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In-silico trial emulation to predict the cardiovascular protection of new lipid-lowering drugs: an illustration through the design of the SIRIUS programme

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Introduction

Inclisiran, an siRNA targeting hepatic PCSK9 mRNA, administered twice-yearly (after initial and 3-month doses), substantially and sustainably reduced LDL-cholesterol (LDL-C) in Phase III trials. Whether lowering LDL-C with inclisiran translates into a reduced risk of major adverse cardiovascular events (MACE) is not yet established. In-silico trials applying a disease computational model to virtual patients receiving new treatments allow to emulate large scale long-term clinical trials. The SIRIUS in-silico trial programme aims to predict the efficacy of inclisiran on CV events in individuals with established atherosclerotic cardiovascular disease (ASCVD).

Methods and results

A knowledge-based mechanistic model of ASCVD was built, calibrated, and validated to conduct the SIRIUS programme (NCT05974345) aiming to predict the effect of inclisiran on CV outcomes. The SIRIUS Virtual Population included patients with established ASCVD (previous myocardial infarction (MI), previous ischemic stroke (IS), previous symptomatic lower limb peripheral arterial disease (PAD) defined as either intermittent claudication with ankle-brachial index <0.85, prior peripheral arterial revascularization procedure, or vascular amputation) and fasting LDL-C ≥ 70 mg/dL, despite stable (≥ 4 weeks) well-tolerated lipid-lowering therapies.

SIRIUS is an in-silico multi-arm trial programme. It follows an idealized crossover design where each virtual patient is its own control, comparing inclisiran to (i) placebo as adjunct to high-intensity statin therapy with or without ezetimibe, (ii) ezetimibe as adjunct to high-intensity statin therapy, (iii) evolocumab as adjunct to high-intensity statin therapy and ezetimibe.

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The co-primary efficacy outcomes are based on the time to the first occurrence of any component of 3P-MACE (composite of CV death, nonfatal MI, or nonfatal IS) and time to occurrence of CV death over 5 years.

Perspectives/ conclusion

The SIRIUS in-silico trial programme will provide early insights regarding potential effect of inclisiran on MACE in ASCVD patients, several years before the availability of the results from ongoing CV outcomes trials (ORION-4 and VICTORION-2-P).

Clinical trial registration

Clinicaltrials.gov identifier: NCT05974345

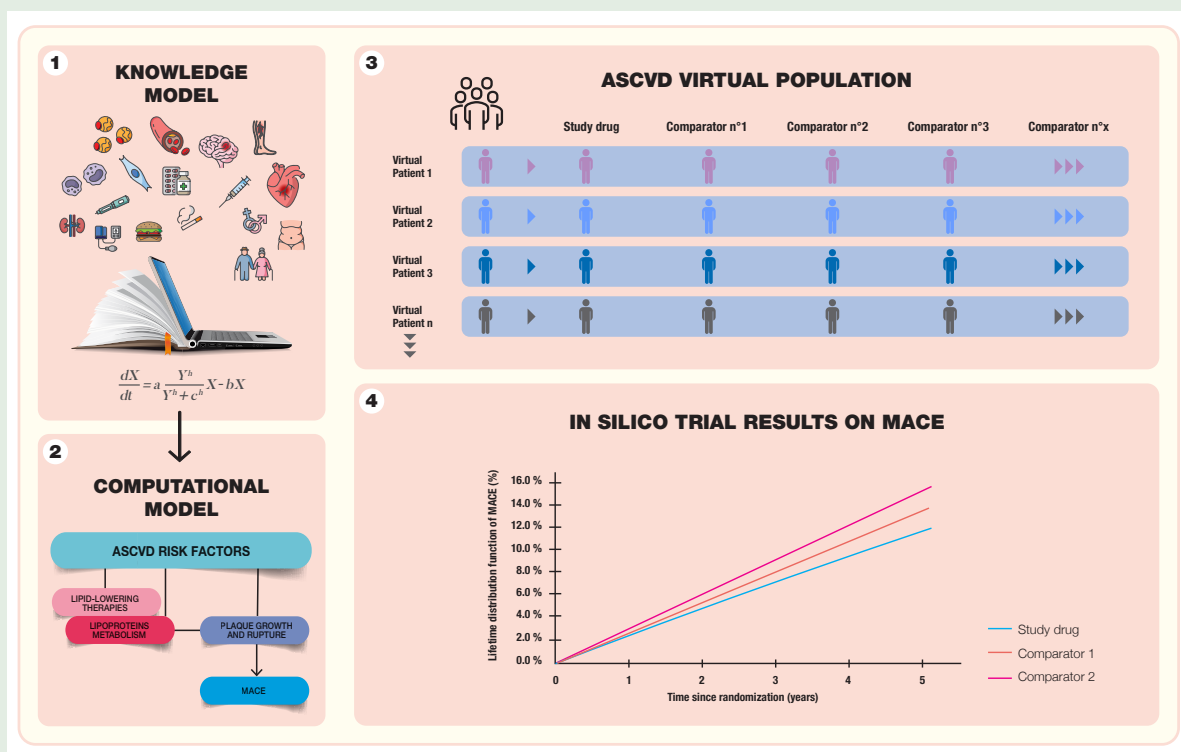
Lay summary

The SIRIUS in-silico trial programme is a knowledge-based computer model built to simulate the biological and clinical long-term effects of inclisiran, an siRNA targeting hepatic PCSK9 mRNA, on virtual patients with cardiovascular disease.

Key findings

- The model accurately replicates the biological processes of cardiovascular disease and the impact of lipid-lowering therapies, allowing for the prediction of randomized clinical trials.
- Simulating clinical trials with virtual patients can provide insights into the efficacy and safety of new treatments before the results of randomized clinical trials, potentially speeding up the drug development process.

Graphical Abstract



In-silico modelling applying a disease knowledge-based mechanistic computational model to virtual patients receiving various treatments allow to emulate large-scale long-term in-silico clinical trials. The ASCVD and therapeutics knowledge-based mechanistic model captures, by using mathematical equations, the functional relationships between the biological entities involved in pathophysiology of ASCVD and the mechanisms of action of lipid lowering therapies. **A knowledge model** corresponds to an extensive bibliographical review to collect and organize knowledge on biochemical and cellular processes of interest, pathophysiological mechanisms involved in the development and progression of ASCVD and mechanisms of action of the drugs of interest. The knowledge model is then translated into mathematical equations to obtain **a computational model**. The ASCVD knowledge-based mechanistic model is applied to an ASCVD virtual population which corresponds to a collection of virtual patients generated *in-silico* to capture realistic inter-patient variability. Each virtual patient corresponds to a set of values for each model parameter. The ASCVD model is applied to the ASCVD virtual population (in which each patient is his own control) allowing to emulate multi-arm, large scale and long-term in-silico trials.

