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Volanesorsen to treat severe hypertriglyceridaemia: A pooled analysis of randomized controlled trials

Ilenia Calcaterra[1] 🟢 | Roberta Lupoli[2] | Alessandro Di Minno[3] |
Matteo Nicola Dario Di Minno[1]

[1]Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy

[2]Department of Molecular Medicine and Medical Biotechnology, Federico II University, Naples, Italy

[3]Department of Pharmacy, Federico II University, Naples, Italy

Correspondence
Ilenia Calcaterra, Department of Clinical Medicine and Surgery Federico II University Via S. Pansini 5, 80131 Naples, Italy.
Email: ileniacalcaterra@hotmail.it

Abstract

Background: Patients with severe hypertriglyceridaemia (sHTG) are often refractory to lipid-lowering therapy. Apolipoprotein (Apo) CIII inhibition could be promising to treat subjects with sHTG. The antisense oligonucleotide against APOC3 mRNA volanesorsen was recently introduced to treat sHTG. We performed a systematic review and meta-analysis of RCTs on the efficacy and safety of volanesorsen as compared to placebo treatment in patients with severe HTG.

Methods: Studies were systematically searched in the PubMed, Web of Science and Scopus databases according to PRISMA guidelines. The last search was performed on 7 February 2022.

Results: Four studies showed significant reduction in TG after 3 months of treatment with volanesorsen as compared with placebo (MD: -73.9%; 95%CI: -93.5%, -54.2; $p<.001$ $I^2=89.05\%$; $p<.001$); VLDL-C level (MD: -71.0%; 95%CI: -76.6%, -65.4%; $p<.001$ $I^2=94.1\%$; $p<.001$); Apo-B48 level (MD: -69.03%; 95%CI: $-98.59.4\%$, -39.47%; $p<.001$, $I^2=93.51\%$; $p<.001$) and Apo-CIII level (MD: -80.0%; 95%CI: -97.5%, -62.5; $p<.001$ $I^2=94.1\%$; $p<.001$) with an increase in HDL-C level (MD: $+45.92\%$, 95%CI: $+37.24\%$, $+54.60\%$; $p<.001$ $I^2=94.34\%$; $p<.001$) and in LDL-C level (MD: $+68.6\%$, 95%CI: $+7.0\%$, $+130.1\%$; $p<.001$ $I^2=96.18\%$; $p<.001$) without a significant elevation of Apo-B100 level (MD: $+4.58\%$, 95%CI: -5.64%, $+14.79\%$; $p=.380$ $I^2=95.09\%$; $p<.001$) in 139 volanesorsen patients as compared to 100 placebo-treated controls. Most of adverse events were mild and related to local injection site reactions.

Conclusions: In patients with severe HTG, volanesorsen is associated with a significant reduction in TG, VLDL-C, Apo-B48 and non-HDL-C and increment of HDL-C as compared to placebo. Documented efficacy is accompanied by an acceptable safety profile.

KEYWORDS
apolipoprotein CIII, familial chylomicronaemia syndrome, hereditary lipid disorder, severe hypertriglyceridaemia, volanesorsen

1 | INTRODUCTION

Hypertriglyceridaemia (HTG) is a subset of dyslipidaemia characterized by elevated plasma triglyceride (TG) levels,[1] due to hereditary disorders (familial chylomicronaemia syndrome, familial combined hyperlipidaemia and familial HTG) and several pathologic settings (diabetes, metabolic syndrome, insulin resistance, thyroid disorders).[2,3]

Plasma TG concentration is a biomarker of circulating TG-rich lipoproteins (TRL) [chylomicrons and very low-density lipoprotein (VLDL)] and their metabolic remnants.[4] TRL are known to have a pathogenic role in the development of acute pancreatitis (chylomicrons)[5,6] and of cardiovascular (CV) disease (VLDL and remnants).[7]

Apolipoprotein (Apo) CIII is a glycoprotein (consisting of 79 amino acids) mainly synthesized in the liver and, to a lesser extent, in the bowel. In normolipidemic subjects, Apo-CIII is mainly bound to HDL,[8] whereas in HTG subjects, the most part of Apo-CIII is bound to TRLs. Probably, Apo-CIII may regulate TG exchange between HDL and TRLs, with a mechanism that is still unknown.[9,10] In addition, Apo-CIII is an inhibitor of lipoprotein lipase (LPL) and is able to prevent TRL remnant uptake by hepatic lipoprotein receptors.[11] This demonstrates that Apo-CIII is involved in different steps of TG metabolisms.

Data from Mendelian randomization studies showed that loss-of-function mutations in the gene encoding Apo-CIII (*APOC3*) are associated with low triglyceride level and a decreased CV risk. On the contrary, overexpression of *APOC3* is associated with HTG.[12,13] Furthermore, data from epidemiological studies evidenced the association of Apo-CIII with CV risk, particularly in patients with diabetes.[10,14,15,16,17]

Although available lipid-lowing therapies (fibrates, statins and omega-3 fatty acids) decrease triglyceride level,[10,14] a not negligible part of HTG subjects does not achieve therapeutic goals, mostly in FCS subjects.[18] Then, targeting Apo-CIII could be promising to treat refractory HTG and to prevent acute pancreatitis (AP) in FCS subjects.

