ESC European Society of Cardiology

European Journal of Preventive Cardiology (2025) **00**, 1–10
https://doi.org/10.1093/eurjpc/zwaf783

FULL RESEARCH PAPER
Cardiovascular disease

Prediction of inclisiran efficacy in patients with established atherosclerotic cardiovascular disease: the SIRIUS *in-silico* modelling of cardiovascular outcomes

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Received 3 September 2025; revised 17 November 2025; accepted 17 December 2025; online publish-ahead-of-print 26 December 2025

Aims Inclisiran, an siRNA-targeting hepatic PCSK9 mRNA, reduces low-density lipoprotein cholesterol (LDL-C), but its effect on major adverse cardiovascular event (MACE) remains unconfirmed. The SIRIUS *in-silico* modelling programme aimed to predict the efficacy of inclisiran on MACE in virtual patients with atherosclerotic cardiovascular disease (ASCVD).

Methods and results The SIRIUS simulation (NCT05974345) used a validated mechanistic model of ASCVD and lipid-lowering therapy (LLT) effects in a virtual population with established ASCVD and LDL-C ≥ 70 mg/dL. Each virtual patient served as their own control to compare inclisiran vs. placebo as an adjunct to high-intensity statin therapy, alone or with ezetimibe over 5 years. The model did not account for non-adherence, recurrent events, or adverse effects. Among 204 691 virtual patients, inclisiran was predicted to reduce LDL-C by 49.7% vs. placebo (from 91.1 to 48.3 mg/dL). Relative to placebo, inclisiran was predicted to lower 5 years risk of 3-point MACE by 25.2% (11.3% vs. 14.9%), myocardial infarction by 34.8% [5.7% vs. 8.6%; hazard ratio (HR) 0.65], ischaemic stroke by 26% (2.6% vs. 3.4%; HR 0.74), and major adverse limb event by 34.1% (0.5% vs. 0.8%; HR 0.66). A 7.1% relative reduction of cardiovascular death was predicted (4.2% vs. 4.5%; HR 0.93).

Conclusion SIRIUS is the first *in-silico* simulation using a knowledge-based mechanistic model to predict the efficacy of LLT on cardiovascular outcomes in ASCVD. These findings offer an early model-based prediction of inclisiran's potential cardiovascular benefit ahead of Phase 3 outcome trials.

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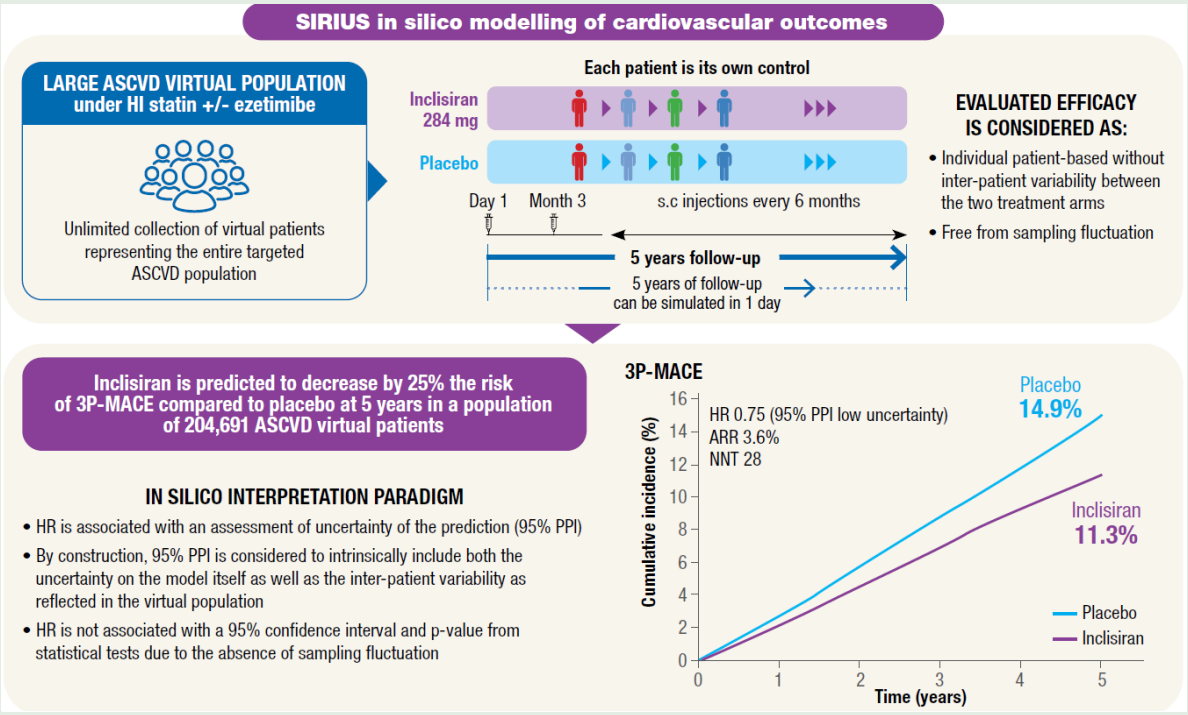
Lay summary

