Efficacy and Safety of Volanesorsen (ISIS 304801): the Evidence from Phase 2 and 3 Clinical Trials

Efficacy and safety of volanesorsen (ISIS 304801): a systematic review and meta-analysis of phase 2 and 3 clinical studies.

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ABSTRACT

AIM: To assess the efficacy and safety of volanesorsen (ISIS 304801) through a systematic review of the literature and a meta-analysis of the available phase 2 and phase 3 clinical studies.

METHODS: A systematic literature search in SCOPUS, PubMed-Medline, ISI Web of Science and Google Scholar databases was conducted up to August 13th, 2019, in order to identify clinical trials assessing the effect of volanesorsen on laboratory parameters and its safety profile. Effect sizes for changes in lipids were expressed as percentage mean differences (MD) and 95% confidence intervals (CI). For safety analysis, odd ratios (OR) and 95%CI were calculated using the Mantel-Haenszel method.

RESULTS: Data were pooled from 3 clinical studies comprising 11 arms, which included overall 156 subjects, with 95 in the active-treated arm and 61 in the placebo one.

Meta-analysis of data suggested that volanesorsen significantly affected plasma levels of triglycerides (TG) [MD= -67.90%, 95%CI: -85.32,-50.48, *P*< 0.001], high-density lipoprotein cholesterol (HDL-C) [MD= 40.06%, 95%CI: 32.79,47.34, *P*< 0.001], very-low-density lipoprotein cholesterol (VLDL-C) [MD= -72.90%, 95%CI: -82.73,-63.07, *P*< 0.001], apolipoprotein B (Apo B) [MD= 8%, 95%CI: 2.17,13.84, *P*= 0.007], Apo B-48 [MD= -64.63, 95%CI: -105.37,-23.88, *P*= 0.002], Apo C-III [MD= -74.83%, 95%CI: -85.93,-63.73, *P*< 0.001], and VLDL ApoCIII [MD= -83.69%, 95%CI: -94.08,-73.29, *P*< 0.001], without significantly impacting plasma total cholesterol (TC) [MD= -0.65%, 95%CI: -10.70,9.40, *P*= 0.900], non HDL-C [MD= -18.89%, 95%CI: -40.96,3.19, *P*= 0.094], and LDL-C [MD= 47.01%, 95%CI: -1.31,95.33, *P*= 0.057] levels.

Treatment with volanesorsen was positively associated with a higher risk of injection site-reaction (OR= 32.89, 95%CI: 7.97,135,74, *P*< 0.001) and with an increased risk of upper respiratory tract infections (OR= 10.58, 95%CI: 1.23,90.93, *P*< 0.05).

CONCLUSION: Volanesorsen has favourable effects on lipid profile. Further well-designed studies are needed to explore its longer-term safety.

KEY WORDS: Volanesorsen; Efficacy; Safety; Meta-analysis.

Background

Hypertriglyceridemia is a very common metabolic lipid disorder associated to increased cardiovascular disease risk.[1] It is usually multifactorial and easily controlled by therapeutic life-style, and common drugs like fibrate and full-dosed polyunsaturated fatty acids. [2] Severe forms of hypertriglyceridemia (serum triglycerides – TG≥10 mmol/L [>885 mg/dL]; prevalence in general population of ~1 in 600), are often associated with an increased risk of acute pancreatitis that is usually not adequately controlled by standard approaches.[3] Among them, the hyperchylomicronemia syndrome is characterized by very high serum TG levels during fasting conditions (usually >16.95 mmol/L [1500 mg/dL]), abdominal pain, acute pancreatitis, eruptive xanthomas, and/or lipemia retinalis. Clinical phenotype is usually caused by impaired plasma clearance of triglyceride-rich lipoproteins (TRLs) resulting from genetic defects in lipoprotein lipase enzyme (LPL) and/or some associated proteins (familial chylomicronemia syndrome, FCS),[4] familial partial lipodystrophy [5] or the presence of secondary forms of hypertriglyceridemia (i.e. uncontrolled diabetes, alcohol abuse, iatrogenic dyslipidaemias).[6] The most used TG-lowering drugs (mainly fenofibrate or elevated doses of polyunsaturated fatty acids) are usually well tolerated, but their efficacy is limited, especially when a reduction of more than 40% in plasma TGs is required such as in the genetic forms of hypertriglyceridemia including FCS.[7]