The antisense oligonucleotide (ASO) against APOC3 mRNA volanesorsen (previously called ISIS 304801, ISIS-ApoCIIIRx and IONIS-ApoCIIIRx) was recently introduced to treat sHTG.[19,20]

The aim of the present study is to perform a systematic review and meta-analyses of randomized controlled studies to assess the safety and efficacy of volanesorsen 300 mg in sHTG patients.

2 | METHODS

A protocol for this review was prospectively developed, detailing the specific objectives, the criteria for study selection, the approach to assess study quality, the outcomes and the statistical methods.

2.1 | Search strategy

To identify all available studies, a detailed search pertaining safety and efficacy of volanesorsen in patients with HTG was conducted according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines.[21] A systematic search was performed in the electronic databases (PubMed, Web of Science, Scopus, EMBASE), using the following search terms in all possible combinations: *volanesorsen, ISIS 304801, ISIS-ApoCIIIRx, IONIS-ApoCIIIRx, triglycerides, apolipoprotein CIII, very low-density lipoprotein, chylomicron, hypertriglyceridaemia, familial chylomicronaemia syndrome, antisense oligonucleotide, ASO, anti-APOC3 mRNA*. The last search was performed on 7 February 2022. The search strategy was developed without any language or publication year restriction.

In addition, the reference lists of all retrieved articles were manually reviewed. Two independent authors (IC and RL) analysed each article and performed the data extraction independently. In case of disagreement, a third investigator was consulted (MNDDM). Discrepancies were solved by consensus. Selection results showed a high inter-reader agreement ($\kappa = 1.000$) and have been reported according to PRISMA flow chart (Figure S1).

2.1.1 | Data extraction and quality assessment

According to the pre-specified protocol, all Phase 2–Phase 3 randomized controlled trial studies evaluating the efficacy or safety of volanesorsen in patients with HTG were included. Only studies including data on volanesorsen 300 mg were included, considering that other dosages included in some registrative trials will not be licensed for the use in clinical practice. Non-randomized controlled trials, case-reports, case-series, reviews and animal studies were excluded. We included in the analysis, all studies providing values (means with standard deviation or standard error) of TG or Apo-CIII or low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein cholesterol (HDL-C) or very-low-density lipoprotein cholesterol (VLDL-C) or apolipoprotein B (Apo-B) or apolipoprotein B48 (Apo-B48) or non-HDL-C or rate of side effects (any adverse events, serious adverse events probably related to drug administration, injection site reaction, muscle-related adverse events, reduction

in platelet count) in patients receiving volanesorsen or placebo treatment. In each study, data regarding sample size, major clinical and demographic variables, values of changes in TG, Apo-CIII, TC, LDL-C, HDL-C, VLDL-C, non-HDL-C, TG, Apo-B100 and Apo-B48 and side effects were extracted.

As primary efficacy outcome, we evaluated percent mean change in TG at 3 months and the incidence of AP in subjects receiving volanesorsen and in the placebo-treated control group. As secondary efficacy outcomes, we evaluated changes in Apo-CIII, TC, LDL-C, HDL-C, VLDL-C, non-HDL-C, TG, Apo-B and Apo-B48 at 3 months (12 weeks) in subjects receiving volanesorsen and in the control group.

As safety outcomes, we evaluated the incidence of any adverse event, severe adverse events potentially related to drug administration, injection site reaction, muscle-related side effects and reduction in platelet count, in subjects receiving volanesorsen and in the control treatment group.

Given the characteristics of the included studies, the evaluation of methodological quality of each study was performed with the Cochrane risk of bias assessment tool[21] and results are reported in Figure S2.

2.1.2 | Statistical analysis and risk of bias assessment

Statistical analysis was carried out using Comprehensive Meta-analysis (Version 3, Biostat, Englewood NJ). Changes in TG, Apo-CIII, TC, LDL-C, HDL-C, VLDL-C, non-HDL-C, TG, Apo-B and Apo-B48 have been expressed as % change from baseline values in patients with volanesorsen as compared to control treatment group and differences amongst cases and controls were expressed as mean difference (MD) with pertinent 95% confidence intervals (95%CI). The impact of volanesorsen treatment on the incidence of AP was expressed as risk ratio (RR) with pertinent 95%CI.

The overall effect was tested using Z scores and significance was set at $p < .05$. Statistical heterogeneity between studies was assessed with chi-square Cochran's Q test and with I^2 statistic, which measures the inconsistency across study results and describes the proportion of total variation in study estimates that is due to heterogeneity rather than sampling error. In detail, I^2 values of 0% indicate no heterogeneity, 25% low, 25%–50% moderate and 50% high heterogeneity.[23]

Publication bias was assessed by Egger's test and represented graphically by funnel plots of the standard difference in means versus the standard error. Visual inspection of funnel plot asymmetry was performed to address for possible small-study effect, as well as Egger's test to address publication bias, over and above any subjective evaluation. $p < .10$ was considered statistically significant.[24] To be as conservative as possible, the random-effect method was used to consider the heterogeneity amongst included studies.

2.1.3 | Meta-regression analyses

We hypothesized that differences amongst included studies may be affected by demographic variables (mean age, male gender) and clinical data (body mass index (BMI), % in Apo-CIII level reduction, % of LDL-C level changes, % of HDL-C level changes, statin use and % of mutation in *LPL gene*. To assess the possible effect of such variables in explaining different results observed across studies, we planned to perform meta-regression analyses after implementing regression models with changes in TG as dependent variable (y) and the above-mentioned covariates as independent variables (x).