Keywords

Atherosclerotic cardiovascular disease • Knowledge-based mechanistic model • Lipid lowering therapy • In silico trial • Inclisiran • PCSK9 siRNA

Introduction

Lipid-lowering therapies (LLTs) are a cornerstone of the prevention of atherosclerotic cardiovascular disease (ASCVD).¹ Recent data from randomized clinical trials (RCT) evaluating drugs as an add-on to baseline statin therapies have led to guidelines changes, now recommending to lower patients' low-density lipoprotein cholesterol (LDL-C) below 55 mg/dL in patients in secondary ASCVD prevention.² To achieve such target levels of LDL-C, addition of ezetimibe and/or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors to high-intensity statin therapy is often necessary. Following the discovery of PCSK9 gene and function in the early 2000s, a first generation of PCSK9 inhibitors, monoclonal antibodies (mAbs) that target the protein in the plasma, have been successfully developed and are now available in routine clinical practice.^{3,4} PCSK9 mAbs (alirocumab and evolocumab) require a subcutaneous injection every 2 or 4 weeks depending on dosing. More recently, inclisiran has emerged as an alternative approach to antagonize PCSK9 using small interfering ribonucleic acid (siRNA)-based technology which degrades PCSK9 messenger RNA (mRNA) in the liver, inhibiting PCSK9 synthesis in the hepatocytes.⁵ This novel mechanism allowing twice-yearly dosing (after initial and 3-month doses) has been shown to substantially, sustainability and safely reduce LDL-C in phase 3 trials.^{6,7} It has the appeal of reduced injection burden and potentially improved adherence compared with monoclonal antibodies.

Despite consistent evidence of inclisiran-induced reduction in LDL-C across a broad range of populations and a well-documented correlation between LDL-C levels and CV risk, large CV outcomes phase III trials are needed to document the long-term efficacy and safety of inclisiran.⁸ Two phase III trials, ORION-4 (NCT03705234) and VICTORION-2-Prevent (NCT05030428) are ongoing to assess the impact of inclisiran on major adverse cardiovascular (CV) events (MACE) in patients with established ASCVD, and results are expected in 2026 and 2027, respectively.

In-silico trials apply computational disease and treatment models to virtual patients. It is an emerging approach to complement RCT and rapidly generate missing comparative efficacy data.^{9,10} In the last years, both the European Medicines Agency (EMA) and the Federal Drug Administration (FDA) started receiving and accepting evidence generated in-silico, i.e. through modelling and simulation.^{11,12} Modelling growth and rupture of atherosclerotic plaques along with LLT mechanisms of action enables us to explore the effect of these therapies on ASCVD events. It is a valuable tool to predict the clinical benefit of new therapeutic strategies and analyze their effects on subgroups of patients.¹³

Based on this approach, we designed the SIRIUS in-silico trial programme to predict the efficacy of inclisiran on MACE in participants with ASCVD using a multi-scale mechanistic computational model.

Trial design and method

Design of an ASCVD knowledge-based mechanistic model

Quantitative systematic pharmacology-based model includes data-based model (e.g. pharmacokinetic model) and knowledge-based model.¹⁴

According to our definition, data refers to any numerical value (non-clinical and clinical), whereas knowledge refers to a qualitative information extracted from the scientific literature describing a functional relationship between biological entities (e.g. cholesterol can be either synthesized *de novo* within cells or brought by ingestion of certain foods, the latter being the main source in human). In our approach, we used knowledge-based model to describe the mechanisms underlying the

dynamic of the disease, the occurrence of an event, and thus allows mechanistic and quantitative causality to be determined in relation to simulation results and outcome prediction.

The first step consisted in designing a knowledge model describing functional relationships between biological entities involved in the pathophysiology of ASCVD and the mechanisms of action of LLTs. A multidisciplinary team of biomodelers (EP, EB, JPB, EC, AD, SG-N, RK, SP, YW) and scientific experts in the field of ASCVD (see list in [supplemental data section](#)) made an extensive bibliographical review to collect and organize knowledge on biochemical and cellular processes of interest, pathophysiological mechanisms involved in the development and progression of the disease, and mechanisms of action of the drugs of interest. Knowledge gaps and resulting modelling hypotheses were resolved by the experts to ensure model biological and clinical coherence. A proprietary dedicated knowledge management platform provided by Novartis was used to ensure traceability between all the elements extracted from the scientific literature and their source articles (details regarding the references used to establish the model can be obtained upon reasonable request).

Following the validation of the knowledge model, the second step was the translation of the knowledge model into mathematical equations to obtain a mechanistic computational model ([Graphical abstract](#)).

The ASCVD model is a combination of several interdependent pathophysiological submodels ([Figure 1](#)):

- the lipoprotein submodel, which focuses on lipoprotein metabolism and homeostasis with the impact of lipid-modifying traditional risk factors such as diabetes, body mass index (BMI), and sex (see [Supplementary material online, Figure S1](#)). Plasma biological entities explicitly modelled are free PCSK9, LDL-C, high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), and lipoprotein (a) [Lp(a)] particles. Additional biological entities [total cholesterol, apolipoprotein B (ApoB), and triglycerides] are derived from these variables using published formulae.
- the atherosclerotic plaque growth and rupture submodel. The model simulates the evolution of 1 to 3 atheromatous plaques in the coronary, carotid, and peripheral vascular beds for each virtual patient (at most one per vascular bed). The number and type of vascular beds affected are determined by medical history (type of pre-existing ASCVD). Plaque location (coronary, carotid, or peripheral arteries) and evolution over simulation time determine whether plaque rupture occurs, and therefore the time and type of clinical event (myocardial infarction (MI), ischemic stroke (IS) or major adverse limb event (MALE) respectively). Due to modelling constraints, all simulated IS and MALE are linked to a carotid or peripheral artery atheroma plaque rupture, respectively. In this submodel, infiltration of plasma lipid and lipoproteins into the intima is enhanced by (i) systemic inflammation, (ii) diabetes, (iii) smoking status, (iv) increasing age, and (v) elevated blood pressure to account for disruption of the endothelium barrier aggravated by these risk factors (see [Supplementary material online, Figure S2](#)).

The occurrence of death following IS and MI is determined on the basis of an exponential law inspired by the Cox model published by Wilson et al, 2012¹⁵ and built with data from the REduction of Atherothrombosis for Continued Health (REACH) registry taking into account the impact of numerous risk factors [sex, smoking, diabetes, BMI, aspirin, heart failure, medical history, atrial fibrillation,