The first genetic therapy for LPL deficiency, alipogene tiparvovec (a nonreplicating adeno-associated viral vector that delivers copies of the LPL gene to muscle tissue), has been withdrawn from the market in 2017 because of the limited risk/benefit profile associated to invasiveness and elevated costs.[8] thus boosting the development of novel TG-lowering drugs. [9] Volanesorsen, is a second-generation antisense oligonucleotide that, by coupling to apolipoprotein C-III (ApoCIII) messenger RNA, inhibits APOCIII protein synthesis.[10] APOCIII plays a critical role in TRLs metabolism (TRLs) by inhibiting

lipoprotein lipase and hepatic lipase activity but also by controlling hepatic lipoprotein biosynthesis.[11]

Animal models lacking the APOC3 gene exhibit reduced plasma TG levels, whereas the overexpression of APOC3 leads to increased plasma TG levels. In humans, loss-of-function mutations in APOC3 are associated with reduced plasma TG levels and reduced risk for ischemic vascular disease and coronary heart disease [30,31]. Based on these observations, volanesorsen has been tested to control plasma TG levels and was approved in May 2019 (in the European Union) for the treatment of adult FCS patients based on the results from the first clinical trials.[12] As the available clinical data on volanesorsen have been sampled in a number of relatively small phase 2 and phase 3 randomized controlled trials (RCTs) enrolling patients affected by different form of hypertriglyceridemia (FCS, severe hypertriglyceridemia, and metabolic dyslipidemia with type 2 diabetes)[13], we aimed to perform a systematic review and meta-analysis on clinical evidence available to date to better define its efficacy and tolerability profile.

Methods

The study was designed according to guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement,[14] and was registered in the PROSPERO database (Registration number CRD42019121525). Due to the study design (meta-analysis), neither Institutional Review Board (IRB) approval, nor patient informed consent was required.

- Search Strategy

PubMed, SCOPUS, Google Scholar and ISI Web of Science by Clarivate databases were searched, with no language restriction, using the following search terms: ("Volanesorsen" or "ISIS 304801") AND ("Trial" OR "Study"). The wild-card term "*" was used to increase the sensitivity of the search strategy, which was limited to studies in humans. The reference list of identified papers was manually checked for additional relevant articles. In particular, additional searches for potential trials included the references of review articles on that issue, and the abstracts from selected congresses on the subject of the meta-analysis. Literature was searched from inception to August 13th, 2019.

All paper abstracts were screened by two reviewers (FF and DN) in an initial process to remove ineligible articles. The remaining articles were obtained in full-text and assessed again by the same two researchers who evaluated each article independently and carried out data extraction and quality assessment. Disagreements were resolved by discussion with a third party (AFGC).

- Study Selection Criteria

Original studies were included if they met the following criteria: (i) being a phase 2 or 3 clinical trial with either multicentre or single-centre design, (ii) investigating the effect of ISIS 304801 on plasma lipids, (iii) testing the safety of ISIS 304801 short and middle-term administration. (iv) reporting all the adverse events occurred during the treatment. Studies that lacked a properly controlled design for ISIS 304801 treatment were excluded.

- Data extraction

Data abstracted from the eligible studies were: i) study registration code; ii) first author's name; iii) year of publication; iv) study design; v) main inclusion criteria and underlying disease; vi) treatment duration; vii) study groups; viii) number of participants in the active and control group; ix) age and sex of study participants. All data extraction and database typing were reviewed by the principal investigator (AFGC) before the final analysis, and doubts were resolved by mutual agreement among the authors.

- Quality assessment

A systematic assessment of risk of bias in the included studies was performed using the Cochrane criteria.[15] The following items were used: adequacy of sequence generation, allocation concealment, blinding addressing of dropouts (incomplete outcome data), selective outcome reporting, and other probable sources of bias.[16] Risk-of-bias assessment was performed independently by 2 reviewers (FF and AFGC); disagreements were resolved by a consensus-based discussion.

- Data synthesis

Meta-analysis was entirely conducted using Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ).[17]

Net changes in the investigated parameters (change scores) were calculated by subtracting the value at baseline from the one after intervention, in the active-treated group and in the control one. All values were collated as percent change from baseline. Standard deviations (SDs) of the mean difference were obtained as following reported by Follman and colleagues: SD= square root $[(SD_{pre\text{-}treatment})^2 + (SD_{post\text{-}treatment})^2 – (2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient (R) = 0.5.[18] If the outcome measures were reported in median and interquartile range (or 95% confidence interval (CI)), mean and SD values were estimated using the method described by Wan *et al.*.[19] Where standard error of the mean (SEM) was only reported as dispersion measure, SD was