The SIRIUS *in-silico* simulation used a validated knowledge-based mechanistic computational model to computationally simulate inclisiran efficacy on 5-year MACE in 204 691 virtual patients with atherosclerotic cardiovascular disease (ASCVD).

- Mean predicted percentage reduction in LDL-C with inclisiran vs. placebo was 49.7% at 5 years, with a 25.2% reduction in 3-point MACE.
- This simulation provides early insights into the potential effect of inclisiran on cardiovascular event reduction in advance of results from ongoing Phase 3 trial.

Graphical Abstract



Keywords Inclisiran • PCSK9 inhibition • Atherosclerotic cardiovascular disease • LDL-C • Secondary prevention • *In-silico* modelling

Introduction

Pharmacological proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition is recommended in combination with a healthy diet to reach low-density lipoprotein cholesterol (LDL-C) targets and reduce the risk of cardiovascular events in patients with atherosclerotic cardiovascular disease (ASCVD) who are not at goal on statins at the maximum-tolerated dose in combination with ezetimibe.[1] Monoclonal antibodies inhibiting PCSK9, such as evolocumab and alirocumab, have demonstrated their clinical benefits by reducing ASCVD events in secondary prevention.[2,3] Inclisiran is a small, interfering ribonucleic acid (siRNA) that prevents hepatic PCSK9 expression and its subsequent secretion in the circulation.[4] With twice-yearly administration (after the initial and 3-month dose), it has proven both its safety and efficacy to reduce plasma PCSK9 and LDL-C in high-risk patients.[5,6] In ORION-8, an open-label extension study with a mean follow-up of 3.7 years, mean percentage LDL-C reduction was 49.4% [95% confidence interval (CI) −50.4, −48.3] with inclisiran, without attenuation of LDL-C lowering over time.[7] Two cardiovascular outcomes trials are ongoing, ORION-4 (NCT03705234) and VICTORION-2-Prevent (NCT05030428), which are evaluating whether lowering LDL-C with inclisiran reduces risk of major cardiovascular adverse events (MACE) in ASCVD patients.

In-silico predictive modelling programmes form part of the model-informed drug-development approach proposed by the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA), in complement to clinical trials, to support drug development.[8,9] These *in-silico* modelling programmes apply a mechanistic computational disease and treatment model to virtual patients to predict and analyse the effect of drugs or interventions.[10,11] To date, no such approach has been applied to model the cardiovascular outcomes of lipid-lowering therapy (LLT) in ASCVD. The aim of the SIRIUS *in-silico* modelling programme is to emulate the effect of inclisiran on 3-point MACE, defined as the first occurrence of cardiovascular death, non-fatal myocardial infarction, or non-fatal ischaemic stroke, in a virtual population with established ASCVD and LDL-C ≥ 70 mg/dL.[12,13]