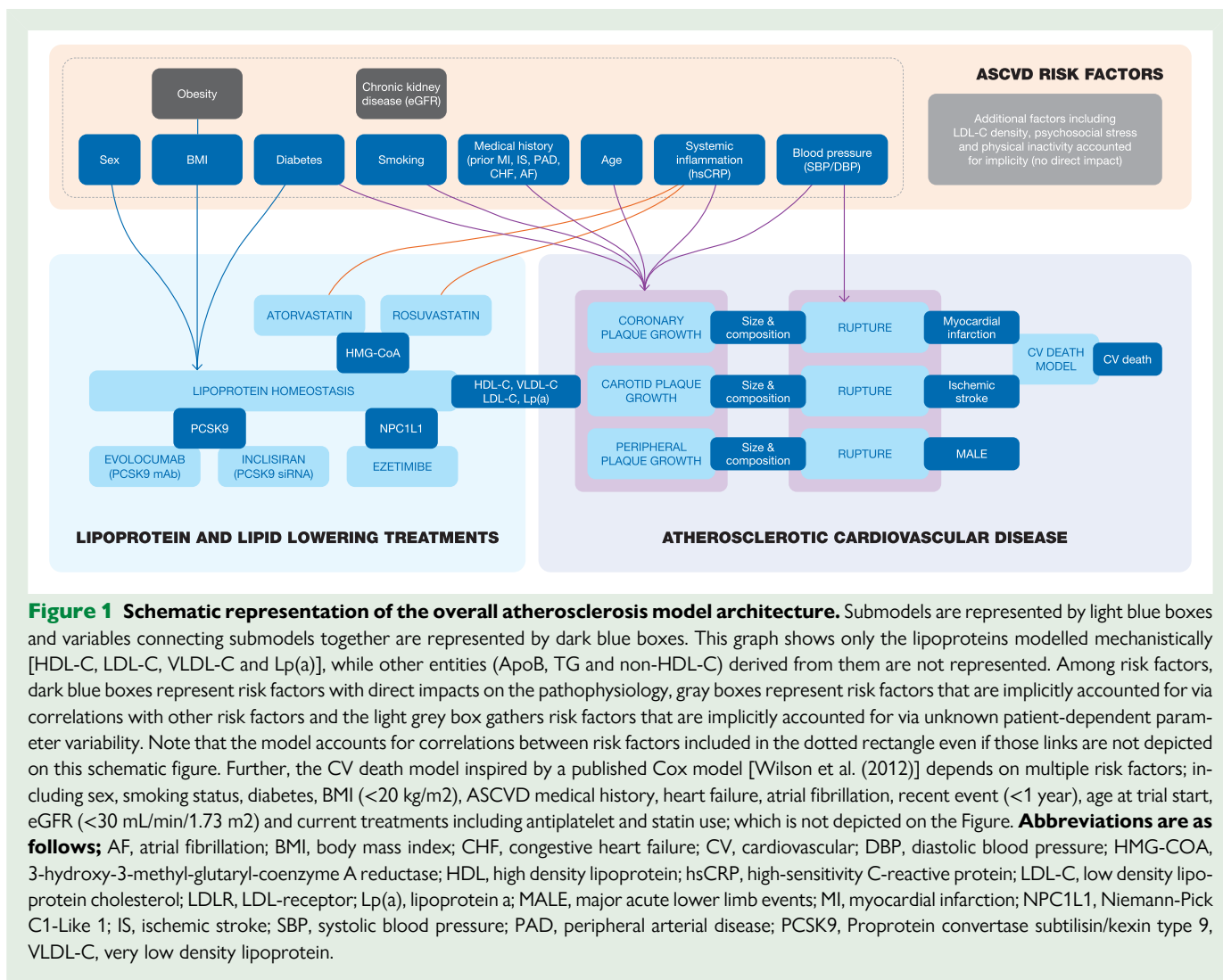


Figure 1 Schematic representation of the overall atherosclerosis model architecture. Submodels are represented by light blue boxes and variables connecting submodels together are represented by dark blue boxes. This graph shows only the lipoproteins modelled mechanistically [HDL-C, LDL-C, VLDL-C and Lp(a)], while other entities (ApoB, TG and non-HDL-C) derived from them are not represented. Among risk factors, dark blue boxes represent risk factors with direct impacts on the pathophysiology, gray boxes represent risk factors that are implicitly accounted for via correlations with other risk factors and the light grey box gathers risk factors that are implicitly accounted for via unknown patient-dependent parameter variability. Note that the model accounts for correlations between risk factors included in the dotted rectangle even if those links are not depicted on this schematic figure. Further, the CV death model inspired by a published Cox model [Wilson et al. (2012)] depends on multiple risk factors; including sex, smoking status, diabetes, BMI (<20 kg/m²), ASCVD medical history, heart failure, atrial fibrillation, recent event (<1 year), age at trial start, eGFR (<30 mL/min/1.73 m²) and current treatments including antiplatelet and statin use; which is not depicted on the Figure. **Abbreviations are as follows;** AF, atrial fibrillation; BMI, body mass index; CHF, congestive heart failure; CV, cardiovascular; DBP, diastolic blood pressure; HMG-COA, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HDL, high density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; LDLR, LDL-receptor; Lp(a), lipoprotein a; MALE, major acute lower limb events; MI, myocardial infarction; NPC1L1, Niemann-Pick C1-Like 1; IS, ischemic stroke; SBP, systolic blood pressure; PAD, peripheral arterial disease; PCSK9, Proprotein convertase subtilisin/kexin type 9, VLDL-C, very low density lipoprotein.

clinical event in the previous year, age, and estimated glomerular filtration rate (eGFR)] already included in the mechanistic model. CV death from other causes than fatal MI and IS are not mechanistically modelled and are predicted through the adapted previous Cox model.¹⁵ This model of plaque growth and rupture is directly impacted by plasma lipoprotein levels, the output variable of the lipoprotein submodel.

- (c) treatment submodels, i.e. atorvastatin and rosuvastatin, ezetimibe, evolocumab, and inclisiran, describe pharmacokinetic properties of corresponding drugs and their mechanism(s) of action, including interactions with the various pharmacological targets impacting lipoprotein metabolism.

The impact of ASCVD risk factors on disease progression, as well as prevalence and correlations between risk factors in the ASCVD secondary prevention population, are integrated in lipoprotein, plaque growth, and rupture submodels. The selection of risk factors accounted for explicitly in the model was restricted based on available literature and calibration data. Their mechanistic impacts were limited to a few key phenomena clearly established in the literature, with associated modelling hypotheses thoroughly discussed with the Scientific Committee. A subset of risk factors was also accounted

for, implicitly via their correlations with other risk factors or variability of model parameters within the virtual population (Figure 1). All modelling hypothesis of model structure will be displayed in a technical publication (Wang et al., manuscript in progress).

Calibration of the model

The model needs to be calibrated and its credibility assessed (essentially a validation process) before simulations can be run to answer the questions identified in the SIRIUS study.

Calibration is the iterative process of adjusting model parameter values to ensure that the model reproduces realistic behaviors over time.¹⁶ In the computational model, there are parameters whose values are known and measurable, but there are also parameters whose values have never been published (e.g. LDL-C infiltration rate constant in the intima of blood vessels). Values for these parameters were determined by calibration, iteratively adapting their values until the model correctly reproduced several selected behaviors.

The model was calibrated to ensure that it could reproduce the pharmacokinetics of atorvastatin,^{17,18} rosuvastatin,¹⁹ ezetimibe,²⁰ lipids and lipoproteins changes under combinations of LLT including inclisiran⁶ (Figure 2A) and evolocumab²¹ (Figure 2B), and occurrence of clinical

events at the population- and subgroup-level observed in the FOURIER RCT²¹ (Figure 2C).

Calibration goodness of fit was assessed using visual predictive checks and pre-defined acceptability error margins around observed data. Acceptability thresholds were set-up as 5% points of biological outcome relative changes and 1% point of clinical outcome incidence rates. Those thresholds were fixed and discussed with the scientific committee that judged that it was an acceptable difference, from a clinical perspective. All calibration results will be displayed in the technical publication (Wang et al., manuscript in progress).

