

# Design, Protocol, and Rationale of the Clinical Trial with a Novel Statin-Free Artisterol Gel Suspension in Subjects with Suboptimal Cholesterol Levels

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## Keywords

Artisterol, cardiovascular risk, dietary supplement, hypercholesterolemia, nutrition adjuvant

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## Abstract

### Introduction

Elevated low-density lipoprotein cholesterol (LDL-C) is a causal, modifiable risk factor for atherosclerotic cardiovascular disease (ASCVD). In individuals at low-to-moderate cardiovascular risk who do not meet criteria for lipid-lowering pharmacotherapy, or who are not willing to use statins or are intolerant, non-pharmacological options are clinically relevant; however, efficacy must be demonstrated for the finished formulation rather than extrapolated from its individual ingredients.

### Material and methods

CHOLAMAZEN (ClinicalTrials.gov NCT07405814) is an international, randomized, double-blind, placebo-controlled, parallel-group trial conducted in Italy and Poland. A total of 106 adults aged 18-70 years with previously untreated mild-to-moderate hypercholesterolemia will be randomized 1:1, stratified by sex, to receive Artisterol or matching placebo once daily for 12 weeks. Each 10-ml liquid stick pack of Artisterol provides 1,200 mg plant sterols, 500 mg artichoke (*Cynara scolymus*) leaf extract, and 5 mg thiamine (vitamin B1).

### Results

The primary endpoint is the absolute mean change in LDL-C from baseline to Week 12. Secondary endpoints include changes in total cholesterol, HDL-C, non-HDL-C, triglycerides, apolipoprotein B, fasting plasma glucose and hepatic transaminases, together with adverse-event monitoring and compliance assessment. Exploratory analyses will evaluate LDL-C and secondary outcomes across baseline, Week 6 and Week 12.

### Conclusions

This protocol is designed to determine whether a non-monacolin, statin-free liquid formulation containing plant sterols, artichoke leaf extract and thiamine lowers LDL-C versus placebo and is safe and well tolerated over 12 weeks. Reporting follows Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2025, and trial results will be reported according to the registered endpoints.

## Design, Protocol, and Rationale of the Clinical Trial with a Novel Statin-Free Artisterol Gel Suspension in Subjects with Suboptimal Cholesterol Levels

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**ABSTRACT:**

**Introduction:** Elevated low-density lipoprotein cholesterol (LDL-C) is a causal, modifiable risk factor for atherosclerotic cardiovascular disease (ASCVD). In individuals at low-to-moderate cardiovascular risk who do not meet criteria for lipid-lowering pharmacotherapy, or who are not willing to use statins or are intolerant, non-pharmacological options are clinically relevant; however, efficacy must be demonstrated for the finished formulation rather than extrapolated from its individual ingredients.

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**Results:** The primary endpoint is the absolute mean change in LDL-C from baseline to Week 12. Secondary endpoints include changes in total cholesterol, HDL-C, non-HDL-C, triglycerides, apolipoprotein B, fasting plasma glucose and hepatic transaminases, together with adverse-event monitoring and compliance assessment. Exploratory analyses will evaluate LDL-C and secondary outcomes across baseline, Week 6 and Week 12.

**Conclusions:** This protocol is designed to determine whether a non-monacolin, statin-free liquid formulation containing plant sterols, artichoke leaf extract and thiamine lowers LDL-C versus placebo and is safe and well tolerated over 12 weeks. Reporting follows Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2025, and trial results will be reported according to the registered endpoints.

**Key words:** Artisterol, cardiovascular risk, dietary supplement, hypercholesterolemia, nutrition adjuvant.

## INTRODUCTION

Elevated low density lipoprotein cholesterol (LDL-C) is a causal and modifiable risk factor for atherosclerotic cardiovascular disease, and the relationship between the absolute reduction in LDL-C and the reduction in major adverse cardiovascular events (MACEs) is continuous and proportional [1].

Contemporary European guidance therefore recommends a risk-based approach in which therapeutic lifestyle change and dietary counselling form the foundation of management, with pharmacological lipid-lowering therapy (LLT) added according to estimated total cardiovascular risk and the distance from the LDL-C goal [1]. A clinical gap remains for individuals at low-to-moderate absolute cardiovascular risk in primary prevention whose LDL-C stays above target despite lifestyle measures, and for those unable or unwilling to use statins because of intolerance or refusal [2,3]. In these groups, long-term pharmacotherapy may not be indicated, and additional non-pharmacological approaches are of interest as an adjunct to, not a substitute for, evidence-based lipid-lowering therapy [3,4].