3 | RESULTS

After excluding duplicate results, the search retrieved 49 articles. Of these studies, 41 were excluded because they were off the topic after scanning the title and/or the abstract, because they were reviews/comments/case-reports, or they lacked data of interest. Four were excluded after full-length paper evaluation because of reporting on overlap population ($n = 3$), including healthy volunteers in the frame of a phase 1 RCT ($n = 1$) (Figure S1).

Overall, four studies[3,25,26,27] enrolling 139 patients receiving volanesorsen and 100 placebo-treated controls were included in the final analysis. One study included patients with clinical diagnosis of FCS, based on phenotype and LPL activity level, with history of recurrent pancreatitis receiving stable doses of maximally tolerated lipid-lowering therapies (fibrates, polyunsaturated fatty acids, statins).[25]

A total of three studies included patients with HTG,[3,26,27] with one of them specifically including subjects with type 2 diabetes,[26] and one also including seven FCS subjects.[27]

Three studies used volanesorsen on top of maximal standard therapy. One study included one arm with monotherapy (volanesorsen alone) and one arm with volanesorsen as on top therapy.

Fasting TG level required for inclusion was ≥750 mg/dl for Witztum 2019 et al, from 350 mg/dl to 2000 mg/dl for Gaudet 2015 et al, from 200 mg/dl to 500 mg/dl for Digenio 2016 et al. and >500 mg/dl for Gouni-Berthold et al.

TABLE 1 Characteristics of studies included

Study	Trial phase	Population	N of patients	Background lipid-lowering therapy	Baseline TG cut-off	Age	Male gender	BMI
Witztum 2019 APPROACH NCT02211209	3	FCS	33 VLN 33 PBO	Add on to LLT	≥750 mg/dl	46 (20–75)	48.5%	25.0
Digenio 2016 NCT01647308	2	HTG and type 2 diabetes	10 VLN 5 PBO	Add on to LLT	>200 mg/dl <500 mg/dl	56.5 (7.5)	20%	33.1
Gaudet a 2015 NCT01529424	2	Severe HTG	11 VLN 16 PBO	No background LLT	>350 mg/dl <2000 mg/dl	52.8±10.4 48.6±11.9	72.7%	31.1
Gaudet b 2015 NCT01529424	2	Severe HTG	10 VLN 8 PBO	Add on to fibrate	>350 mg/dl <2000 mg/dl	54.0±13.8 58.9±16.6	70%	32.9
Gouni-Berthold 2021 COMPASS NCT02300233	3	Severe HTG (including FCS)	75 VLN 38 PBO	Add on to LLT	>500 mg/dl	50 (26–73) 53 (22–71)	76%	31.2

Abbreviations: BMI, body mass index; FCS, familial chylomicronaemia syndrome; HTG, hypertriglyceridaemia; LLT, lipid-lowering therapy; PBO, placebo; VLN, volanesorsen.

All the four studies were randomized controlled trials and major characteristics of the study populations are shown in Table 1.

3.1 | Efficacy and safety outcomes

The four studies included in the analysis[3,25,26] showed a more significant reduction in TG after 3 months of treatment with volanesorsen as compared with placebo (MD: −73.9%; 95%CI: −93.5%, −54.2; $p < .001$ $I^2 = 89.05\%$; $p < .001$, Figure 1).

TG reduction was accompanied by a significant reduction in VLDL-C level (MD: −71.0%; 95%CI: −76.6%, −65.4; $p < .001$ $I^2 = 94.1\%$; $p < .001$,); in Apo-B48 level (MD: −69.03%; 95%CI: −98.59.4%, −39.47%; $p < .001$, $I^2 = 93.51\%$; $p < .001$, Figure 1) and Apo-CIII level (MD: −80.0%; 95%CI: −97.5%, −62.5; $p < .001$ $I^2 = 94.1\%$; $p < .001$).