Credibility assessment of the model

Following model calibration, additional steps of credibility assessment were performed. Credibility assessment refers to the evaluation of trust in the predictive capability of a computational model. It is established through collection of evidence including model verification, evaluation of the goodness-of-fit of calibration results, qualitative and quantitative validation, and robustness analysis. Validation assesses the degree to which a model accurately represents the real world within its context of use (secondary ASCVD prevention).¹⁶ Evaluation of robustness allows an assessment of the ability of a model to accurately predict beyond the context of use for which it has been calibrated and validated for. Validation and robustness analysis were performed by comparing simulated results to real observed data, while parameter values of the model were fixed (unlike during calibration, where they were modified iteratively).

Data used for validation and robustness assessment must differ from data used for calibration, to avoid any risk of tautology.

In the validation step, we verified that the model was able to reproduce the results of the ORION-11,²² FOURIER-OLE,²³ and ODYSSEY-OUTCOMES²⁴ studies. For robustness assessment of the model, studies with inclusion criteria differing slightly from the context of use in SIRIUS were used: IMPROVE-IT²⁵ which differs from SIRIUS in terms of the temporality of the qualifying event, and CANTOS²⁶ which explored the importance of residual inflammatory risk in the post-MI population.

Validation of a hazard ratio (HR) prediction was assessed based on three criteria: (i) inclusion of the observed HR in the simulated 95% prediction percentile interval (PPI, please refer to the uncertainty management section below), (ii) inclusion of the predicted HR in the 95% confidence interval (CI) and (iii) HR precision corresponding to proportion of the predicted 95% PPI included in the observed 95% CI above 30% (threshold chosen empirically). For the results deviating from the validation acceptability criteria, an adjudication was carried out with the scientific committee to understand the mechanistic rationale explaining this result, the impact on the credibility of the model, and the interpretation of SIRIUS simulations.

Given the results obtained during validation and robustness assessment [available in Wang et al. (2024) manuscript in progress], the ASCVD model was deemed successfully validated for its use to answer the SIRIUS research questions with confidence.

Virtual population

A Virtual Population (VPop) refers to a collection of virtual patients generated in-silico to capture realistic inter-patient variability. Each virtual patient corresponds to a set of model-dependent patient descriptors. A virtual patient is not an independent model entity, but rather a set of parameter values required to run the model. Each virtual patient has different parameter values for the set of 'patient-dependent'

parameters (e.g. age, sex, lipoprotein infiltration rate). The generation of a VPop requires a predefined list containing the disease model parameters that vary within a population, as well as a thorough characterization of these parameters (mean values, type of distribution, parameters of distribution, multivariate distribution also called joint distribution). The SIRIUS VPop is computer-generated, according to predefined parameter distributions, as to present baseline characteristics representative of a contemporary ASCVD population, mainly based on the FOURIER and ORION 11 RCTs, and recent European observational studies as well as data from the French health data system (SNDS) on LLT prescription.^{21,22,27} Inclusion criteria combine: virtual patients with ASCVD (defined as any of the following: previous MI, previous IS, previous symptomatic peripheral arterial disease (PAD) defined as either intermittent claudication with ankle-brachial index (ABI) < 0.85, prior peripheral arterial revascularization procedure, or amputation due to atherosclerotic disease) and fasting LDL-C \geq 70 mg/dL under stable (\geq 4 weeks) well-tolerated lipid-lowering therapy corresponding to the background LLT to be administered during the future trial [i.e. high intensity (HI) statin with or without ezetimibe]. Virtual patients with acute coronary syndrome, IS, peripheral arterial revascularization procedure, or amputation due to atherosclerotic disease < 4 weeks prior to the first study visit are excluded.

From the entire SIRIUS VPop, two subpopulations are extracted according to the background therapy: (1) patients receiving HI statin alone and (2) patients receiving HI statin with ezetimibe, to evaluate the efficacy of inclisiran vs. ezetimibe and evolocumab, respectively. The efficacy of inclisiran vs. placebo is also evaluated in these two subpopulations.

Study protocol

The in-silico study protocol for the SIRIUS programme was published on clinical trial (NCT05974345) on 26 July 2023.

The main objective of the in-silico study SIRIUS is to predict the efficacy of inclisiran 300 mg subcutaneous (s.c.), administered on day 1, month 3 (day 90), and every 6 months thereafter in combination with currently available LLT, on 3-point major adverse cardiovascular events (3P-MACE) defined as the composite of CV death, nonfatal MI or nonfatal IS, and on CV death in a secondary prevention ASCVD population with elevated LDL-C. Three trials are performed in the SIRIUS programme comparing Inclisiran to 1) placebo as adjunct to HI statin therapy (corresponding to atorvastatin \geq 40 mg/day or rosuvastatin \geq 20 mg/day) with or without ezetimibe 10 mg/day, 2) ezetimibe 10 mg/day as adjunct to HI statin therapy (corresponding to atorvastatin \geq 40 mg/day or rosuvastatin \geq 20 mg/day), 3) evolocumab 140 mg s.c. every 2 weeks as adjunct to HI statin therapy and ezetimibe 10 mg/day (Figure 3). The duration of the follow-up is 5 years.

Secondary objectives include predicting the efficacy of inclisiran therapy in combination with currently available LLTs on fatal and nonfatal MI, fatal and nonfatal IS and MALE in a secondary prevention ASCVD population with elevated LDL-C as well as quantifying the link between reduction in LDL-C levels and reduction in the incidence of clinical events.

As exploratory objectives, the relative contribution of the decrease in LDL-C and Lp(a) on the efficacy of inclisiran therapy in combination with currently available LLTs on 3P-MACE reduction in a secondary prevention ASCVD population with elevated LDL-C is investigated. In addition, efficacy of inclisiran therapy in combination with currently available LLT on 3P-MACE, CV death, fatal and nonfatal MI, fatal and nonfatal IS and MALE are investigated for several subgroups of interest stratified by age, sex, baseline eGFR, medical history (prior MI, prior stroke or prior PAD), number of vascular beds affected, diabetes