Red yeast rice (RYR), whose active constituent monacolin K is structurally identical to lovastatin, has historically been used as a statin-like alternative [1,4]. Because its pharmacology mirrors that of synthetic statins, it carries comparable risks of muscle symptoms, transaminase elevation and cytochrome P 450 (CYP) 3A4-mediated interactions, and fungal fermentation may allow citrinin contamination. Consequently, the European Food Safety Authority (EFSA) and some national European regulators restricted permitted monacolin content in food supplements and in February 2025 recommended avoiding its use because of safety concerns [5,6]. Although many experts dispute that interpretation of the data, monacolin-K preparations are expected to disappear from the market by late 2027 [7,8]. There is, therefore, interest in non-monacolin, statin-free formulations.

Two classes of bioactive have been studied most extensively in this setting. Plant sterols reduce intestinal cholesterol absorption and lower LDL-C in a dose-dependent manner in randomized controlled trials and meta-analyses [4,9]. Artichoke leaf extract (*Cynara scolymus* L.) has been associated with modest reductions in total cholesterol and LDL-C, although the evidence base is smaller and more heterogeneous [4,10,11]. Combination nutraceuticals containing artichoke extract with RYR or berberine have produced LDL-C reductions of approximately 16–18% [12,13] in relatively small, randomized trials, whereas a randomized placebo-controlled trial of a solid-capsule combination of plant sterols, bergamot, artichoke leaf extract and

hydroxytyrosol found no significant change in LDL-C, total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides or apolipoproteins versus placebo [14]. Taken together, these results indicate that the lipid-lowering effect of a fixed combination cannot be predicted from the activity of its individual ingredients, and that formulation and delivery may materially influence the result.

Artisterol is a non-monacolin, statin-free liquid formulation combining plant sterols, artichoke leaf dry extract and vitamin B1 in a 10 mL stick pack. The rationale for the trial is therefore to test whether this specific finished formulation produces a clinically relevant short-term reduction in LDL-C with acceptable safety and tolerability in appropriately selected participants with hypercholesterolemia, rather than to extrapolate efficacy from its individual components.

These observations informed the design of the present trial. It is hypothesized, but not established, that presenting the bioactives pre-dispersed in a liquid stick-pack suspension rather than in a compressed solid dose may favour dispersion in the upper gastrointestinal tract; this is a formulation rationale and is not evidence of superior bioavailability, tolerability or adherence. The trial is designed to determine whether daily administration of this fixed combination lowers LDL-C relative to placebo over 12 weeks, and whether it is safe and well tolerated.

### **Investigational Product and Mechanistic Rationale**

The components of Artisterol are intended to act exogenous (intestinal absorption) and endogenous (hepatic synthesis and clearance) pathways of cholesterol homeostasis [13]. The mechanisms outlined below are presented as biological plausibility derived from studies of the individual constituents; they have not been demonstrated for the finished product, and the trial is not designed to establish the mechanism of action or the contribution of individual components.

Phytosterols, including sitosterol, campesterol, and stigmasterol, are plant-derived steroid alcohols that are structurally analogous to cholesterol, differing only in their side-chain alkylation patterns [4,15,16]. Due to their structural similarity, phytosterols possess a higher affinity for mixed micelles within the intestinal lumen than dietary or biliary cholesterol [4,13-16]. Upon ingestion, phytosterols compete with cholesterol for integration into these mixed micelles. The displaced, unmicellized free cholesterol remains insoluble and is eliminated in

the stool, reducing intestinal cholesterol absorption by 30% to 40% [4]. In response to this reduced enterocyte cholesterol delivery, hepatocytes upregulate the expression of surface LDL receptors, which increases the clearance of circulating apolipoprotein B-containing particles and lowers plasma LDL-C levels [12].

However, isolated inhibition of intestinal cholesterol absorption can trigger a compensatory upregulation of hepatic *de novo* cholesterol synthesis, a feedback mechanism mediated by sterol regulatory element-binding protein 2 (SREBP-2). On this basis, Artisterol also contains a dry extract of artichoke leaf (*Cynara scolymus* L.) [9], which is rich in bioactive polyphenolic constituents including chlorogenic acid, cynarin, and the flavonoid luteolin [11]. In preclinical and *in vitro* work, luteolin has been reported to inhibit hepatic HMG-CoA reductase activity indirectly [10] and to downregulate hepatic nuclear factor 4- $\alpha$  (HNF4 $\alpha$ ), with consequent effects on SREBP-2 transcription and on the assembly and secretion of apolipoprotein B-100 (ApoB), the primary structural protein of very-low-density lipoproteins (VLDL) and LDL particles [17]. Cynarin has been reported to stimulate choleresis, which could increase the hepatic utilisation of cholesterol for bile acid synthesis [11]. These effects are plausible but unproven in humans for this formulation and should be regarded as hypotheses rather than as demonstrated mechanisms of Artisterol.