In parallel, we observed an increase in HDL-C level (MD: +45.92%, 95%CI: +37.24%, +54.60%; $p < .001$ $I^2 = 94.34\%$; $p < .001$) and in LDL-C level (MD: +68.6%, 95%CI: +7.0%, +130.1%; $p < .001$ $I^2 = 96.18\%$; $p < .001$) without a significant elevation of Apo-B100 level (MD: +4.58%, 95%CI: −5.64%, +14.79%; $p = .380$ $I^2 = 95.09\%$; $p = .380$) and in volanesorsen patients as compared to placebo group.
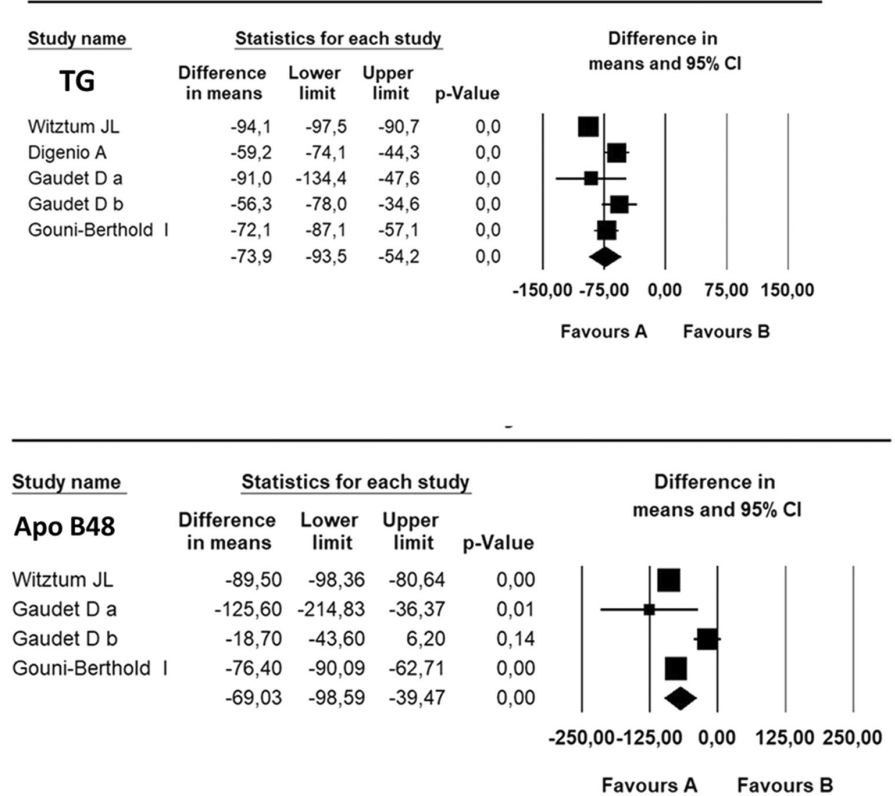
Two studies reported data on the incidence of AP.[25,27] No difference was found in the history of AP events in the volanesorsen group ($n = 108$) of treatment versus placebo ($n = 71$) (RR: 0.873; 95%CI: 0.680, 1.120; Z-value −1.068; $p = .286$). We found a significant reduction in AP incidence during study observation (RR: 0.102; 95%CI: 0.013, 0.814; Z-value −2.154; $p = .031$) in the volanesorsen-treated group versus placebo.

Meta-regression models showed a direct association between % reduction in Apo-CIII level and TG % reduction (Z-value 2.93; $p = .003$) (Figure 2). Higher age was associated with a less significant reduction in TG (Z-value 5.97; $p < .001$). A higher % TG reduction was associated with LDL % augmentation (Z-value 5.88; $p < .001$) No impact of male gender, LPL mutation status, % change of HDL and statin use was observed on % reduction of TG (Figure 3, Table 2).

Visual inspection of funnel plots suggested the absence of publication bias and of small-study effect for all efficacy outcomes considered (Figure S3), confirmed by Egger's test (p always >.10).

In the four studies included,[3,25,26,27] the most frequent adverse events (AEs) were local injection site reactions, which were mostly mild. Overall, injection site reactions occurred in 14% (95%CI: 12.8, 15.9) of all drug administrations.

FIGURE 1 Changes in triglycerides (TG) and apolipoprotein B48 (Apo-B48) after 3 months of treatment with volanesorsen as compared to the control treatment group. TG, triglycerides; Apo B48, apolipoprotein B48

Study name	Difference in means	Lower limit	Upper limit	p-Value
TG				
Witztum JL	-94,1	-97,5	-90,7	0,0
Digenio A	-59,2	-74,1	-44,3	0,0
Gaudet D a	-91,0	-134,4	-47,6	0,0
Gaudet D b	-56,3	-78,0	-34,6	0,0
Gouni-Berthold I	-72,1	-87,1	-57,1	0,0
	-73,9	-93,5	-54,2	0,0

Study name	Difference in means	Lower limit	Upper limit	p-Value
Apo B48				
Witztum JL	-89,50	-98,36	-80,64	0,00
Gaudet D a	-125,60	-214,83	-36,37	0,01
Gaudet D b	-18,70	-43,60	6,20	0,14
Gouni-Berthold I	-76,40	-90,09	-62,71	0,00
	-69,03	-98,59	-39,47	0,00

FIGURE 2 Meta-regression analyses. Correlation between Apo-CIII mean % reduction and TG mean % reduction. TG, triglycerides; Apo-CIII, apolipoprotein CIII
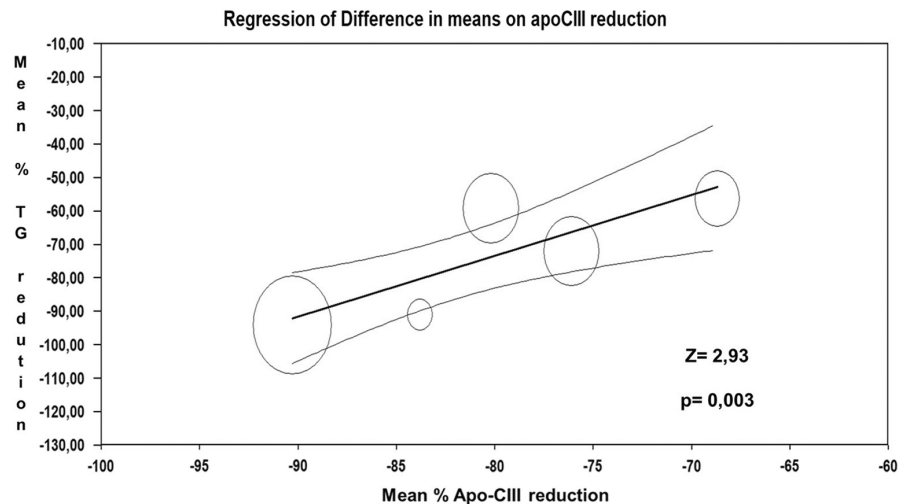
Moreover, treatment with volanesorsen was associated with a higher rate of nausea (OR: 6.04; 95%CI:1.23–29.7) and platelet count decreased (OR: 12.7; 95%CI 1.56, 103.9) as compared to placebo treatment. No difference was found in the incidence of the other AEs between volanesorsen-treated subjects and controls (Table S1).