Methods

Model construction

A knowledge-based mechanistic computational model simulating functional relationships between the biological entities involved in the pathophysiology of ASCVD was built collaboratively by scientific experts in the field of ASCVD (D.A., P.A., F.B., B.C., G.M., and P.G.S.) and by Nova In Silico (Lyon, France). Details on the modelling approach and ASCVD

computational model structure are provided in Angoulvant *et al.*[13] and Wang *et al.*[12] Briefly, the model encompasses lipoprotein metabolism and cholesterol homeostasis, dynamics of atherosclerotic plaque growth with lipoprotein infiltration in the intima, and evolution of lipidic, necrotic, and fibrotic tissues, as well as plaque rupture leading to myocardial infarction, ischaemic stroke, or major adverse limb event (MALE), depending on plaque location. Myocardial infarction and ischaemic stroke were generated mechanistically via plaque rupture pathways, while cardiovascular death (fatal myocardial infarction, fatal ischaemic stroke, or other causes of cardiovascular death) was derived from an external Cox model adapted from Wilson *et al.*, conditioned on simulated event history and risk factors.[14] This hybrid approach ensures internal consistency across endpoints while allowing mortality prediction based on well-validated survival relationships. The ASCVD model also included age, sex, body mass index, medical history of diabetes mellitus, blood pressure, smoking status, systemic inflammation, and known mechanisms of action of lipid-lowering therapies (LLT) including atorvastatin, rosuvastatin, ezetimibe, evolocumab, and inclisiran. The mechanistic model has been encoded and solved on Nova In Silico proprietary software Jinkō, which relies on the programming language Haskell and the ODE solver Sundials.

The credibility of the ASCVD model was assessed following the guidelines recommended by the FDA[15] and EMA.[8] A clear context of use and questions of interest were defined, and model risks were assessed. Model credibility was established through multiple lines of evidence, including assessment of model plausibility, transparency of model structure, verification of code and calculations, quality check credibility goals, sensitivity analyses, calibration goodness-of-fit, and population-based validation and robustness evidence.[16] All supporting evidence was documented in a comprehensive credibility report.

Calibration of the ASCVD model and virtual population was performed at the population and subgroup levels to fit with observed summarized data extracted from 36 articles, including data from the FOURIER[2] (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk), ORION-1,[17] and ORION-10[5] studies, in terms of plasma lipoproteins decrease under combinations of LLT and associated reduction in cardiovascular events.

The credibility of the ASCVD model was successfully demonstrated according to a pre-defined protocol that ensured a clear separation between datasets used for calibration and those reserved for validation (ORION-11,[18] FOURIER-OLE,[19] and ODYSSEY OUTCOMES[3]) and robustness analysis (IMPROVE-IT[20] and CANTOS[21]), while also defining explicit validation metrics. Among 829 comparative metrics assessing simulated vs. observed data, 735 met the pre-defined acceptability criteria (88.7%), establishing the model's accuracy and building confidence in its predictive capability within the SIRIUS programme. An overview of the credibility assessment of the ASCVD computational model is shown in Supplementary material online, *Figure S1*, while more details on the credibility assessment of the ASCVD computational model are provided in Angoulvant *et al.*[13] and Wang *et al.*[12]

Study objectives and virtual population

An overview of the study design has been published.[13] The main objectives of the SIRIUS *in-silico* simulation (NCT05974345) were to emulate the 5-year efficacy of inclisiran vs. placebo, in combination with high-intensity statin treatment, with or without ezetimibe, on (i) 3-point MACE and (ii) cardiovascular death, in a virtual population with established ASCVD and LDL-C ≥ 70 mg/dL. Three-point MACE was defined as the first occurrence of cardiovascular death, non-fatal myocardial infarction, or non-fatal ischaemic stroke. Inclisiran 284 mg was administered subcutaneously on Day 1, Month 3 (Day 90), and every 6 months thereafter. High-intensity statin therapy was simulated using four possible regimens, comprising atorvastatin at doses of ≥40 or 80 mg/day or rosuvastatin at doses of ≥20 or 40 mg/day, thereby introducing variability in the background LLT among virtual patients. Ezetimibe was administered at a standardized dose of 10 mg/day.[13]

The secondary objectives included predicting the efficacy of inclisiran in combination with high-intensity statin with or without ezetimibe on fatal and non-fatal myocardial infarction, fatal and non-fatal ischaemic stroke, and MALE (including acute lower limb ischaemia, lower limb amputation, or revascularization due to ischaemia).

As exploratory objectives, the efficacy of inclisiran in combination with high-intensity statin with or without ezetimibe on plasma lipoprotein outcomes of interest was investigated. In addition, the predicted efficacy of inclisiran on 3-point MACE was investigated for several subgroups, stratified by age group, sex, baseline estimated glomerular filtration rate (eGFR), medical history [previous myocardial infarction, stroke, or peripheral artery disease (PAD)], number of vascular beds affected having led to a clinical event, diabetes mellitus status, smoking status, uncontrolled high blood pressure (as a proxy for hypertension status), and number of risk factors.