The theoretical rationale for the combination is that concurrent action on intestinal absorption and on hepatic synthesis and clearance may limit the counter-regulatory response to absorption blockade alone [9]. Whether this translates into an additive or synergistic effect on LDL-C in humans is not established and cannot be determined from the present two-arm design.

Thiamine hydrochloride (Vitamin B1) is a cofactor for enzymes of glucose and amino acid metabolism [4,18], including pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. Its inclusion is based on this general metabolic role; no specific lipid-lowering or cardiovascular effect of thiamine and the dose used is claimed or being tested in this trial.

Presentation of the bioactives in a 10 mL liquid stick-pack suspension rather than as a compressed solid dose is a formulation choice intended to avoid the solid dissolution step and to allow the constituents to be delivered already dispersed. It is hypothesized that this may facilitate contact with the intestinal contents. Superior bioavailability, gastrointestinal tolerability or adherence relative to solid dosage forms has not been demonstrated for this product and is not assessed as an endpoint in this trial.

## MATERIAL AND METHODS

Study CINARL118125 (CHOLAMAZEN) is an international, randomized, double-blind, placebo-controlled study of adults, consisting of two randomized parallel arms, conducted in two centers [16]. The location of the participating centres is Italy and Poland. Subjects will be administered their assigned investigational product for 12-weeks [16]. The composition of the active investigational product (Artisterol) is presented in **Table 1**.

The study is a double-blind study. The identity of the specific product is blinded to participants, support staff, investigators, the site team, the CRO managing the study (except specified delegated statistical staff) and the sponsor. The colour and taste of the products are similar, and the products are packaged and labelled identically except for distinguishable product codes printed on the packaging. Unblinding will be performed only in case of emergency or at database lock for the final statistical analyses.

Participants attend 4 clinic visits — Screening (V1), Baseline/Enrolment (V2), Week 6 (V3) and Week 12/End of Treatment/End of Study (V4) — and complete two at-home procedures during which a diary is completed by the subject. Week 6 corresponds to study day 42 ( $\pm 5$  days) and Week 12 to study day 84 ( $\pm 5$  days). Randomization is performed at V2. Blood samples are collected at all four visits; LDL-C is measured at screening (V1), baseline (V2), Week 6 (V3) and Week 12 (V4), while total cholesterol, HDL-C, non-HDL-C, triglycerides, apolipoprotein B, fasting plasma glucose, AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyl transferase) are measured from baseline (V2) to Week 12 (V4). Vital signs, pregnancy testing in women of childbearing potential, compliance records, concomitant medication/treatment/diet records and adverse event records are collected at each clinic visit. Physical examination is performed at baseline and after 12 weeks of intervention. Safety and tolerability endpoints are monitored until the last consumption of investigational product (**Figure 1** and **2**). A schedule of enrolment, interventions and assessments in the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2025 [19] format is presented in **Table 2**.

### Eligibility Criteria and Study Population

Eligible participants are female or male, 18-70 years old, with mild-to-moderate hypercholesterolemia previously untreated, defined as fasting plasma LDL-C levels between 115 mg/dL and 160 mg/dL; the upper limit may be extended to 190 mg/dL for subjects who

refuse alternative treatment or are intolerant to statins or other lipid-lowering therapies [16,20]. If previously treated, a washout period of at least 3 months for any prior lipid-lowering therapy is required [16,20]. Participants must be in primary prevention for cardiovascular disease, provide written informed consent before any study-specific procedures, be willing and able to comply with scheduled visits and protocol requirements, including the study-specific dietary recommendations, and be considered healthy or clinically stable based on medical history, physical examination, and assessment of clinically relevant laboratory findings by the investigator. Females of childbearing potential must have a negative pregnancy test at screening and agree to use a highly effective method of contraception from screening through the final study visit [16,20].

**Key exclusion criteria** are pregnancy or lactation; specific feeding regimens not stabilized between screening and baseline, especially those in conflict with the study-specific Dietary Recommendation; therapies or medications that may affect lipid levels without a 3-month washout; known or suspected allergy to the content of the investigational product; previous or ongoing severe medical or laboratory abnormality that in the investigator's judgement makes the subject inappropriate for entry; long-term treatment unless stable for 30 days; a history of acute myocardial infarction, severe cardiac conditions or other atherosclerosis-related cardiovascular disease; a history of alcoholism or drug use, or severe psychotic disorders (stable anti-depressive treatment permitted); inability to understand and follow study procedures; current participation in another interventional clinical study, with historical participation allowed after a 3-month washout; and family or hierarchical relationships with research team members at the clinical site [16,20].