4 | DISCUSSION

In the present meta-analysis on Phase 2 and Phase 3 RCTs, we evaluated the efficacy and safety of volanesorsen in patients with HTG. The only previous meta-analysis available on this topic included two Phase 2 and one Phase 3 studies on volanesorsen given at heterogeneous dosages, often other than 300 mg.[28] Comparing different dose could lead to inconsistent results because in a study designed for dose finding, some of the included dosage could be ineffective. Moreover, the authors did not perform meta-regression analyses to assess the possible effect of such variables in explaining different results observed across studies. Overall, consistently with our results, they found a significant reduction in TG, ApoCIII, VLDL-C, ApoB48 and an augmentation in HDL-C. They also found a trend in LDL-C augmentation ($p = .057$).
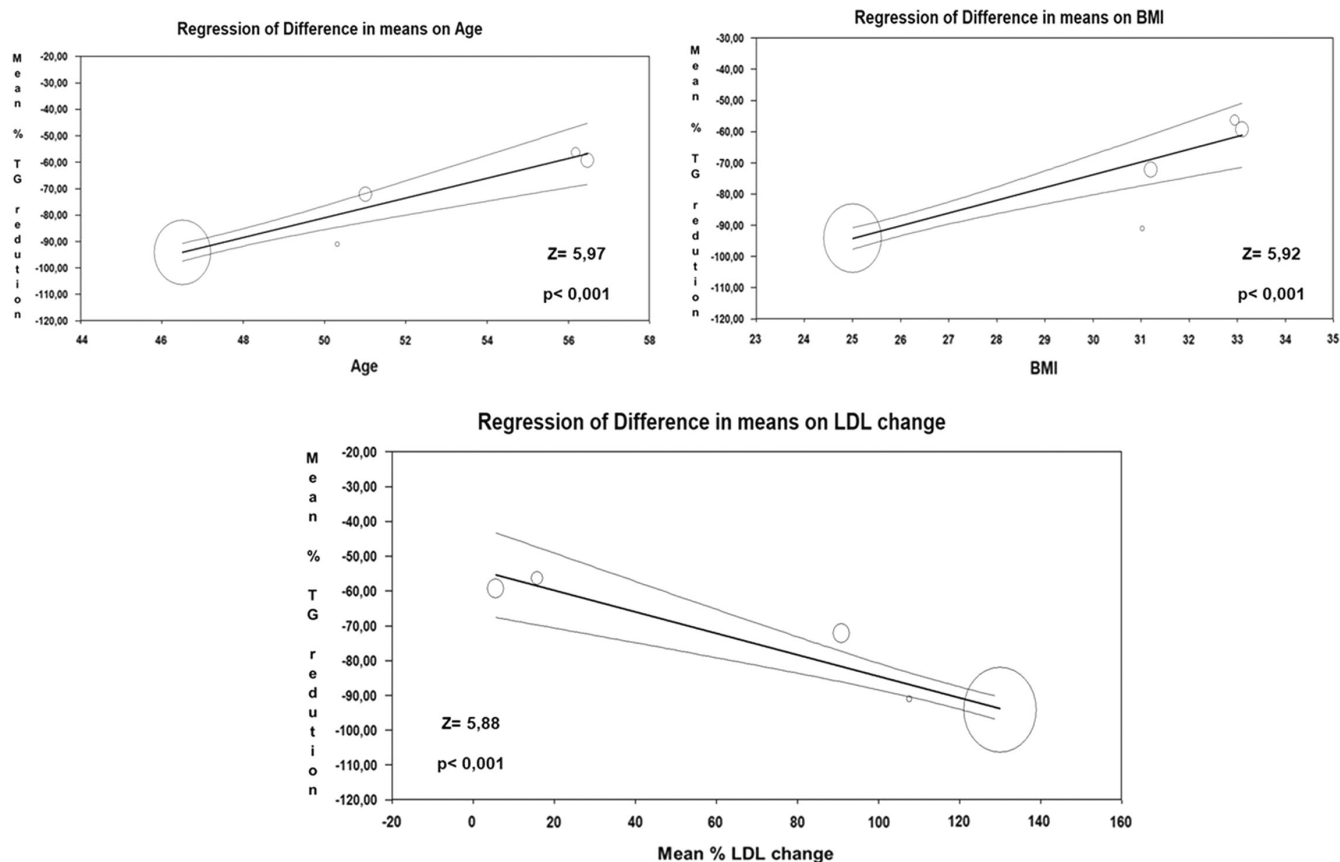
FIGURE 3 Meta-regression analyses. Impact of age and body mass index on difference in triglycerides (TG) between patients receiving volanesorsen and placebo-treated controls. TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; Apo-CIII, apolipoprotein CIII; *LPL*, lipoprotein lipase gene; BMI, body mass index

TABLE 2 Meta-regression analyses

		Age	Male	BMI	Statin use	*LPL* mutation status	Apo-CIII % changes	LDL-C % changes	HDL-C % changes
TG	*Z* value	5.97	−0.30	5.92	1.01	−1.66	2.93	5.88	1.05
	p value	**<.001**	.768	**<.001**	.313	.098	**.003**	**<.001**	.295

Notes: Impact of age, male gender, body mass index (BMI), statin use, LPL gene mutation status, % changes in apolipoprotein CIII (Apo-CIII), % changes in low-density lipoprotein cholesterol (LDL-C) and % changes in high-density lipoprotein cholesterol (HDL-C) on the difference in % changes of TG between patients receiving volanesorsen acid and control treatment group.

