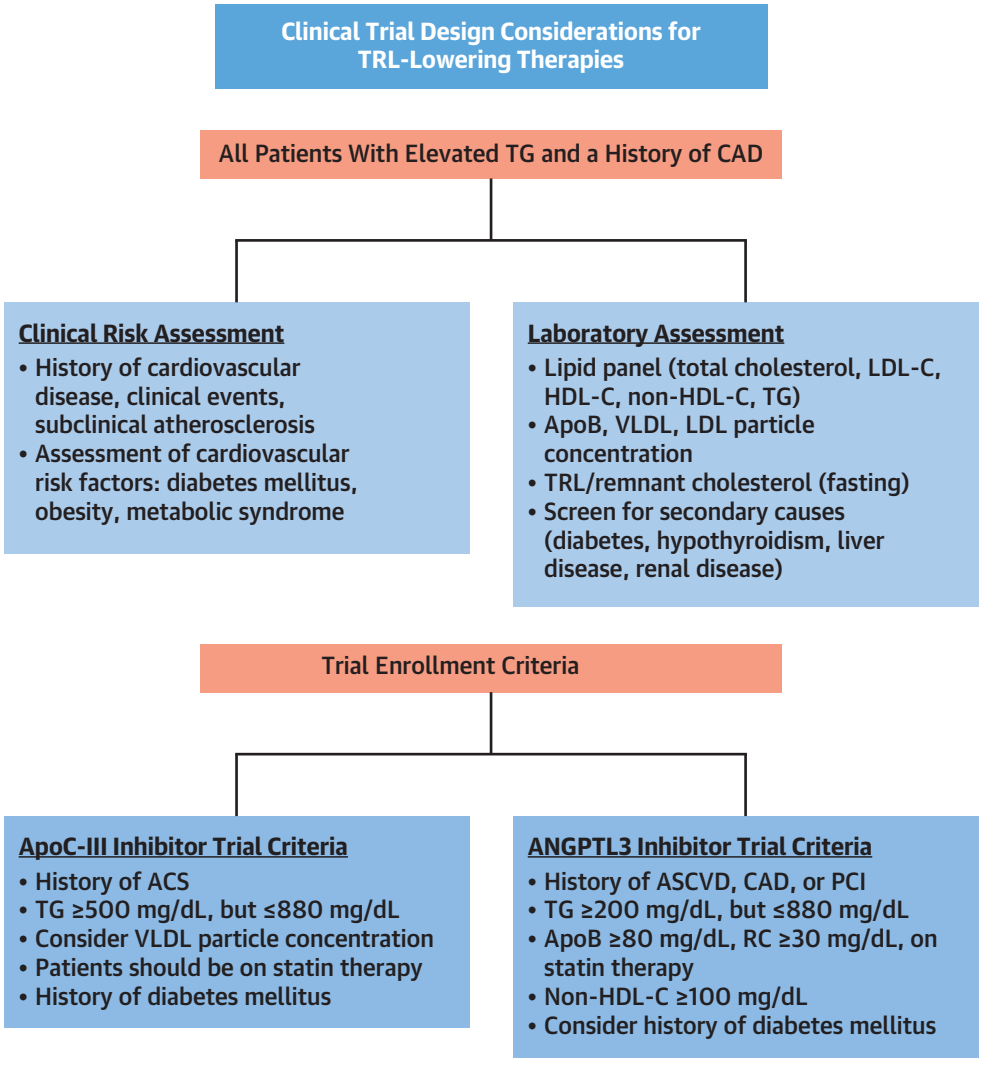
with APOC3 inhibition. The differences in TRL lowering in patients with FCS vs MCS or unspecified severe HTG should influence trial design (Central Illustration).

One major consideration in designing clinical trials for TRL-lowering therapies is utilizing apoB, non-HDL-C, and/or RC. Previous trials have focused on lowering TGs, and more recently, in patients with low HDL-C and high TGs.[12] However, these studies have failed to demonstrate a benefit in all-comers with high TGs, most likely because of a lack of clinically significant reduction in total apoB and/or RC. Using a cutoff value of apoB ≥80 to 85 mg/dL or non-HDL-C ≥100 to 120 mg/dL after maximal LDL-C-lowering therapy may be reasonable, as this would indicate excess residual risk. Additionally, VLDL particle concentration is more significantly associated with increased risk of incident ASCVD than LDL particles.[7] Based on a previous MR analysis, we expect a 10-mg/dL reduction in apoB and 70-mg/dL reduction in TGs to reduce MACE by 23%.[32] However, MR analyses are limited in that we are using lifetime genetic exposures to predict outcomes of short-term therapies.