### Statistical Analysis Plan

*The sample size* was calculated to detect a 5% absolute difference between treatment groups in the change from baseline in LDL-C at Week 12 [21], assuming a two-sided alpha level of 0.05 and 90% power. Based on these assumptions, 74 participants (37 per group) are required [21]. To account for an anticipated dropout rate of 30%, the target sample size was increased to 106 participants (53 per group) [21]. Although the study hypothesis anticipates an approximately 15% reduction in LDL-C with Artisterol relative to placebo, the sample size calculation was conservatively based on a smaller between-group difference of 5% to ensure adequate power to detect clinically meaningful treatment effects.

*Randomization.* Participants are randomized in a 1:1 ratio, stratified by sex (male/female), implemented via Viedoc Balance using a dynamic allocation algorithm to ensure balanced assignment across strata. Randomization and the investigational product numbers assigned to each participant are documented (**Figure 1**).

*Analysis populations.* The Enrolled Population includes all patients who have provided informed consent and have been included in the study database. The Intention-to-Treat (ITT) Population is defined as all subjects who were randomized, regardless of whether they completed the study, adhered to the protocol, or received the assigned treatment. The Full Analysis Set (FAS) Population includes all subjects who received one dose of Artisterol or placebo. The Per Protocol (PP) Population includes all subjects who consume at least 80% of the experimental group or placebo doses foreseen by the trial design. The Safety (SAF) Population includes all subjects with documented use of at least one feeding of the study formula or product, classified according to the feeding actually received [21]. Continuous variables will be evaluated for normality, and baseline parameters will be compared using appropriate parametric or non-parametric tests [21].

*Primary analysis.* The primary efficacy analysis will be performed on the FAS. Analyses in the PP population will be considered supportive. The primary analysis will evaluate the absolute mean change in fasting LDL-C (mg/mL) from baseline to Week 12 between the experimental group (Aristerol) and the control group (placebo). An analysis of covariance (ANCOVA) model will be estimated on the changes from baseline, adjusting for baseline LDL-C, and estimates of the treatment effect will be presented with two-sided 95% confidence intervals; a p-value of  $<0.05$  will be considered statistically significant. If assumptions for the ANCOVA are violated, appropriate alternative statistical methods will be applied [21]. A subgroup analysis by sex is planned. Details of the subgroup analyses and statistical methodology, including the handling of stratification factors and covariates, will be specified in the final Statistical Analysis Plan (SAP). Treatment effect estimates and corresponding confidence intervals and p-values will be presented as appropriate. The analysis populations and the role of supportive analyses will be defined in the final SAP.

*Secondary and exploratory analyses.* Secondary lipid, metabolic and liver parameters will be analysed using models analogous to the primary analysis [21]. Exploratory analyses will the trend of LDL-C and the secondary parameters across baseline, Week 6, and Week 12 using a mixed design with a between-subject factor (experimental versus control group) and a within-subject factor (timepoint). Repeated-measures ANOVA will be performed if parametric

assumptions are met, with between-group comparison by independent Student's t-test and within-group comparison with Bonferroni correction. If the assumptions for ANOVA are violated, a Friedman test will be used for within-group changes over time and the Mann-Whitney U test for between-group comparison at each timepoint. Safety parameters will be summarized descriptively [21].

*Multiplicity.* No confirmatory multiplicity adjustment is planned across the secondary endpoints; therefore, associated confidence intervals and p-values will be interpreted descriptively/supportively. Bonferroni adjustment, where used, applies only to the prespecified within-group post-hoc comparisons in the exploratory longitudinal analysis and does not establish confirmatory control across the secondary endpoint family.

*Missing data.* The handling of missing data, including the definition of the estimand, the management of intercurrent events, and any sensitivity analyses, will be prespecified in the final SAP. No unplanned post hoc single-imputation methods will be introduced after database lock.

*Baseline data.* Baseline characteristics will be summarized descriptively by randomized treatment group using appropriate summary statistics. Details of any additional analyses of baseline characteristics will be specified in the final SAP.

## **Data Management and Quality Assurance**

Data collection is performed in accordance with the International Conference on Harmonisation (ICH) E6 (R3) Good Clinical Practice guidelines, the Declaration of Helsinki and applicable personal data protection regulations [22,23]. All data modifications are documented in an audit trail, and the system complies with electronic signature requirements [16].