Abbreviations: Apo-CIII, apolipoprotein CIII; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *LPL*, lipoprotein lipase gene; TG, triglycerides.

Bold Values are to highlight the statistical significant *p* values.

Data from four RCTs included in our analysis showed a ≈74% mean reduction in TG in subjects receiving volanesorsen as compared to placebo-treated controls, accompanied by a significant reduction of ≈71% in VLDL-C. In parallel, we observed a ≈46% increase in HDL-C level and a reduction of ≈69% in Apo-B48 level.

Interestingly, the results of our analysis showed a marked reduction in TG associated with a documented inhibition of Apo-CIII, in a sHTG population, also including FCS subjects, typically refractory to classical lipid-lowering therapy. Indeed, we showed

that reduction in TG was directly associated with Apo-CIII inhibition, thus leading to the promotion of LPL-dependent TG clearance pathway. Although Apo-CIII is known as a direct inhibitor of LPL, the proven efficacy of volanesorsen also in patients with deep LPL impairment suggests the involvement of other LPL-independent pathways.[20]

Further confirming the role of Apo-CIII inhibition with volanesorsen in improving metabolism of TRLs, we found a significant reduction in VLDL-C and Apo-B48 levels. In particular, Apo-B48 indirectly reflects level of

plasma chylomicrons. In FCS subject reduction of plasma chylomicrons level represents an important achievement that may lead to reduction in acute pancreatitis episodes. To support this hypothesis.

We evaluated the incidence of AP, and we found a ≈ 10-fold reduction in risk of AP incidence in patients treated with volanesorsen compared with placebo. Interestingly, there was no difference in history of AP between the volanesorsen-treated group and control group. In the two studies reporting this outcome, no episodes of AP were found in volanesorsen-treated patients. Decrease in pancreatitis incidence and related pancreatic pain leads to a reduction of disease burden in FCS subjects. This is documented by ReFOCUS study demonstrating that, in 22 FCS subjects, treatment with volanesorsen had a positive impact on patients' quality of life and improvement in patients' activities of daily living across all domains assessed.[29] To the best of our knowledge, a paediatric investigation plane of volanesorsen is running out and some studies are planned for child and adolescent with FCS open-label trial to evaluate pharmacokinetics (PK), pharmacodynamics (PD) and safety of volanesorsen in children from 2 years to less than 18 years of age with FCS. Volanesorsen could represent a promising therapeutic option also for child with severe pancreatitis to prevent acute pancreatitis and chronic pain reducing the development of chronic consequences.

Furthermore, we observed that reduction in TG and related lipoproteins and apo, is accompanied to a concomitant increase in HDL level. This finding also supports the efficacy of Apo-CIII inhibition in TG metabolism improvement and it can be interpreted as an epiphenomenon of a metabolic switch characterized by a reduction of TRLs level.[30]

A further important aspect to consider is the potential effect of Apo-CIII inhibition on CV risk reduction. Indeed, Apo-CIII is involved in multiple steps of atherogenic process, regulating monocyte adhesion to endothelial cells (EC) and smooth muscle cell proliferation, enhancing oxidative stress, interacting with LDL and influencing the relative degrees of atherogenicity of these particles.[31] Supporting this hypothesis, a recent in vitro and in vivo study showed that statin therapy-induced Apo-CIII level reduction can decrease vascular adhesiveness by down-regulation of VCAM-1 in human saphenous vein ECs or human coronary artery ECs. This study is an important demonstration of potential therapeutic Apo-CIII-induced suppression of vascular inflammation.[32]

Moreover, reduction of TRLs remnants is another aspect to consider. TRLs remnants are known to be able to pass arterial wall and to be uptaken by macrophages, generating foam cells and, thus, triggering the atherogenic process.[31,33,34,35] According to this, we found a significant reduction in Apo-B48 that also reflects the level of TRL remnants. This is an important finding, especially in patients with sHTG associated with diabetes and metabolic syndrome.

On the other hand, we have found a modest increment in LDL-C but not in Apo-B100. These results could be considered as an epiphenomenon of activation of LPL-independent pathway of TG metabolism in FCS subjects. From a pathophysiological point of view, in subjects with severe impairment of LPL function, removal of TRLs occurs through an LPL-independent pathway mainly due to uptake of TRLs by the liver.[20,35] Thus, Apo-CIII inhibition could enhance the liver's ability to clearance TRLs from the plasma improving attachment of Apo-B and Apo-E to hepatic receptors, thus reducing TG also in absence of LPL activity. This might lead to an increase in the circulation of LDL particles.[30]

Another important aspect to consider is that LDL particle size was not evaluated in the included studies. Evaluating LDL size could be useful to potentially distinguish between large LDL of de novo synthesis and small dense LDL that have a greater atherogenic effect.[37-39]

However, the complete underlying mechanism is still largely unclear and further studies are needed to definitely address this issue.