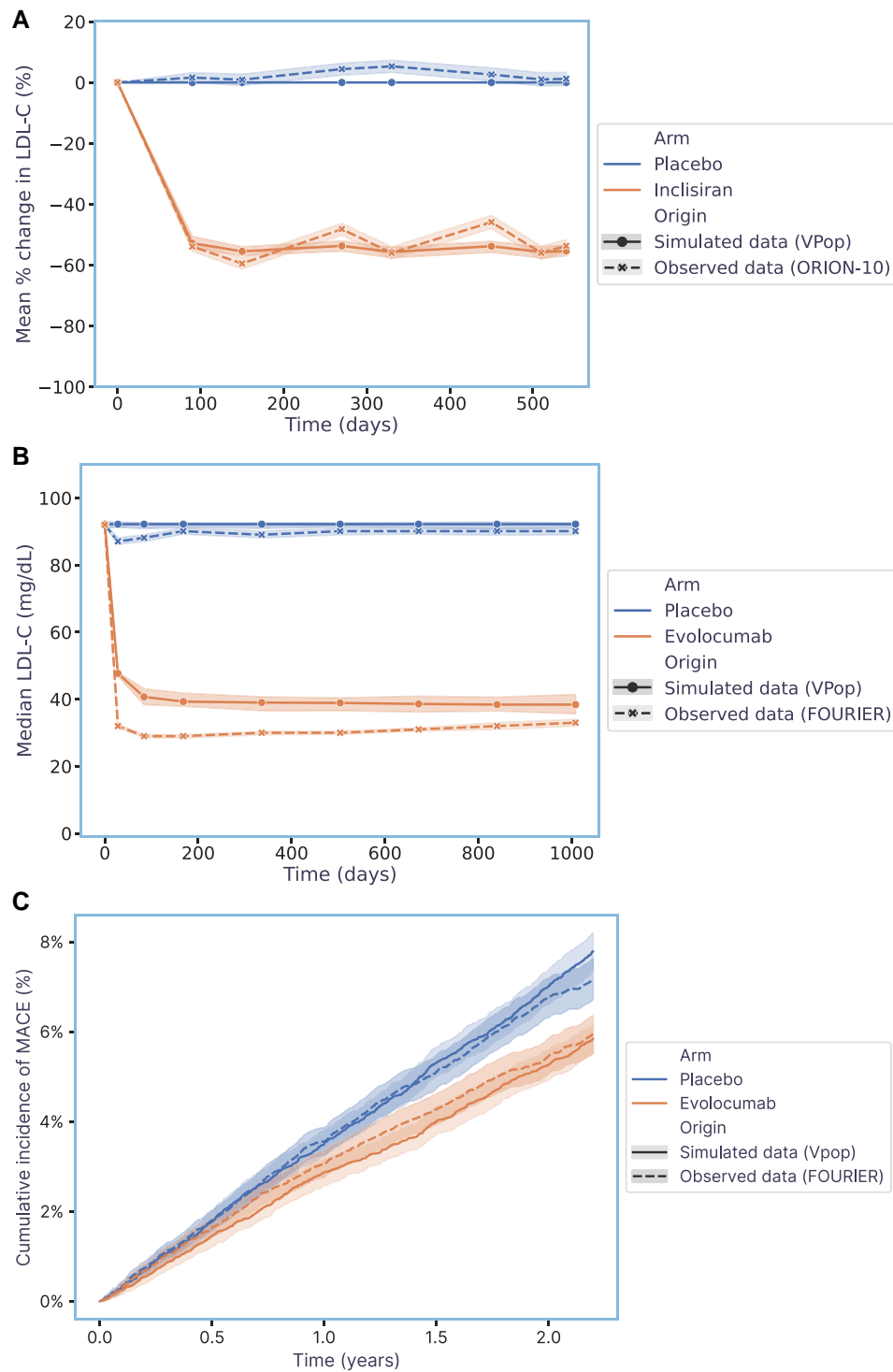


Figure 2 Goodness of fit of calibration results obtained with the ASCVD model and VPop. Comparison between simulated and observed data of (A) mean LDL-C relative change from baseline over time for LDL-C under placebo or inclisiran treatment, (B) median LDL-C level over time under placebo or evolocumab treatment, (C) cumulative incidence of MACE over time under placebo or evolocumab treatment. Simulated data were obtained by applying the placebo scenario and (A) inclisiran scenario or (B–C) evolocumab scenario to the calibrated ASCVD model and VPop and are shown in solid lines. Observed data are extracted from ORION-10 RCT⁶ for (A) and from FOURIER RCT²¹ for (B–C) and are shown in dashed lines. Placebo arms are shown in blue and treatment arms in orange, shading area represents the 95%CI for observed data and the 95%PPI for simulated data.

Abbreviations: MACE, major adverse cardiovascular event; CV death, cardiovascular death; MI, myocardial infarction; IS, ischemic stroke; MALE, major adverse limb event, RCT: Randomized controlled trial.