All virtual patients had established ASCVD, defined as any of the following: Previous myocardial infarction, previous ischaemic stroke, previous symptomatic PAD (defined as intermittent claudication with ankle-brachial index <0.85, previous peripheral artery revascularization procedure, or amputation due to atherosclerotic disease), with fasting LDL-C ≥ 70 mg/dL on stable (≥4 weeks) high-intensity statin therapy alone or in combination with ezetimibe. Virtual patients with acute coronary syndromes, ischaemic stroke, peripheral artery revascularization procedure, or amputation due to atherosclerotic disease during the 4 weeks before the first study visit were excluded.

The SIRIUS virtual population was computer-generated according to pre-defined rules, as to present baseline characteristics representative of a contemporary ASCVD population. The virtual population baseline characteristics were inspired mainly by the FOURIER and ORION-11 studies and recent European observational studies, as well as data from the French social security database (*Le Système National des Données de Santé*) on LLT prescription to reflect current trends in the use of LLT.[2,18,22,23]

The virtual population accounts for inter-patient variability by varying certain model parameters among virtual patients (value randomly drawn for each virtual patient within a pre-defined distribution). Intra-patient variability was not addressed in this project because the model is intended for making predictions at the population or subgroup level and not at the individual level. However, we could consider that intra-patient variability is included in the virtual population variability, as inter-patient variability certainly provides more variability than intra-patient variability.

Each virtual patient was duplicated and used as its own control, receiving in one arm inclisiran as add-on to high-intensity statin alone or in combination with ezetimibe, and in the other arm placebo as add-on to high-intensity statin alone or in combination with ezetimibe. Lack of adherence to treatment and discontinuation of background LLT were not simulated in either arm. Placebo effect was not mechanistically modelled, but it was calibrated during model building. The model did not account for recurrent events or adverse effects, which were beyond the scope of this analysis.

The size of the population was defined to ensure prediction convergence and good representativeness of each subgroup. The SIRIUS virtual population included 204 691 virtual patients.

Statistical analysis

The analysis included the use of summary statistics for continuous variables [mean with standard deviation (SD) or median with quartile (Q)1, Q3] and percentages for discrete variables.

Efficacy results in terms of cardiovascular events are reported as 5-year incidence rates, hazard ratios (HR), relative and absolute risk reductions (RRR and ARR, respectively), and number-needed-to-treat (NNT), and are illustrated using cumulative incidence curves. In addition, the *in-silico* approach (with each virtual patient being its own control) allowed precise determination of the number of events prevented by inclisiran (computed by the difference between number of events in the control arm minus number of events in the inclisiran arm).

The uncertainty related to model prediction for a given measurement was approximated using the 95% percentile prediction interval (PPI) generated with a bootstrap method.[24,25] The PPI is an overall quantification of prediction uncertainty, including both uncertainties on the model itself and uncertainties on parameter values, as well as inter-patient variability

as reflected in the virtual population. The number of bootstrap iterations was selected after having performed stabilization tests. One hundred bootstraps are estimated to be large enough to reach a convergence of the 95% PPI with reasonable computational times. Sample size was chosen to reflect the order of magnitude of the populations usually included in randomized clinical trials exploring the effect of LLT on cardiovascular events (13 780 being chosen according to FOURIER,[2] which corresponds to the number of patients in one arm). Thus, one hundred samples of 13 780 patients were randomly drawn with replacement from the SIRIUS total virtual population ($n = 204\,691$). The measure of interest (e.g. HR) was computed for each of these 100 samples. The 95% PPI corresponds to the extraction of the 2.5–97% empirical quantiles from the distribution of the measure of interest obtained with the 100 samples (see Supplementary material online, *Figure S2*).

PPI numerical values were translated qualitatively into uncertainty levels qualified as low, medium, or high according to the width of the 95% PPI (<0.2, 0.2–0.5, and >0.5, respectively).[13] As no guideline exists to define such categories, they were consensually defined by the research team.