As to safety data, we found from the available data that injection site reactions were the most frequently reported adverse event. In two of the included studies,[25,27] cases of decreased platelet count are reported. The underlying mechanisms of this side effect are still unknown and further studies are needed to address this issue. However, available data suggest that volanesorsen is quite well tolerated and cases of thrombocytopenia were found to be dose related and reversible. Based on this, volanesorsen received approval by the food drug administration (FDA) for FCS subjects' treatment.[40]

Some potential limitations of our study need to be discussed. Studies included in our meta-analysis have different inclusion and exclusion criteria and subjects enrolled in the studies included in our meta-analysis have different disease settings.

Small number of included studies and the respective exiguous sample could represent a limitation of the present meta-analysis. However, FCS is a rare disease with a prevalence of ≈ 1:1,000,000 and sHTG refractory to standard therapy is not a common condition.[2]

However, since meta-analysis is performed on aggregate data and some missing information is present in each study, the meta-regression approach allowed for the adjustment for some, but not all, potential confounders. Thus, ad hoc designed studies are needed to confirm in real-word settings safety and efficacy of volanesorsen.

In conclusion, whilst waiting for results of the ongoing trial evaluating the impact of volanesorsen treatment on HTG clinical outcomes and complications, (APPROACH open-label extension (NCT02658175), Volanesorsen Early Access Program for Patients With FCS (NCT03544060) BROADEN study (NCT02527343) conducted in patient with familial partial lipodystrophy, results of the present meta-analysis of RCTs suggest that volanesorsen is an effective lipid-lowering agent for hypertriglyceridaemic subjects management and a good treatment alternative both for patients with FCS and for those with severe hypertriglyceridaemia due to non-monogenic causes.

AUTHORS CONTRIBUTIONS

I.C and R.L. conceived and designed the study, performed statistical analysis, interpreted results and drafted the manuscript. A.D.M. and M.D.M. supervised the project, drafted the manuscript and performed critical revision. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

All authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

ORCID

Ilenia Calcaterra https://orcid.org/0000-0001-8077-2809

REFERENCES

1. Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet*. 2009;10:109-121. doi:10.1038/nrg2481
2. Moulin P, Dufour R, Averna M, et al. Identification and diagnosis of patients with familial chylomicronaemia syndrome (FCS): expert panel recommendations and proposal of an "FCS score". *Atherosclerosis*. 2018;275:265-272. doi:10.1016/j.atherosclerosis.2018.06.814
3. Gaudet D, Alexander VJ, Baker BF, et al. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438-447. doi:10.1056/NEJMoa1400283
4. Hegele RA, Ginsberg HN, Chapman MJ, et al. The polygenic nature of hypertriglyceridaemia: implications for definition, diagnosis, and management. *Lancet Diabetes Endocrinol*. 2014;2:655-666. doi:10.1016/S2213-8587(13)70191-8
5. Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: role in pancreatitis. *J Clin Lipidol*. 2012;6:409-412. doi:10.1016/j.jacl.2012.06.005
6. Lindberg DA. Acute pancreatitis and hypertriglyceridemia. *Gastroenterol Nurs*. 2009;32:75-82; quiz 83-74. doi:10.1097/SGA.0b013e31819de3e0
7. Mason RP, Libby P, Bhatt DL. Emerging mechanisms of cardiovascular protection for the Omega-3 fatty acid Eicosapentaenoic acid. *Arterioscler Thromb Vasc Biol*. 2020;40:1135-1147. doi:10.1161/ATVBAHA.119.313286
8. Malmendier CL, Lontie JF, Grutman GA, Delcroix C. Metabolism of apolipoprotein C-III in normolipemic human subjects. *Atherosclerosis*. 1988;69:51-59. doi:10.1016/0021-9150(88)90288-2
9. Huff MW, Fidge NH, Nestel PJ, Billington T, Watson B. Metabolism of C-apolipoproteins: kinetics of C-II, C-III1 and C-III2, and VLDL-apolipoprotein B in normal and hyperlipoproteinemic subjects. *J Lipid Res*. 1981;22:1235-1246.
10. Ooi EM, Barrett PH, Chan DC, Watts GF. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci*. 2008;114:611-624. doi:10.1042/CS20070308
11. Chan DC, Chen MM, Ooi EM, Watts GF. An ABC of apolipoprotein C-III: a clinically useful new cardiovascular risk factor? *Int J Clin Pract*. 2008;62:799-809. doi:10.1111/j.1742-1241.2007.01678.x
12. Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjaerg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32-41. doi:10.1056/NEJMoa1308027
13. The TG; HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22-31. doi:10.1056/NEJMoa1307095
14. Gouni-Berthold I. The role of antisense oligonucleotide therapy against apolipoprotein-CIII in hypertriglyceridemia. *Atheroscler Suppl*. 2017;30:19-27. doi:10.1016/j.atherosclerosissup.2017.05.003
15. Wyler von Ballmoos MC, Haring B, Sacks FM. The risk of cardiovascular events with increased apolipoprotein CIII: a systematic review and meta-analysis. *J Clin Lipidol*. 2015;9:498-510. doi:10.1016/j.jacl.2015.05.002
16. Onat A, Hergenc G, Sansoy V, et al. Apolipoprotein C-III, a strong discriminant of coronary risk in men and a determinant of the metabolic syndrome in both genders. *Atherosclerosis*. 2003;168:81-89. doi:10.1016/s0021-9150(03)00025-x
17. Gerber Y, Goldbourt U, Segev S, Harats D. Indices related to apo CII and CIII serum concentrations and coronary heart disease: a case-control study. *Prev Med*. 2003;37:18-22. doi:10.1016/s0091-7435(03)00051-3
18. Warden BA, Duell PB. Volanesorsen for treatment of patients with familial chylomicronemia syndrome. *Drugs of Today*. 2018;54:721-735. doi:10.1358/dot.2018.54.12.2899384
19. Macchi C, Sirtori CR, Corsini A, Santos RD, Watts GF, Ruscica M. A new dawn for managing dyslipidemias: the era of rna-based therapies. *Pharmacol Res*. 2019;150:104413. doi:10.1016/j.phrs.2019.104413
20. Gaudet D, Brisson D, Tremblay K, et al. Targeting APOC3 in the familial chylomicronemia syndrome. *N Engl J Med*. 2014;371:2200-2206. doi:10.1056/NEJMoa1400284
21. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097. doi:10.1371/journal.pmed.1000097
22. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928. doi:10.1136/bmj.d5928
23. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557-560. doi:10.1136/bmj.327.7414.557