estimated using the following formula: SD= SEM x square root (n), being n the subjects' number. To avoid a double-counting problem, in trials comparing multiple treatment arms versus a single control group, the number of subjects in the control group was divided by the required comparisons. Studies' findings were combined using a fixed-effect model or a random-effect model (using the DerSimonian-Laird method) and the generic inverse variance method, based on the level of interstudy heterogeneity, which was quantitatively assessed using the Higgins index (I^2).[20] Effect sizes for lipids changes were expressed as percentage mean differences (MD) and 95%CI. For safety analysis, odd ratios (OR) and 95%CI intervals were calculated using the Mantel-Haenszel method.[21] Safety analysis was performed by excluding studies with zero events in both arms. If one or more outcomes could not be extracted from a study, the study was removed only from the analysis involving those outcomes. Adverse events were considered for the analysis only if occurring in at least two of the included clinical trials. Events declared as unlikely related to the intervention were excluded. Sensitivity analysis was conducted using the leave-one-out method (i.e. removing one study at a time and repeating the analysis) in order to evaluate the influence of each study on the overall effect size.[22] Two-sided *P*-values ≤0.05 were considered as statistically significant for all tests.

- Meta-regression analysis

As potential moderator for the treatment response, ISIS 304801 weekly administered dose was entered into a fixed- or random-effect unrestricted maximum likelihood meta-regression model to explore its association with the estimated effect sizes on lipids.

- Publication biases

Potential publication biases were explored using visual inspection of Begg's funnel plot asymmetry, Begg's rank correlation test, and Egger's weighted regression test.[23] The Duval & Tweedie "trim and fill" method was used to adjust the analysis for the effects of publication biases.[24] Two-sided *P* values ≤0.05 were considered statistically significant.

Results

- Flow and characteristics of the included studies

After database searches performed strictly according to inclusion and exclusion criteria, 42 published articles were identified, and the abstracts reviewed. Of these, 32 were excluded because they were non-original articles. All the other 10 studies met the inclusion criteria and were carefully assessed and reviewed. On the basis of the established eligibility criteria, seven additional studies were excluded because of incomplete data (n= 2) or substantial sample overlap (n= 5). Finally, three studies were eligible and were included in the meta-analysis.[25-27] The study selection process is shown in Figure S1. Data were pooled from three clinical trials comprising 11 treatment arms, which included 156 subjects, with 95 in the active-treated arm and 61 in the control one.

Eligible studies were published between 2015 and 2019. Follow-up periods ranged between 13 and 52 weeks and several treatment schedules were tested. All selected trials were designed with parallel groups and were multicentre [25] or single-centre [26,27] clinical studies. Enrolled subjects were patients affected by familial chylomicronemia syndrome,[25], with diabetes [26] or with moderately high levels of TG.[27] The baseline characteristics of patients included in the studies are summarized in Table 1.

- Risk of bias assessment

All the included studies were characterized by enough information regarding sequence generation, allocation concealment, personal and outcome assessments. Other domains, such as incomplete outcome data and selective outcome reporting, may have different risks of bias for different outcomes within each study. Details of the quality of bias assessment are reported in Table 2.

- Effect of volanesorsen on laboratory parameters

Meta-analysis of data suggested that volanesorsen significantly affected plasma levels of triglycerides (TG) [MD= -67.90%, 95%CI: -85.32,-50.48, $P< 0.001$; $I^2= 67.4\%$], high-density

lipoprotein cholesterol (HDL-C) [MD= 40.06%, 95%CI: 32.79,47.34, *P*< 0.001; I^2= 0%], very-low-density lipoprotein cholesterol (VLDL-C) [MD= -72.90%, 95%CI: -82.73,-63.07, *P*< 0.001; I^2= 0%] (Figure 1), apolipoprotein B (Apo B) [MD= 8%, 95%CI: 2.17,13.84, *P*= 0.007; I^2= 46.5%], Apo B-48 [MD= -64.63, 95%CI: -105.37,-23.88, *P*= 0.002; I^2= 84.7%], Apo C-III [MD= -74.83%, 95%CI: -85.93,-63.73, *P*< 0.001; I^2= 68.5%], and VLDL ApoCIII [MD= -83.69%, 95%CI: -94.08,-73.29, *P*< 0.001; I^2= 0%] (Figure 2), without significantly impacting total cholesterol (TC) [MD= -0.65%, 95%CI: -10.70,9.40, *P*= 0.900; I^2= 0%], non HDL-C [MD= -18.89%, 95%CI: -40.96,3.19, *P*= 0.094; I^2= 85.2%], and LDL-C [MD= 47.01%, 95%CI: -1.31,95.33, *P*= 0.057; I^2= 90%] plasma levels (Figure 3). The effect sizes were robust in the leave-one-out sensitivity analysis (Figure S2-S4) and not mainly driven by a single study. Furthermore, volanesorsen weekly dose did not have any relation to the observed effect sizes (*P*> 0.05 for all dose comparisons) exception for Apo C-III (Figure S5).