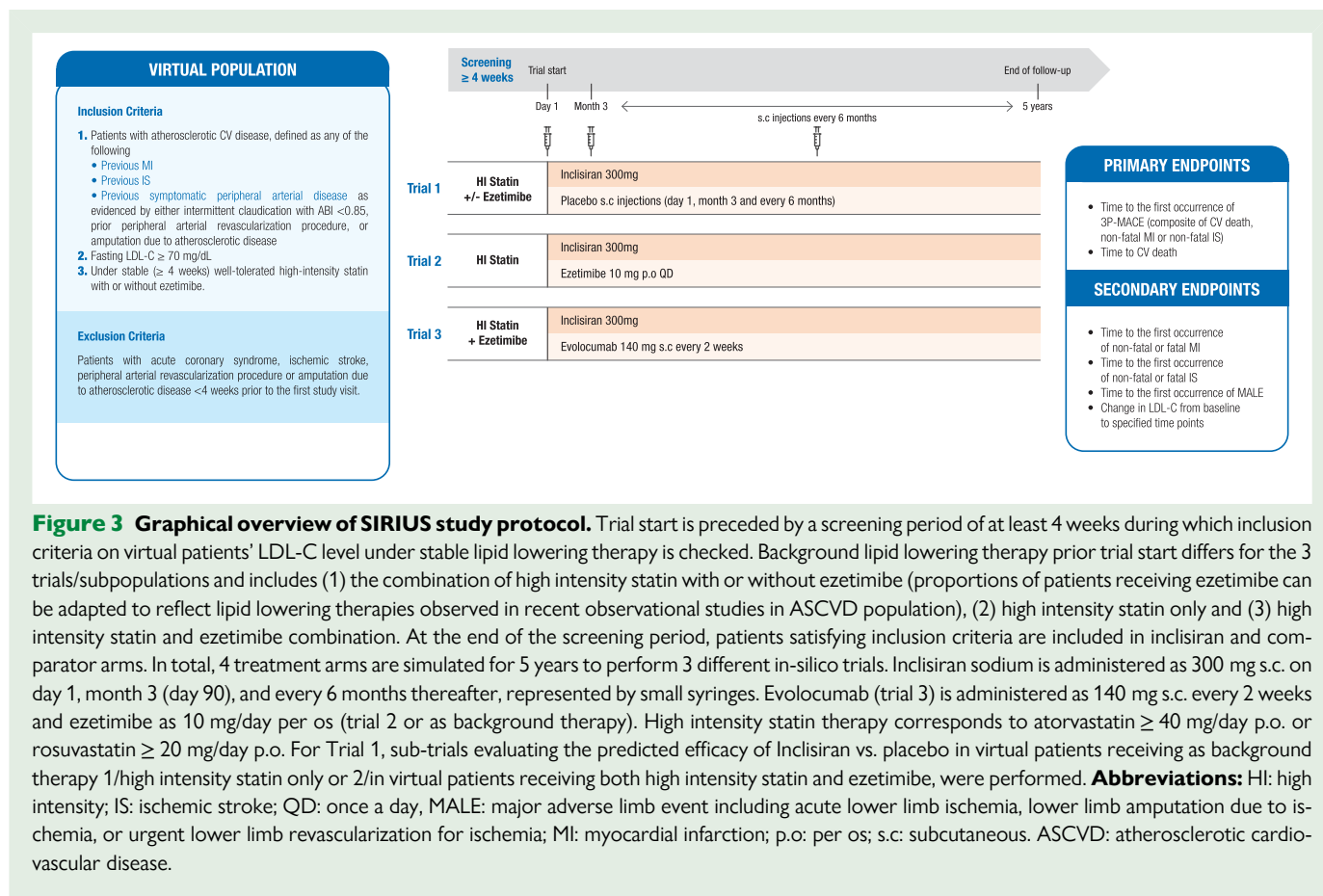


Figure 3 Graphical overview of SIRIUS study protocol. Trial start is preceded by a screening period of at least 4 weeks during which inclusion criteria on virtual patients' LDL-C level under stable lipid lowering therapy is checked. Background lipid lowering therapy prior trial start differs for the 3 trials/subpopulations and includes (1) the combination of high intensity statin with or without ezetimibe (proportions of patients receiving ezetimibe can be adapted to reflect lipid lowering therapies observed in recent observational studies in ASCVD population), (2) high intensity statin only and (3) high intensity statin and ezetimibe combination. At the end of the screening period, patients satisfying inclusion criteria are included in inclisiran and comparator arms. In total, 4 treatment arms are simulated for 5 years to perform 3 different in-silico trials. Inclisiran sodium is administered as 300 mg s.c. on day 1, month 3 (day 90), and every 6 months thereafter, represented by small syringes. Evolocumab (trial 3) is administered as 140 mg s.c. every 2 weeks and ezetimibe as 10 mg/day per os (trial 2 or as background therapy). High intensity statin therapy corresponds to atorvastatin \geq 40 mg/day p.o. or rosuvastatin \geq 20 mg/day p.o. For Trial 1, sub-trials evaluating the predicted efficacy of Inclisiran vs. placebo in virtual patients receiving as background therapy 1/high intensity statin only or 2/in virtual patients receiving both high intensity statin and ezetimibe, were performed. **Abbreviations:** HI: high intensity; IS: ischemic stroke; QD: once a day, MALE: major adverse limb event including acute lower limb ischemia, lower limb amputation due to ischemia, or urgent lower limb revascularization for ischemia; MI: myocardial infarction; p.o: per os; s.c: subcutaneous. ASCVD: atherosclerotic cardiovascular disease.

mellitus status, smoker status, uncontrolled high blood pressure (as a proxy for hypertension status), number of risk factors, or baseline Lp(a) levels. The efficacy of inclisiran on the various subgroups of interest are explored via forest plot analyses for 3P-MACE, CV death, fatal and nonfatal MI, fatal and nonfatal IS, and MALE.

Outcomes

The co-primary outcomes are based on time to the first occurrence between trial start and end of 5 years of follow-up of any component of the 3P-MACE and time to CV death. Secondary and exploratory outcomes comprise clinical and biological outcomes: based on time to the first occurrence between trial start and end of follow-up of MI (fatal or nonfatal), IS (fatal or nonfatal), MALE (including acute lower limb ischemia, lower limb amputation due to ischemia, or urgent lower limb revascularization for ischemia), lipid and lipoprotein change in LDL-C, HDL-C, non-HDL-C, Lp(a), ApoB, and triglycerides concentrations from baseline to specified time points (baseline, 30, 90, and every 6 months afterward) and time adjusted percentage changes in LDL-C from baseline between day 90 and the end of the follow-up period.

Virtual population size

Theoretically, there is no limitation in the generation of Virtual Patients. However, in practice, computational time and costs to run the model and analyze the results impose limitations. For the in-silico paradigm approach, population size will be chosen large enough to ensure the stability of model predictions in terms of CV event occurrence at the

population level. Further, although it will not be a direct criterion to determine the VPop size, population size would also ideally be chosen large enough to make predictions at the subgroup level for subgroups of interest.

Study management and scientific committee

Novartis was the SIRIUS programme sponsor. The SIRIUS in-silico trial is conducted by Novartis Pharma SAS in collaboration with the biomodelling centre Novadiscovery and under the guidance and leadership of the Scientific Committee.

Model design, calibration, and credibility assessment, virtual population design, and model simulations are performed by Novadiscovery using its modelling and trial simulation platform. Simulated data analyses are performed by Novadiscovery using R scripts, JinkoStats, and R studio software.

The scientific committee composed of external medical experts listed in the [supplemental data](#) was set up to advise on the in-silico study protocol and analysis plan. Their tasks were to review and formulate recommendations on the knowledge models, answer questions on knowledge gaps, and validate biological hypotheses and modelling choices. They also provided advice on the model calibration and validation strategy and results (i.e. assess adequation between simulation outputs and clinical observations), adjudicated validation results and identified implications for model and prediction credibility, and provided clinical advice on the analysis of simulation results.

Protection of human subjects and management and reporting of adverse events/adverse reactions

As part of this in-silico clinical trial, RCT data used to calibrate, validate the ASCVD and VPop were perfectly anonymized. Principles related to data protection are not applicable in this case. No safety data are generated as adverse events and reactions are not accounted for by the model.

Statistical considerations

In the in-silico paradigm interpretation, we assume that since we are not working in a sample of the population of interest but in a representation of the whole ASCVD population itself, the sampling theory-based statistical tests cannot apply. Thus, efficacy results are expressed as HR or incidence curves over time without 95% CI and *P*-value. Moreover, an in-silico trial allows evaluation of treatment efficacy that is not biased by inter-patient variability and the potential unbalance of the randomization process since each virtual patient is under its own control. In the in-silico paradigm, the distinction between superiority and non-inferiority trials, accounting for multiplicity, endpoint hierarchization are not applicable, as the efficacy value calculated by the in-silico trial, i.e. the mean absolute benefit value, directly illustrates the difference in efficacy of the treatments compared. For the same reasons, constraints on the number of subgroup analyses to be carried out, and on their possible *post-hoc* nature, are no longer relevant in this approach. The classic verbatim of primary or secondary criteria are used in this protocol for convenience, but they are not associated with statistical constraints of prioritization or alpha risk allocation (Figure 4).

The model allows for computing and comparing the absolute risk reduction between two treatments and so for deriving the number needed to treat (NNT) value. In addition, the in-silico approach with all virtual patients being their own control, allows to precisely determine the number of events prevented by inclisiran (simply computed by the difference between the number of events occurring in the control arm minus the number of events in the inclisiran arm).

Uncertainty management

A certain number of contingencies and inaccuracies may arise from the model predictions. These uncertainties may be due to the knowledge model structure (knowledge hypothesis, mathematical solutions) and model parameters. Model uncertainties were assessed and quantified all along the model development process (see supplemental data for details and Wang et al, manuscript in progress).

The uncertainty of the model's prediction for a given measurement such as HR is approximated by calculating the 95% PPI generated using bootstrapping. Bootstrapping is an approach that calculates the distribution of a statistical metric by drawing (with replacement) a certain number of samples of a pre-determined size from a population (Figure 4).

In the case of the SIRIUS study, the 95% PPI associated with the metric of interest (e.g. HR) was obtained by deriving/calculating this measure for 100 (*n*) populations of 13 780 patients (*n* chosen to reflect the order of magnitude of the populations usually included in RCTs exploring the effect of LLT on cardiovascular events; 13 780 being chosen according to FOURIER RCT where 13 780 and 13 784 patients were enrolled in the placebo and evolocumab arms respectively) randomly subsampled from the total population with replacement. This 95% PPI derived by bootstrapping should not be confused with a 95% CI,

the former measuring the degree of uncertainty in the model's predictions, the latter measuring, in a real-life context, the uncertainty linked to sampling. By construction, 95% PPI includes both uncertainties on the model itself and uncertainties on parameter values as well as inter-patient variability as reflected in the virtual population.