The Clinical Data Manager validates and cleans the data as specified in the Data Validation Plan, and data are reviewed through regular Data Review meetings [16]. Adverse events and serious adverse events are coded using MedDRA valid during the clinical part of the study. The electronic data capture database will be locked after review, query resolution, signature of the eCRF and determination that the clinical database is ready for analysis. Further operational detail is provided in the Data Management Plan and may be made available as supplementary material. No patient-reported outcomes (PROs) are collected or analyzed as formal efficacy endpoints; compliance and dietary adherence records are the only subject-reported variables [16].

## RESULTS

### Objectives and Endpoints

The primary endpoint is to assess the effect of the investigational product on LDL-C levels by measuring the absolute mean change from baseline to Week 12. The study hypothesis is a minimum 15% reduction in LDL-C for the investigational product compared with placebo [16].

Secondary endpoints are the absolute mean changes from baseline to Week 12 in total cholesterol, HDL-C, non-HDL-C and triglycerides; the absolute mean change in apolipoprotein B; the absolute mean changes in fasting plasma glucose, ALT, ASP and GGT; and the assessment of tolerability through monitoring of adverse events, comprising physical examination at baseline and after 12 weeks of intervention, reported adverse events including type, incidence, severity, seriousness and relation to the intervention, and reported concomitant medications including name, dose and duration [16]. A subgroup analysis by sex is planned.

The exploratory endpoint is the change over time (baseline, Week 6, Week 12) in LDL-C and secondary biomarkers, assessed using repeated-measures analyses [16].

### Safety, Pharmacovigilance, and Ethical Considerations

The study is conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki, the ICH Guideline for Good Clinical Practice, and applicable regulatory requirements, with ethics committee approval obtained prior to enrolment [22,23]. A serious adverse event (SAE) or serious adverse reaction (SARs) is defined as any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, is a congenital anomaly or birth defect, or is otherwise judged an important medical event by the investigator. Investigators record any adverse event on the adverse event page of the eCRF [16].

Upon awareness of SAEs or Adverse Reactions (ARs), the investigator must submit within 24 hours a serious event report form to the sponsor safety monitoring team and pharmacovigilance representatives and enter the data in the eCRF, regardless of suspected causality [16]. All such events occurring until the last study visit and up to 30 days after the last intake of investigational product are reported in the same way. Suspected unexpected, serious adverse reactions that are fatal or life-threatening are reported to the ethics committee within 7 days, with additional

relevant information within a further 8 days; other suspected unexpected, serious adverse reactions are reported within 15 days. Pregnant participants will trigger immediate specific reporting protocols, and all safety monitoring continues until trial completion or resolution [16]. The sponsor maintains ongoing safety evaluations, ensuring compliance with local regulatory standards such as the Vigierbe database in Italy where applicable [16]. Safety and tolerability endpoints are monitored until the last consumption of investigational product.

## DISCUSSION

*Expected contribution.* Evidence for combination nutraceuticals in mild hypercholesterolaemia is mixed. Randomized trials of artichoke extract combined with red yeast rice or with berberine have reported LDL-C reductions in the region of 16–18% [12,13], whereas a randomized placebo-controlled trial of a solid-capsule combination of plant sterols, bergamot, artichoke leaf extract and hydroxytyrosol found no significant effect on LDL-C or other lipid parameters [14]. These divergent results support the reviewer's point that efficacy must be demonstrated for a specific finished formulation rather than inferred from its constituents. The present trial will provide randomized, placebo-controlled data on whether this particular non-monacolin liquid formulation lowers LDL-C over 12 weeks and whether it is safe and well tolerated in appropriately selected participants.

*Potential implications.* These results could meaningfully inform the debate on nutraceuticals for patients with lipid disorders. Current European and US guidelines do not recommend dietary supplements and provide little detail on this topic [1,24]. Well-designed, high-quality studies of combined nutraceuticals as dietary adjuvants could improve adherence and lipid outcomes in individuals for whom statin therapy is not yet indicated. Such evidence would also inform decisions about starting statins in low- and moderate-risk patients - an area the guidelines largely overlook. If evidence-based nutraceuticals help optimize diet and lifestyle in these individuals, more patients might reach LDL-C targets and experience slower atherosclerosis progression, potentially delaying the need for pharmacotherapy [25-27].