24. Sterne JA, Egger M, Smith GD. Systematic reviews in health care: investigating and dealing with publication and other biases in meta-analysis. *BMJ*. 2001;323:101-105. doi:10.1136/bmj.323.7304.101

25. Witztum JL, Gaudet D, Freedman SD, et al. Volanesorsen and triglyceride levels in familial chylomicronemia syndrome. *N Engl J Med*. 2019;381:531-542. doi:10.1056/NEJMoa1715944

26. Digenio A, Dunbar RL, Alexander VJ, et al. Antisense-mediated lowering of plasma apolipoprotein C-III by Volanesorsen improves dyslipidemia and insulin sensitivity in type 2 diabetes. *Diabetes Care*. 2016;39:1408-1415. doi:10.2337/dc16-0126

27. Gouni-Berthold I, Alexander VJ, Yang Q, et al. Efficacy and safety of volanesorsen in patients with multifactorial chylomicronaemia (COMPASS): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol*. 2021;9:264-275. doi:10.1016/S2213-8587(21)00046-2

28. Fogacci F, Norata GD, Toth PP, Arca M, Cicero AFG. Efficacy and safety of Volanesorsen (ISIS 304801): the evidence from phase 2 and 3 clinical trials. *Curr Atheroscler Rep*. 2020;22:18. doi:10.1007/s11883-020-00836-w

29. Arca M, Hsieh A, Soran H, Rosenblit P, O'Dea L, Stevenson M. The effect of volanesorsen treatment on the burden associated with familial chylomicronemia syndrome: the results of the ReFOCUS study. *Expert Rev Cardiovasc Ther*. 2018;16:537-546. doi:10.1080/14779072.2018.1487290

30. Frikke-Schmidt R. HDL cholesterol and apolipoprotein A-I concentrations and risk of atherosclerotic cardiovascular disease: human genetics to unravel causality. *Atherosclerosis*. 2020;299:53-55. doi:10.1016/j.atherosclerosis.2020.03.005

31. Rocha NA, East C, Zhang J, McCullough PA. ApoCIII as a cardiovascular risk factor and modulation by the novel lipid-lowering agent volanesorsen. *Curr Atheroscler Rep*. 2017;19:62. doi:10.1007/s11883-017-0697-3

32. Zheng C, Azcutia V, Aikawa E, et al. Statins suppress apolipoprotein CIII-induced vascular endothelial cell activation and monocyte adhesion. *Eur Heart J*. 2013;34:615-624. doi:10.1093/eurheartj/ehs271

33. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298:299-308. doi:10.1001/jama.298.3.299

34. Chait A, Ginsberg HN, Vaisar T, Heinecke JW, Goldberg IJ, Bornfeldt KE. Remnants of the triglyceride-rich lipoproteins, diabetes, and cardiovascular disease. *Diabetes*. 2020;69:508-516. doi:10.2337/dbi19-0007

35. Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. *Circulation*. 2004;109:1918-1925. doi:10.1161/01.CIR.0000125278.58527.F3

36. Aalto-Setala K, Fisher EA, Chen X, et al. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. *J Clin Invest*. 1992;90:1889-1900. doi:10.1172/JCI116066

37. Di Minno MND, Gentile M, Di Minno A, et al. Changes in carotid stiffness in patients with familial hypercholesterolemia treated with Evolocumab(R): a prospective cohort study. *Nutr Metab Cardiovasc Dis*. 2020;30:996-1004. doi:10.1016/j.numecd.2020.02.018

38. Anesi A, Di Minno A, Calcaterra I, et al. An untargeted lipidomic analysis reveals depletion of several phospholipid classes in patients with familial hypercholesterolemia on treatment with Evolocumab. *Biomedicine*. 2021;9:1941. doi:10.3390/biomedicines9121941

39. Di Minno A, Gentile M, Iannuzzo G, et al. Endothelial function improvement in patients with familial hypercholesterolemia receiving PCSK-9 inhibitors on top of maximally tolerated lipid lowering therapy. *Thromb Res*. 2020;194:229-236. doi:10.1016/j.thromres.2020.07.049

40. Paik J, Duggan S. Volanesorsen: first global approval. *Drugs*. 2019;79:1349-1354. doi:10.1007/s40265-019-01168-z

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.