APOC3 inhibitors. APOC3 inhibition chiefly attenuates TGs, with modest effects on apoB-containing lipoproteins. As apoC-III has been suggested to contribute to a proatherogenic, proinflammatory, and prothrombotic state,[68] initial trials may focus especially on patients with acute coronary syndrome (ACS). In the setting of ACS, amelioration of plaque inflammation, monocyte activation, and microvascular thrombosis through APOC3 inhibition might be expected to yield a greater cardiovascular benefit. These trials should enroll patients with adequate background statin therapy, ideally toward a target LDL-C ≤70 mg/dL.

Studies have shown that patients with type 1 and 2 diabetes both demonstrate higher circulating plasma apoC-III levels than those in body mass index-matched control patients without diabetes.[38] In patients with diabetes, apoC-III interferes with pancreatic beta-cell function, contributing to insulin resistance, islet cell inflammation, and derangement of glycemic control.[69] Conversely, administration of a glucagon like peptide 1 (GLP1) analog in these populations demonstrated reduction of apoC-III as their glycemic profiles improved, suggesting that glucose homeostasis serves as an important regulator of apoC-III secretion.[38] The recruitment of ACS patients with type 2 diabetes should be considered in APOC3 inhibitor trials, because these populations are likely

CENTRAL ILLUSTRATION **Clinical Trial Considerations for APOC3 Inhibitors and ANGPTL3 Inhibitors**



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Patients should be assessed for residual risk. Non–high-density lipoprotein cholesterol (HDL-C), remnant cholesterol, and apolipoprotein (apo) B should be assessed. Patients on statins with elevated apoB, triglycerides (TGs), and non–HDL-C should be considered for triglyceride-rich lipoprotein (TRL) trials. ACS = acute coronary syndrome; ASCVD = atherosclerotic cardiovascular disease; CAD = coronary artery disease; LDL = low-density lipoprotein; LDL-C = low-density lipoprotein cholesterol; PCI = percutaneous coronary intervention; VLDL = very low-density lipoprotein.

to have higher levels of circulating apoC-III and TRLs and stand to demonstrate greater ASCVD risk reduction.

ANGPTL3 inhibitors. In contrast to APOC3 inhibition that yields modest reduction in LDL-C and apoB levels, ANGPTL3 inhibition seems to reduce LDL-C, total apoB, and TGs more consistently. Therefore, a trial focused on ASCVD risk reduction using ANGPTL3 inhibition is more likely to utilize different enrollment criteria compared with APOC3 inhibitors (Central Illustration). We recommend enrolling patients with known ASCVD, including patients with stable CAD who underwent revascularization, with residual combined hyperlipidemia despite maximal LDL-C lowering therapy with statins and/or ezetimibe. TG cutoffs for recruitment in ANGPTL3 inhibitor trials may be less important such that patients with even mild HTG may benefit, especially in the setting of residual elevated apoB and LDL-C. We recommend utilizing thresholds of TG ≥200 mg/dL,

apoB ≥80 mg/dL, and non-HDL-C ≥100 mg/dL, consistent with a calculated RC ≥30 mg/dL. We expect that recruiting a population with a history of clinical ASCVD or stable CAD would likely confer a significant reduction in MACE.

NOVEL TRL-LOWERING THERAPIES ON THE HORIZON

Severe HTG is associated with multiple other metabolic diseases, particularly nonalcoholic fatty liver disease.[70] Fibroblast growth factor-21 (FGF21) is an endogenous hormone that regulates energy expenditure and glucose and lipid metabolism.[71] FGF21 improves insulin sensitivity; increases glucose uptake into adipocytes; and decreases TGs, LDL-C, and hepatic inflammation and fibrosis. Furthermore, FGF21 stimulates lipolysis and assists with weight loss. FGF21 analogues have been developed with glycoPEGylation technology, which prolongs the half-life and limits off-target FGF21 effects.[70] A phase 2 trial (NCT04541186) assessing the efficacy of BIO89-100 (pegozafermin), a FGF21 analogue, in patients with severe HTG revealed that doses of 9, 18, and 27 mg every week and 36 mg every 2 weeks resulted in a median reduction in TG of 57%, 56%, 63%, and 36%, respectively.[72] Additionally, the 27-mg weekly dosing resulted in a 29% reduction in non-HDL-C and 18% reduction in total apoB. Magnetic resonance imaging of the liver revealed significantly decreased perihepatic fat. FGF21 analogues offer an exciting alternative therapy for patients with significant cardiometabolic disease and residual risk for ASCVD.

CONCLUSIONS

Previous trials have failed to demonstrate a consistent reduction in ASCVD risk with reduction in TGs. As new therapies targeted toward TRL metabolism emerge, there is renewed hope that these therapies can lead to a reduction in residual ASCVD risk conferred by an elevation in TRLs. As phase 3 clinical trials are conducted, investigators need to be cognizant of the previous limitations in TG-lowering trials and apply lessons from those trials to design optimal trials targeted at a population that stands to clinically benefit from TRL reduction. For ASCVD risk reduction, therapies should target patients with TGs <880 mg/dL because of more consistent reductions in LDL-C and apoB.

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