*Strengths of the design.* The trial is randomized, double-blind and placebo-controlled, with a placebo of similar colour and taste and identical packaging; randomization is stratified by sex and implemented centrally using a dynamic allocation algorithm; the primary endpoint is objective and centrally measurable; and safety and tolerability are assessed systematically

through physical examination, adverse event reporting and hepatic and glycaemic laboratory parameters.

*Limitations.* Several limitations should be acknowledged. First, the 12-week treatment period is adequate to detect changes in plasma lipid and short-term safety signals but cannot address long-term efficacy, durability of effects, or cardiovascular outcomes [10]. Second, the endpoints are surrogate lipid measures rather than clinical cardiovascular events. Third, the sample size of 106 randomized participants is modest and is powered for the primary LDL-C comparison only; secondary and exploratory endpoints are supportive and are not adjusted for multiplicity. Fourth, the eligible population is heterogeneous, since the LDL-C entry range may extend from 115 to 190 mg/dL and includes both individuals declining other treatment and individuals reporting statin intolerance, which may introduce variability in baseline risk and in treatment response. Fifth, because Artisterol is tested as a fixed combination against placebo, the contribution of the individual components - plant sterols, artichoke leaf extract and vitamin B1 - cannot be determined. Sixth, dietary counselling is qualitative rather than a controlled metabolic diet, which reflects real-world conditions but permits variability in background nutrient intake. Finally, inter-individual variability in cholesterol absorption and synthesis may influence the response to plant sterols.

### ***Conclusions***

Study CINARL118125 (CHOLAMAZEN) will determine whether a non-monacolin, statin-free fixed combination of plant sterols, artichoke leaf dry extract and vitamin B1, administered as a 10 mL liquid stick pack once daily for 12 weeks, reduces LDL-C relative to placebo, and whether it is safe and well tolerated, in adults aged 18–70 years with previously untreated mild hypercholesterolaemia in primary prevention. The trial does not establish, and is not intended to establish, that this formulation is an alternative to evidence-based lipid-lowering pharmacotherapy in individuals for whom such therapy is indicated. Reporting of this protocol follows SPIRIT 2025, and the results will be reported in accordance with the registered endpoints.

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**Trial registration:**\_\_The study is registered under trial number NCT07405814 / CINARL1118125.

**Ethics approval and consent to participate:** The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and applicable national regulatory requirements. The study protocol, informed consent forms, and related study documents were approved by the appropriate ethics committees and regulatory authorities before study initiation. Written informed consent was obtained from all participants before any study-specific procedures were performed.

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**Conflicts of Interest:** Arrigo F.G. Cicero has served as a consultant/received speakers fees from Bayer Healthcare, Dompé, Italfarmaco, Zentiva, ESI, Therascience, Named and Menarini. Maciej Banach has received research grant(s)/support from Amgen, Daiichi Sankyo, Mylan/Viatris, and Sanofi, and has served as a consultant/received speakers fees from Adamed, Amgen, Daiichi-Sankyo, KRKA, Lilly, Mylan/Viatris, NewAmsterdam, Novartis, Novo-Nordisk, Polpharma, Sanofi, Servier, Sobe, Teva, and Zentiva. Helena Krist, Aleksandra Domicic, and Marek Ferkl are the employees of Zentiva.

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FIGURES:

Figure 1. CHOLAMAZEN study design.