In the SIRIUS *in-silico* simulation, which involves a large virtual population considered as the entire population of interest, we assume that sampling-theory–based statistical tests do not apply. Consequently, we do not make any superiority or non-inferiority hypotheses, nor do we have constraints related to multiplicity testing, outcome hierarchization, or number of subgroup analyses to be carried out, and their possible post-hoc nature.[26,27] The classic verbatim of primary or secondary criterion was used in the SIRIUS protocol for convenience, but corresponding predictions were not associated with statistical constraints of prioritization or alpha-risk allocation.[13]

Data management and analyses of model simulations were performed by Nova In Silico using Python scripts.

Results

Baseline characteristics

The SIRIUS virtual population ($n = 204\,691$) comprised 75.0% males and had a mean (SD) age of 62.5 (8.7) years and a mean (SD) body mass index of 29.1 (5.1) kg/m^2. Overall, 82.4% of patients had a history of myocardial infarction, 19.2% had a history of ischaemic stroke, and 13.7% presented symptomatic lower extremity PAD as a qualifying event. Polyvascular ASCVD, defined as more than one qualifying event among myocardial infarction, ischaemic stroke, and PAD, was present in 15.0% of the virtual population. Background comorbidities included diabetes (36.8%), current smoking (28.8%), moderate chronic kidney disease (20.4%; eGFR 30–59 mL/min/1.73 m^2), heart failure (23.7%), and atrial fibrillation (8.7%). All patients were on high-intensity statin therapy (80.3% atorvastatin and 19.7% rosuvastatin). One patient in five (20.6%) received ezetimibe in combination with a high-intensity statin. Despite such optimized lipid-lowering background treatment, the population had a median (Q1, Q3) LDL-C concentration of 91.1 (78.9, 115.7) mg/dL (*Table 1*).

Table 1 Baseline characteristics of the SIRIUS virtual population

Characteristic	$n = 204\,691$
Age, years	62.5 (8.7)
Sex, male	153 475 (75.0)
Body mass index, kg/m^2	29.1 (5.1)
Mean sitting systolic blood pressure, mmHg	129.2 (115.4, 143.0)
Mean sitting diastolic blood pressure, mmHg	79.9 (73.0, 86.8)
Current smoker	58 994 (28.8)
Diabetes mellitus	75 319 (36.8)
High-sensitivity C-reactive protein, mg/L	1.7 (0.7–3.7)
eGFR, mL/min/1.73 m^2	75.8 (62.4–89.2)
<30 mL/min/1.73 m^2	2127 (1.0)
30–59 mL/min/1.73 m^2	41 660 (20.4)
Qualifying cardiovascular event	
ASCVD	204 691 (100)
Myocardial infarction	168 760 (82.4)
Ischaemic stroke	39 371 (19.2)
Symptomatic lower extremity peripheral artery disease	28 072 (13.7)
Polyvascular ASCVD	30 776 (15.0)
Other cardiovascular medical history	
Atrial fibrillation	17 892 (8.7)
Heart failure	48 600 (23.7)
Concomitant treatment	
Antiplatelet drug	190 155 (92.9)
High-intensity statin	204 691 (100)
Atorvastatin (40–80 mg/day)	164 463 (80.3)
Rosuvastatin (20–40 mg/day)	40 228 (19.7)
Ezetimibe	42 238 (20.6)
Lipoprotein and lipid concentrations	
LDL-C mg/dL	91.1 (78.9, 115.7)
Total cholesterol, mg/dL	165.9 (149.0, 187.4)
Non-HDL-C, mg/dL	114.2 (101.4, 138.4)
HDL-C, mg/dL	47.5 (43.4, 51.7)
Apolipoprotein B, mg/dL	79.3 (69.4, 93.7)
Triglycerides, mg/dL	133.9 (95.8, 171.6)
Lipoprotein(a), nmol/L	40.9 (12.4, 140.3)
PCSK9, µg/L	374.7 (123.6)

Values are *n* (%), median (Q1, Q3), or mean (SD). Dichotomous variables are reported as percentage with the characteristic.
ASCVD, atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9.