Visual inspection of Begg's funnel plots did not reveal any asymmetry, suggesting no publication bias for the effect of volanesorsen on the investigated parameters (Figure S6). However, Duval & Tweedie's "trim and fill" method yielded two potentially missing studies on the right-side of the plot which lowered the effect size on TC to 2.22 (95%CI: -6.61,11.06); one potentially missing study on the left-side of the plot which lowered the effect size on HDL-C to 39.61 (95%CI: 32.49,46.73); two potentially missing studies on the left-side of the plot increasing the effect size on non HDL-C to -25.05 (95%CI: -42.56,-7.54); three potentially missing study on the left-side of the funnel increasing the effect size on VLDL-C to -77.48 (95%CI: -86.55,-68.41); four potentially missing studies on the left-side of the plot increasing the effect size on Apo B to 13.78 (95%CI: 8.65,18.91); one potentially missing study on the right-side of the plot which lowered the effect size on Apo B-48 particle number to -71.98 (95%CI: -82.47,-61.49), and three potentially missing studies on the left-side of the plot increasing the effect size on Apo C-III to -90.35 (95%CI:

-81.59,-82.39). Egger's linear regression –though not Begg's rank correlation- confirmed the presence of publication biases for non HDL-C (t= 4.26, *P*= 0.008), VLDL-C (t= 4.05, *P*= 0.01), Apo B (t= 2.75, *P*= 0.04) and Apo C-III (t= 4, *P*= 0.01).

The classic fail-safe N test suggested that 350 studies with negative results would be needed to bring the estimated effect size on TG to a non-significant level (*P*> 0.05); 158 studies with negative results would be needed to bring the estimated effect size on HDL-C to a non-significant level (*P*> 0.05); 267 studies with negative results would be needed to bring the estimated effect size on VLDL-C to a non-significant level (*P*> 0.05); 137 studies with negative results would be needed to bring the estimated effect size on Apo B-48 to a non-significant level (*P*> 0.05); 1338 studies with negative results would be needed to bring the estimated effect size on Apo C-III to a non-significant level (*P*> 0.05); and 310 studies with negative results would be needed to bring the estimated effect size on VLDL Apo C-III to a non-significant level (*P*> 0.05).

- Safety analysis

Primary outcomes were any adverse event (AE) leading to treatment discontinuation, feeling of relaxation, headache, fatigue, asthenia, musculoskeletal pain and pain in extremity, myalgia, arthralgia, nasopharyngitis, chills, upper respiratory tract infections, abdominal pain, nausea, vomiting, diarrhea, diabetes mellitus, epistaxis, petechiae, variation in platelet count and thrombocytopenia. The average percentage of injections leading to at least one injection site-reaction was also considered as primary outcome.

Volanesorsen was positively associated with a higher risk of injection site-reaction (OR= 32.89, 95%CI: 7.97, 135.74, *P*< 0.001; I^2=0%) and increased risk of upper respiratory tract infections (OR= 10.58, 95%CI: 1.23, 90.93, *P*< 0.05; I^2=0%). These findings were robust in the leave-one-out sensitivity analyses (Figure 4, Figure 5). The incidence of the other adverse events did not differ between groups (Table S1), although platelet count

reduction/thrombocytopenia have been not clearly reported in all the available clinical trials.

Visually, the funnel plot of standard error by log odds ratio for the percentage risk of injection site-reaction was symmetric. The absence of publication bias for the analysis was confirmed by Begg's rank correlation ($P > 0.05$), though not by Egger's linear regression ($t = 49.03$, $P < 0.01$).

The classic fail-safe N test suggested that 21 studies with a negative result would be needed to bring the estimated percentage risk of injection site-reaction to a non-significant level ($P > 0.05$).