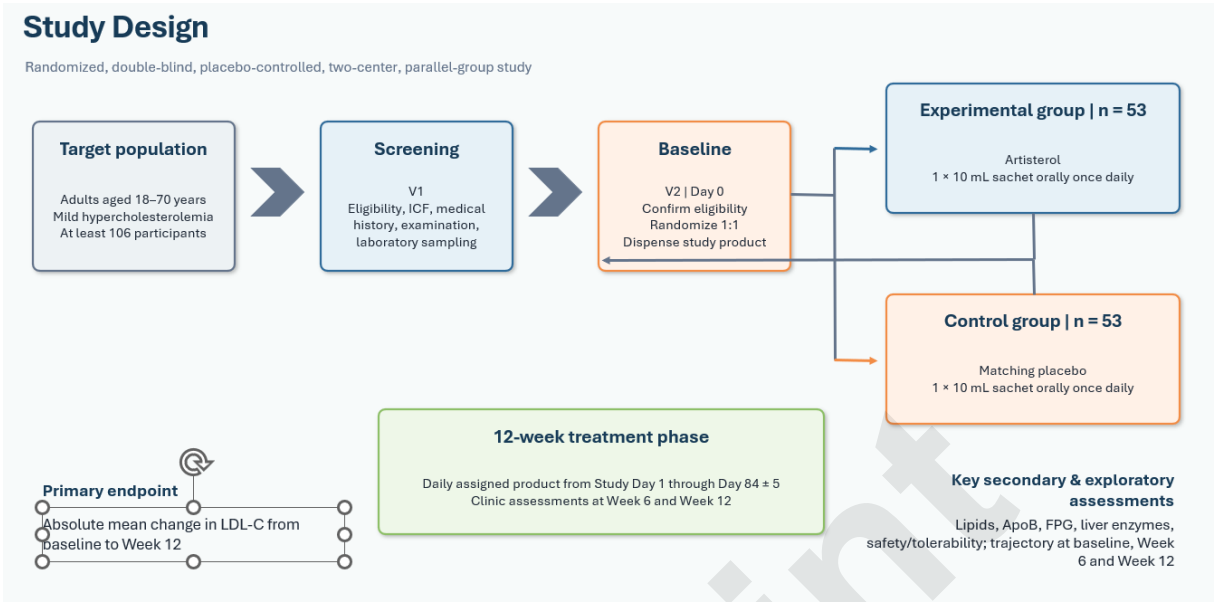
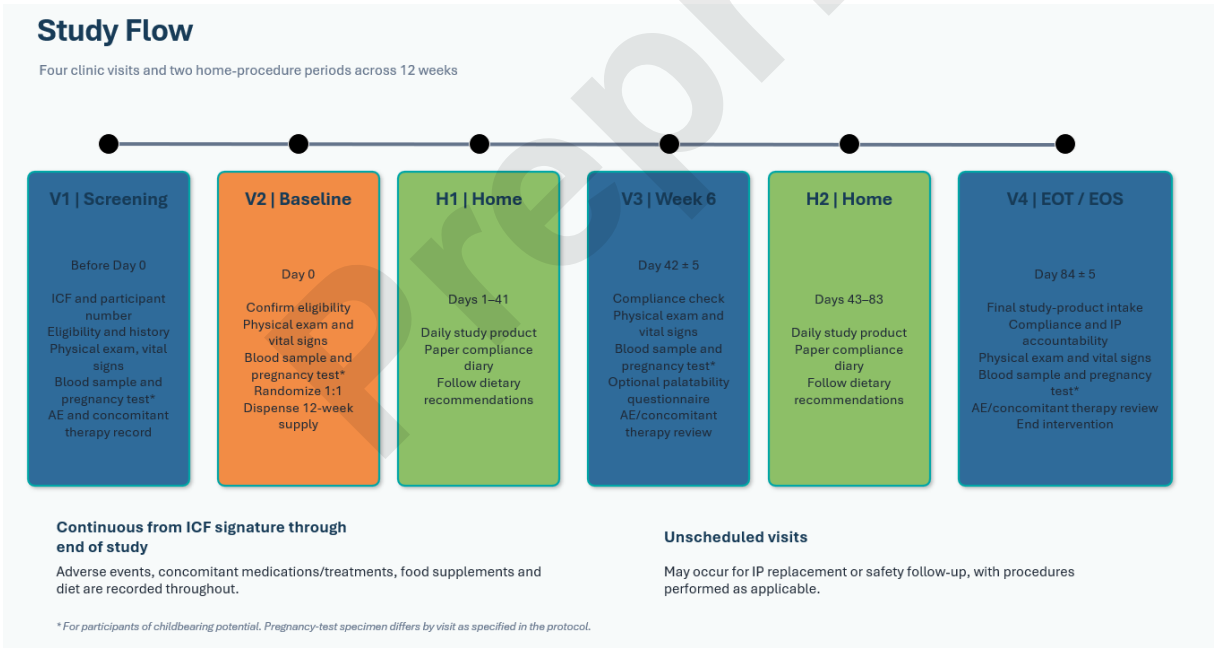


Figure 2. Detailed study Flow.



**TABLES:****Table 1. Composition of Artisterol per 10 mL stick pack (per daily intake)**

| <b>Ingredient category</b> | <b>Component name</b>                                      | <b>Function in formulation</b>                                                                                   | <b>Active group per 10 mL</b> |
|----------------------------|------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|-------------------------------|
| <b>Active ingredients</b>  | Plant sterols                                              | Competition with cholesterol for intestinal micellar incorporation.                                              | 1200.0 mg                     |
| <b>Active ingredients</b>  | Artichoke dry extract ( <i>Cynara scolymus</i> L., leaves) | Proposed biological rationale based on constituent-level evidence; not established for the finished formulation. | 500.0 mg                      |
| <b>Active ingredients</b>  | Thiamine hydrochloride (Vitamin B1)                        | Vitamin/cofactor included in the formulation; no lipid-lowering effect is claimed in this trial.                 | 5.0 mg                        |