To avoid any confusion, PPI numerical intervals will be translated qualitatively into degrees of uncertainty, ranging from low to high based on interval widths (Figure 4).

Discussion

SIRIUS is the first in-silico trial programme deemed to predict the effect of inclisiran on top of standard-of-care LLT on clinical outcomes in an ASCVD virtual population. The ORION-9, -10 and -11, 18-months phase III RCT compared inclisiran to placebo as adjunct to maximally tolerated statin therapy in 3660 participants with ASCVD, ASCVD risk equivalent, and/or heterozygous familial hypercholesterolemia (HeFH). In the phase III studies, treatment with inclisiran sodium 300 mg administered by s.c. injection on day 1, month 3 (day 90) and every 6 months thereafter resulted in placebo-adjusted percentage reductions in LDL-C from baseline at Day 510 of 48% to 52%, with time-adjusted average reductions of 44% to 54% sustained over 18 months.^{6,28} The efficacy of inclisiran was consistent across phase II and phase III studies, and across a broad range of subpopulations.²⁹ The doses and administration schedule of inclisiran in our model strictly follow those of the previously published RCT as well as those used in the ongoing CV outcome trials.³⁰

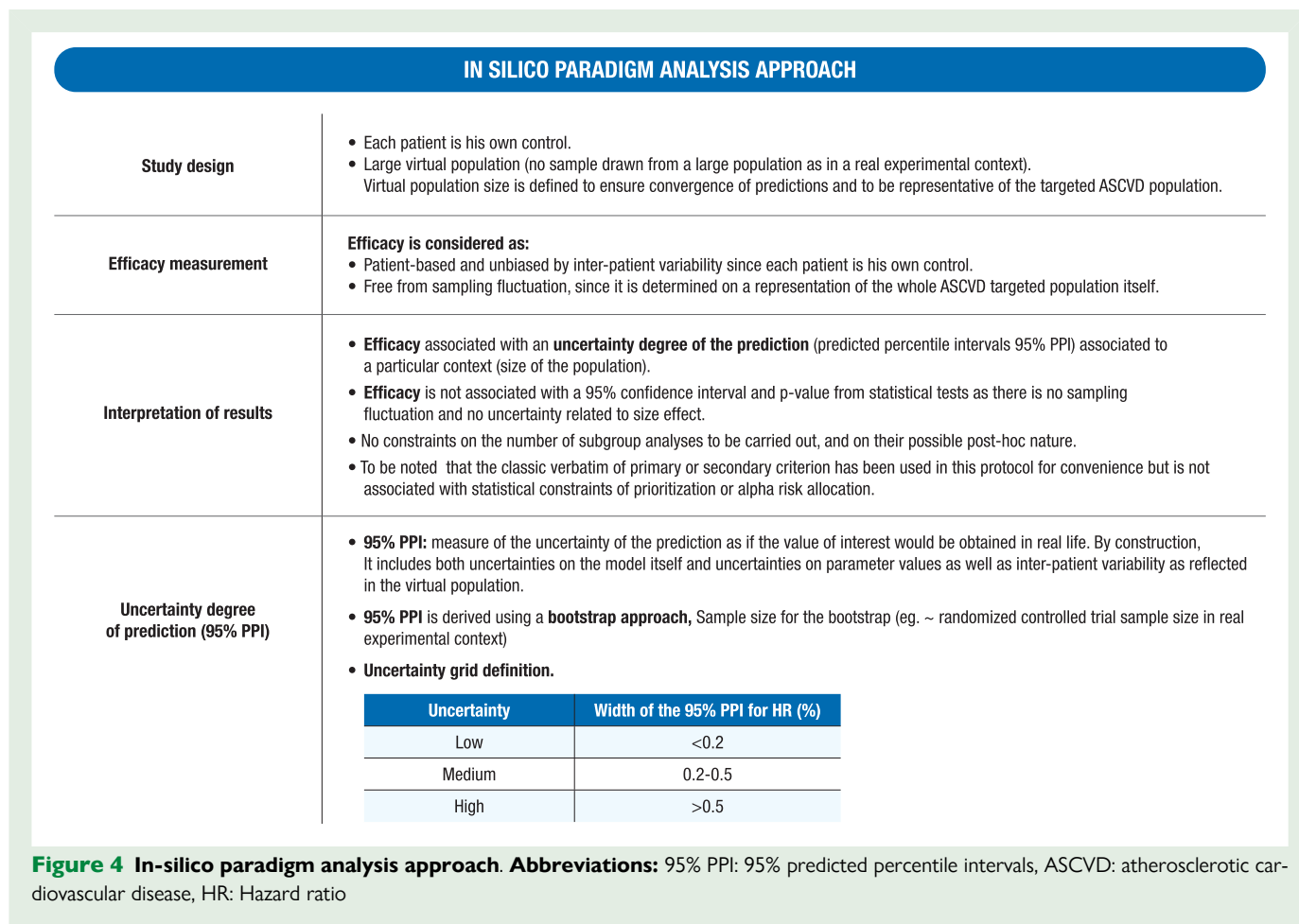
The correlation between LDL-C levels and CV risk has been robustly documented with evidence from >200 studies involving >2 million participants that have shown a dose-dependent log-linear association between the magnitude of exposure to LDL-C and risk of ASCVD.³¹ Moreover, evidence has consistently linked therapeutic reductions in LDL-C with reduced risk of CV events, regardless of the therapeutic class (statins, ezetimibe, PCSK9 blocking mAbs)³² with the only exception of fibrates. Since the results of ORION-4 and VICTORION-2-Prevent, phase III studies assessing the impact of inclisiran on major adverse CV events in participants with established CV disease will not be available before 2026, the results of this in-silico study prediction can contribute to consolidate the knowledge regarding inclisiran's clinical benefit in ASCVD patients.

We chose to generate a VPop matching patient's characteristics of the FOURIER study because it was a study investigating the efficacy of a PCSK9 inhibitor in secondary prevention encompassing the broad range of ASCVD phenotypes (prior MI, IS, and PAD). We also chose to adjust the number of virtual patients receiving atorvastatin vs. rosuvastatin or ezetimibe based on several recent observational studies to better match current practice on LLT following recent guidelines advocating for more intense LLT use in high-risk patients.^{2,27}

Clinical and biological criteria of the SIRIUS in-silico trials are similar to the ones explored in recent major LLT RCT.^{21,24} In the SIRIUS in-silico study, safety criteria were not included, considering that inclisiran safety is already well documented through ORION and VICTORION research programmes and post-market surveillance for drug safety.⁷

In-silico trials present several advantages compared to traditional RCT that we leveraged to design the SIRIUS programme.