Discussion

Familial chylomicronemia syndrome is a rare autosomal recessive disorders characterized by massive refractory hypertriglyceridemia and recurrent pancreatitis, potentially leading to life-threatening multi-organ dysfunction.[28] Current therapies for FCS are poorly effective: diet, fibrates and full-dosed polyunsaturated fatty acids (2-4 gr/day) reduce plasma TGs by around 50%, that, however, is insufficient for most FCS patients.[29] Therefore, pharmacological research is focusing on the development of novel and more powerful TG lowering therapies. Inhibiting ApoC-III represents a novel approach to extensively reduce plasma TG levels and related clinical consequences by improving TRLs clearance. Studies in animal models highlighted the key role of APOCIII on triglyceride metabolism [11] and large Mendelian randomization studies associated loss-of-function mutations in apolipoprotein CIII (APOCIII) with lower plasma TG levels.[30,31] With ample in-vitro and in-vivo evidence for a role of apoCIII in lipoprotein lipase-mediated triglyceride clearance and remnant removal; apoCIII is an attractive target for the treatment of severe hypertriglyceridemia.[11,32]

By analyzing data from 3 randomized controlled clinical studies including a total of 156 subjects, this meta-analysis shows that volanesorsen significantly impacts serum levels of TG (-68%), HDL-C (+40%), VLDL-C (-73%), Apo B-48 (-65%), Apo C-III (-75%) and VLDL Apo C-III (-84%) and Apo B (+8%).

A meta-analysis carried out on 5 retrospective and 7 prospective studies with a total of 3163 cases of cardiovascular events showed that a 5 mg/dL increase in total plasma apoC-III translates into a pool risk estimate of 1.33 (1.07-1.66) for cardiovascular events while the same increase of apoCIII only in the non-HDL fraction translates into a pool risk estimate of 2.48 (1.48-4.32).[33] This highlights the potential clinical relevance of decreasing apoCIII plasma levels.

Recently, the ReFOCUS web-based survey showed that treatment with volanesorsen is able to improve the disease burden in patients affected by FCS, significantly reducing the incidence for pancreatic pain, steatorrhea, physical weakness, fatigue and back pain.[34] This finding is certainly of great interest, and it has expected to be confirmed by further controlled clinical trials.

Additional approaches for patients with severe hypertrygliceridemia with a mechanism of action different from that of APOC-III silencing, such as anti angiopoietin-like protein 3 (ANGPTL3) monoclonal antibodies (evinacumab) [35] or ANGPTL3 gene silencing [36] are under development. These drugs improve plasma TG profile by promoting LPL and hepatic lipase mediated clearance of lipoproteins, normally inhibited by ANGPTL3 and were shown to successfully control TG in hypertriglyceridemic patients.[35] Another promising drug for FCS treatment is pradigastat, a novel diacylglycerol acyltransferase 1 inhibitor which inhibits chylomicron synthesis thus reducing post-prandial lipemia and TG levels.[37]. These later drugs, in addition to plasma TG, also reduce plasma cholesterol and LDL-C levels; thus it will be interesting to compare the efficacy of these strategies on key clinical outcomes, including pancreatitis, as compared to volanesorsen.

Our meta-analysis has some limitations, which reflect some limits of the clinical trials reported to date. Firstly, the sample size is limited, even though it depends on the incidence of FCS, which is an autosomal recessive monogenic disease affecting one to two individuals in every million.[38] Furthermore, it was not possible to carry out a subgroup analysis to evaluate synergistic effect of volanesorsen as add on to other lipid-lowering drugs, since in most studies detailed information on the background treatment of the patients were lacking. The degree of heterogeneity for HDL-C, TG, LDL-C, Apo B-48 and Apo C-III values is another important limitation for the current analysis.

Further research should be focused on the investigation of volanesorsen safety profile, since some critical adverse events emerged in the first trials with volanesorsen, like

platelet count reduction/thrombocytopenia, have been not clearly reported in some of the available clinical trials.

In summary, the meta-analysis of available data indicates that volanesorsen has favourable effects on lipid profile while data are scarce to address the benefit on the incidence of pancreatitis in patients with severe hypertriglyceridemia. Large, well-designed prospective randomized clinical trials are needed to definitively validate volanesorsen treatment as a possible strategy for FCS and related co-morbidities, including pancreatitis, and also to explore its long-term safety.

Figure 1 – Forest plot displaying the percentage mean difference and 95% confidence intervals for the effect of volanesorsen on plasma levels of TG, HDL-C and VLDL-C.

Figure 2 - Forest plot displaying the percentage mean difference and 95% confidence intervals for the effect of volanesorsen on plasma levels of Apo B, Apo B-48, Apo C-III and VLDL Apo C-III.

Figure 3 - Forest plot displaying the percentage mean difference and 95% confidence intervals for the effect of volanesorsen on plasma levels of TC, non HDL-C and LDL-C.

Figure 4 - Forest plot for the risk of upper respiratory tract infections following treatment with volanesorsen. The effect size is expressed as odd ratio and 95% confidence interval,

Figure 5 - Forest plot for the percentage risk of at least one injection site-reaction following treatment with volanesorsen. The effect size is expressed as odd ratio and 95% confidence interval.

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