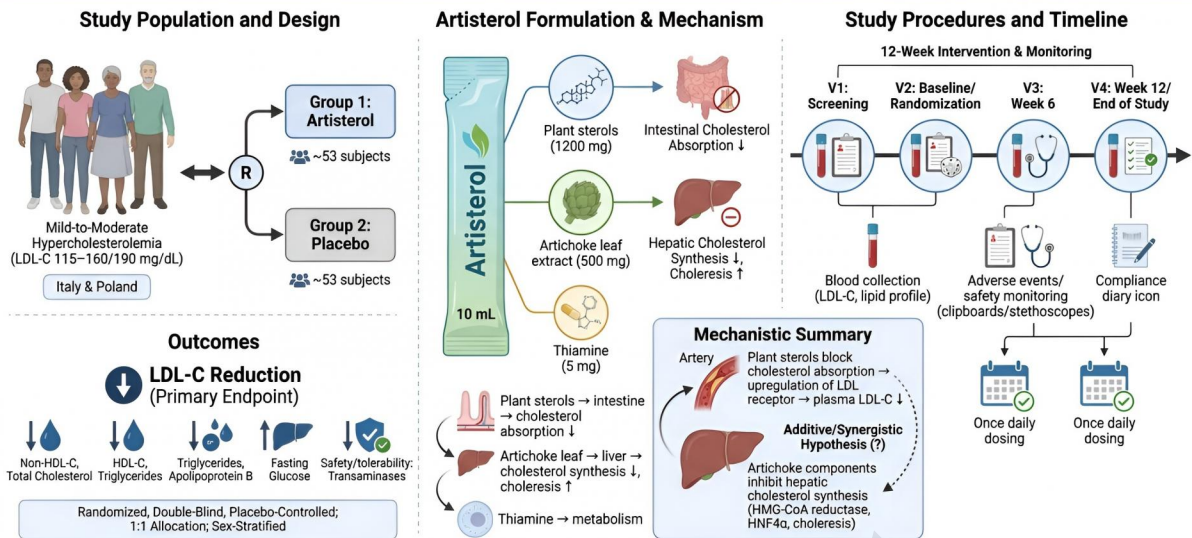
**Table 2. Schedule of enrolment, interventions and assessments**

| <b>Study period</b>                                 | <b>Screening</b> | <b>Baseline</b> | <b>Treatment</b> | <b>Week 6</b> | <b>Treatment</b> | <b>Week 12/EOT</b> |
|-----------------------------------------------------|------------------|-----------------|------------------|---------------|------------------|--------------------|
| <b>Visit</b>                                        | V1               | V2              | H1               | V3            | H2               | V4                 |
| <b>Study day</b>                                    | Screening        | 0               | 1-41             | 42±5          | 43-83            | 84±5               |
| <b>Informed consent</b>                             | X                |                 |                  |               |                  |                    |
| <b>Eligibility assessment</b>                       | X                | X               |                  |               |                  |                    |
| <b>Randomization</b>                                |                  | X               |                  |               |                  |                    |
| <b>Artisterol or placebo once daily</b>             |                  |                 | X                | X             | X                | X                  |
| <b>Dietary recommendations and compliance diary</b> |                  | X               | X                | X             | X                | X                  |
| <b>Physical examination and vital signs</b>         | X                | X               |                  | X             |                  | X                  |

|                                                                                     |   |   |   |   |   |   |
|-------------------------------------------------------------------------------------|---|---|---|---|---|---|
| <b>Blood samples and LDL-C</b>                                                      | X | X |   | X |   | X |
| <b>Total cholesterol, HDL-C, non-HDL-C, triglycerides, ApoB, FPG, ALT, ASP, GGT</b> |   | X |   | X |   | X |
| <b>Adverse events and concomitant medications/diet</b>                              | X | X | X | X | X | X |

**Abbreviations:** ApoB, apolipoprotein B; DBP, diastolic blood pressure; EOT, end of treatment; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; AST (aspartate aminotransferase), ALT (alanine aminotransferase); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure. X indicates that the activity or assessment is performed at the corresponding visit or period.

# CHOLAMAZEN Clinical Trial: Statin-Free Artisterol Suspension for Suboptimal Cholesterol



**CHOLAMAZEN Study (NCT07405814):** Testing a statin-free Artisterol liquid stick pack for 12 weeks in adults with mild-to-moderate hypercholesterolemia. Primary endpoint: LDL-C reduction vs placebo. Secondary: other lipid/liver/glucose metrics, safety & tolerability.