Predicted efficacy of inclisiran on low-density lipoprotein cholesterol reduction

Predicted mean (SD) percentage reduction in LDL-C with inclisiran was 49.7% (25.2), from a median (Q1, Q3) baseline of 91.1 mg/dL (78.9, 115.7) to 48.3 mg/dL (22.5, 70.2) at 5 years (*Figure 1*). Predicted mean (SD) percentage reductions in lipoprotein(a), apolipoprotein B, and high-density lipoprotein cholesterol concentrations with inclisiran were, respectively, −13.3% (10.2), −39.4% (20.4), and −2.7% (3.1) (*Table 2*).

Predicted efficacy of inclisiran on major adverse cardiovascular event

At 5-year follow-up, inclisiran vs. placebo was predicted to reduce the relative risk of 3-point MACE by 25.2%, with a low prediction uncertainty (11.3% vs. 14.9%; ARR 3.6%; NNT 28) (*Table 3* and *Figure 2A*). At 5-year follow-up, inclisiran vs. placebo was predicted to reduce cardiovascular death by a relative 7.1% (4.2% vs. 4.5%; HR 0.93; ARR 0.3%; NNT 323; medium prediction uncertainty) (*Figure 2B*). When separating the cardiovascular death criteria, inclisiran was predicted to reduce the relative risk of cardiovascular death from myocardial infarction by

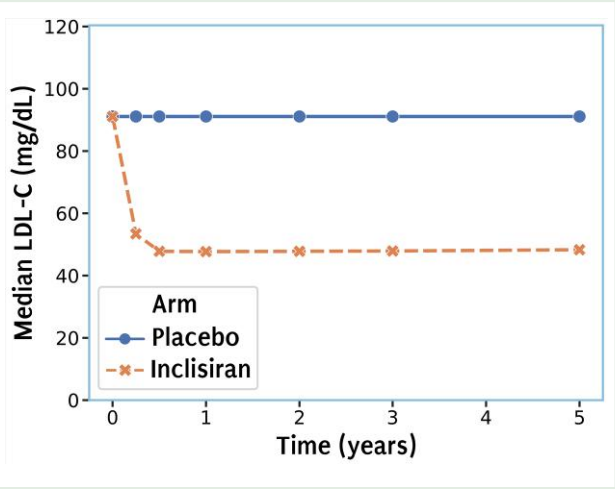


Figure 1 Simulated dynamics of plasma LDL-C concentration over time for inclisiran and placebo applied to the SIRIUS virtual population ($n = 204\,691$). Results are displayed as medians for placebo and inclisiran as add-on to high-intensity statin therapy alone or in combination with ezetimibe. 95% PPI was calculated and translates into low prediction uncertainty at all time points. LDL-C, low-density lipoprotein cholesterol; PPI, percentile prediction interval.

34.3% (0.4% vs. 0.5%; HR 0.66; ARR 0.2%; medium prediction uncertainty) and the relative risk of cardiovascular death from ischaemic stroke by 27.7% (0.4% vs. 0.5%; HR 0.72; ARR 0.1%; high prediction uncertainty). Inclisiran had no predicted effect on death from other cardiovascular causes (3.5% vs. 3.5%; HR 1.00; medium prediction uncertainty) (*Table 3*).

Secondary outcomes

At 5-year follow-up, inclisiran vs. placebo was predicted to reduce the risk of fatal or non-fatal myocardial infarction, fatal or non-fatal ischaemic stroke, and MALE. The predicted 5-year incidence of fatal or non-fatal myocardial infarction was 5.7% in the inclisiran-treated virtual population vs. 8.6% in the placebo-treated virtual population, translating into an RRR of 34.8% (HR 0.65) and an ARR of 2.9% (NNT 34) (*Figure 2C* and *Table 3*). This result was predicted with low uncertainty. The predicted 5-year incidence rate of fatal or non-fatal ischaemic stroke was 2.6% vs. 3.4%, translating into an RRR of 26.0% (HR 0.74) and an ARR of 0.9% (NNT 114) (*Figure 2D* and *Table 3*). This result was predicted with medium uncertainty. The predicted 5-year incidence rate of MALE was 0.5% vs. 0.8%, translating into an RRR of 34.1% (HR 0.66) and an ARR of 0.3% (NNT 357) (*Figure 2E* and *Table 3*). This result was predicted with medium uncertainty.

Subgroup analyses

Inclisiran was predicted to have a consistent effect across all subgroups, with an RRR in 3-point MACE of ~26% for most subgroups vs. placebo (*Figure 3*, Supplementary material online, *Figures S3* and *S4*).