The use of an in-silico clinical trial allows to investigate 5 years treatment and follow-up for all patients which is barely achievable in RCT. For example, in FOURIER, the median follow-up duration was 2.2 years, and in ODYSSEY OUTCOMES it was 2.8 years. Despite a significant



reduction of MACE in the PCSK9 inhibitors arms, both trials failed to show significant CV death reduction, and this was partly attributed to a short follow-up in very well treated patients. Of note a longer treatment duration and follow-up in the FOURIER-OLE study showed significant CV death reduction in the subgroup of patients that received evolocumab for 7 years vs. those who received it for 5 years after 2 years of placebo.²³

In-silico trials are not limited in the number of comparator arms, allowing comparisons to several active comparators such as ezetimibe or evolocumab as proposed in the SIRIUS trial. These comparisons would be difficult to achieve using the classic sampling approach with RCTs, due to the large sample size required or the length of follow-up necessary to demonstrate a hypothesis of superiority or non-inferiority of inclisiran vs. these active comparators. For example, sample sizes required to investigate inclisiran superiority over ezetimibe and non-inferiority vs. evolocumab with a 20% non-inferiority margin were estimated to be 54 724 and 36, 932 patients respectively for a 5-year trial duration with a 1:1 allocation ratio, alpha of 0.025 and beta of 90% and based on 3P-MACE and CV death hazard rate and treatment efficacy hypotheses derived based on available published RCTs data. These comparisons are made possible using the large SIRIUS VPop. In-silico trials can also be used to explore specific populations for which few data are available such as ASCVD patients with low LDL-C baseline, or statin-intolerant patients. These patients are rarely included in real morbi-mortality studies, as long follow-up period or large sample sizes are needed to demonstrate the superiority of the LLT of interest vs. the comparator. These populations might be studied

in the SIRIUS trial, after specific calibration and validation of the model and the virtual population.

In-silico trials allow us to obtain results more rapidly and at lower cost. In addition, it removes randomization bias as each patient is its own control. Moreover, potential confounding variables are controlled as environment in simulations is perfectly defined (compliance, adherence, diet). The limited number of patients in subgroups of RCTs greatly limits the ability to analyze fine-grained patient subgroups differences whereas in an in-silico trial, the large size of the VPop allows a higher number of patients in each subgroup of interest for which stability of predictions is reached.³³

In-silico clinical trials differ from RCTs conducted in real life mainly in that they do not rely on the sampling theory and therefore do not require usual statistical tests. Under the in-silico paradigm, the efficacy of treatments can be computed, and underlying mechanisms and causality explaining the impact of treatments can be explored. Confidence in in-silico paradigm outputs relies on the proper evaluation of the overall credibility of the model through the assessment of the methodological framework and the estimate of model's associated uncertainty along with the representativity of its associated VPop and the use of each virtual subject as its own control.¹³

In-silico trials of the SIRIUS programme are conducted using a validated knowledge-based mechanistic computational model of ASCVD applied to a VPop. The development of this in-silico mechanistic model was performed according to a high-level framework inspired by FDA guidance.³⁴ During the model construction, simplifying knowledge assumptions or modelling hypothesis must sometimes be made due to a

gap of knowledge or the complexity of involved pathophysiological mechanisms. The multidisciplinary scientific committee discussed and validated these hypotheses ensuring the pathophysiological credibility of the model built to answer the questions raised in the SIRIUS protocol. As an illustration, a modelling hypothesis was made considering that all IS occurring in the model are linked to a carotid plaque rupture event. The alternative would have been to mechanistically simulate non-atherosclerotic sub-etiologies of IS, with the need to develop, calibrate, and validate several other submodels (cardio-embolic, lacunar IS). All modelling and knowledge hypothesis of the model will be described in detail in a dedicated publication (Wang et al., 2024 manuscript in progress) and results of SIRIUS trials will be considered and discussed in the light of model structure and credibility assessment results.

Model calibration and validation are key steps performed according to a pre-defined protocol with acceptability criteria to ensure the credibility of predictions generated by the model. The fact that the literature is very dense and rich in the cardiovascular field was a strength for the calibration and validation process of the in-silico model. It reinforces the credibility of predictions on each endpoint of the SIRIUS programme. However, published randomized controlled trials generally focus on time to first event, and only provide details on event recurrences in *post hoc* analysis.^{35,36} Due to this unavailability of relevant published data for calibration/validation about recurrent events, the model does not simulate recurrent events in the same vascular bed and only one atheromatous plaque per vascular bed was modelled. This modelling choice has no impact on the relevance of the analysis as the SIRIUS in-silico trials only assess time to first event occurrence, but it could be a limitation for the exploration of CV death outcomes as recurrent CV events could contribute to it.

The aim of the SIRIUS protocol was to enable predictions at the population or at the subgroup level and not at the individual patient level. The model calibration and validation strategy were built around this purpose and hence the model can only generate predictions at those levels with good reliability. To be able to generate reliable personalized predictions or risk scoring at an individual level, the current model would need to be modified and another set of calibration and validation would be required.

This model and VPop have been specifically developed for the SIRIUS programme. Currently, the model is specifically designed to studying the impact of LLT by including treatment mechanistic submodels for H1 statins, ezetimibe, evolocumab, alirocumab, and inclisiran. However, thanks to the calibration using data from patients receiving other drugs (e.g. antihypertensive drugs, treatments for diabetes), the model can indirectly account for the effects of these additional drugs, despite not explicitly modelling them mechanistically. The impacts of those treatments cannot be specifically studied, but their effects are implicitly integrated into the simulation results.

This knowledge-based model can be evolutive and adapted to allow explicit modelling of other drugs by adding corresponding submodels or other populations of patients such as statin intolerant or primary prevention patients.

Conclusion

SIRIUS trials applying validated knowledge-based computational model of ASCVD and treatments to virtual patients is an innovative approach to complement RCTs. SIRIUS programme will provide insights regarding the potential effect of inclisiran on MACE in ASCVD patients,

several years before the availability of the results from ongoing outcomes trials (ORION-4 and VICTORION-2-P).

Authorship

DA, PA, AB, FB, BC, GM, LP, PGS: contributed to the conception and design of the study and critically analyzed the calibration and validation data. EP, EB, JPB, EC, AD, SG-N, RK, SP, YW built up the in-silico model and performed the calibration and validation processes. DA, LP and EB drafted the manuscript. PA, FB, BC, GM, PGS, JPB, AB, AF critically revised the manuscript. All gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Novartis as sponsor of SIRIUS programme was in charge of designing, reviewing and approving the study protocol and its amendments, designing and approving Statistical Analysis Plan, the study registration and results publication in public registries (<https://clinicaltrials.gov/>), reviewing and approving the knowledge models used to build the in-silico ASCVD model, approving the calibration and validation strategy of the model, transferring anonymized individual data from Novartis clinical studies used for the ASCVD model calibration and validation, reviewing and approving calibration/validation results of the in-silico ASCVD model, reviewing and approving study report regarding model development and SIRIUS in-silico results, participating in article writing and reviewing.

Conflict of interest: Denis Angoulvant reports having received payment or honoraria for lectures, presentations, speakers bureaus or educational events from Amgen, Alnylam, Amarin, Astra Zeneca, Boehringer, BMS, Bouchara Recordati, Pfizer, Novartis, Novo Nordisk, Organon, Sanofi, Servier, Vifor.

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Data availability

Calibration and validation data used to generate the figures presented in the manuscript will be made available in the technical publication by Wang et al (in progress) and upon reasonable request to the corresponding author.

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