Discussion

To our knowledge, this is among the first reports of a knowledge-based *in-silico* modelling of cardiovascular outcomes following LLT in ASCVD virtual patients. We used the SIRIUS simulation to predict the effect of inclisiran in a virtual population with established ASCVD while awaiting the results of the ORION-4 and VICTORION-2-Prevent Phase 3 cardiovascular outcomes trials.[28] *In-silico* modelling of clinical outcomes, applying ASCVD and LLT computational models to virtual ASCVD patients, offers an innovative approach to complement preclinical and clinical evidence and help in designing and derisking future randomized clinical trials in drug development plans.[29] This *in-silico* approach has previously demonstrated its ability to prospectively predict drug efficacy in cancer.[30]

In the SIRIUS simulation, the addition of inclisiran to high-intensity statin alone or in combination with ezetimibe was predicted to substantially lower the risk of cardiovascular events, with a 25% reduction in 3-point MACE at 5 years (cardiovascular death, non-fatal myocardial infarction, non-fatal ischaemic stroke) with a low prediction uncertainty. This prediction is of the same order of magnitude as the 26% MACE reduction reported at 540 days with inclisiran by Ray *et al.*, in a patient-level, pooled analysis of the ORION-9, ORION-10, and ORION-11 trials, in which MACE was evaluated as part of the safety assessments.[31] This finding could be translated into the prevention of 7318 events across 204 691 treated patients, with an NTT of 28 to prevent one additional event in the SIRIUS population.

The magnitude of the predicted inclisiran effect on each individual ASCVD event (myocardial infarction, ischaemic stroke, MALE) was consistent with data from recent clinical trials on alirocumab and evolocumab.[2,3]

It is noteworthy that SIRIUS predicted a reduction in cardiovascular death (RRR 7.1%) at 5 years. Among the causes of cardiovascular death, inclisiran was associated with a predicted RRR of 34.3% for fatal myocardial infarction and 27.7% for fatal ischaemic stroke vs. placebo, while no effect on other cardiovascular causes of death was predicted. These predictions need to be considered in light of modelling hypotheses structuring the ASCVD model. In particular, as opposed to atherosclerotic non-fatal events, cardiovascular death outcomes were not modelled mechanistically but by using statistical Cox models. Moreover, no mechanistic hypothesis was implemented in the model on the potential effect of LLT on causes of cardiovascular death other than fatal myocardial infarction and fatal ischaemic stroke. By reducing the risk of recurrent cardiovascular events such as myocardial infarction, LLT, including inclisiran, could also indirectly help to reduce these other causes of cardiovascular death (potentially due to arrhythmias and heart failure). The present ASCVD model does not simulate recurrent events in a given vascular bed and consequently does not capture this potential beneficial effect on cardiovascular death. Of note, recent cardiovascular outcomes trials of anti-PCSK9 monoclonal antibodies, while demonstrating clear effects on cardiovascular events and particularly myocardial infarction, did not significantly show reductions in cardiovascular death in short-term follow-up studies.[2,3] Significant cardiovascular death reduction was more remarkably observed in long-term follow-up of the open-label extension of the FOURIER study.[19]

SIRIUS predicted a 49.7% mean LDL-C reduction with inclisiran, stable from 1 to 5 years, consistent with data reported from previous trials, namely ORION-8 and VICTORION-INITIATE, which recently evaluated the effectiveness of an 'inclisiran first' implementation strategy compared with usual care in ASCVD.[7,32]

The main advantage of the SIRIUS simulation is that it allows the assessment of an ideal comparison of responses to treatments, with each virtual patient being its own control in a large ASCVD virtual population. SIRIUS was conducted using an ASCVD mechanistic model based on knowledge. This type of model differs from traditional statistical

Table 2 Lipid and lipoprotein results for placebo and inclisiran scenarios simulated in the SIRIUS virtual population

Biological variable	Baseline (common to both arms)	Placebo arm at 5 years	Inclisiran arm at 5 years	Difference in relative change from baseline to 5 years between inclisiran and placebo, %
LDL-C, mg/dL	101.7 (28.1)	101.7 (28.1)	51.4 (31.3)	−49.7 (25.2)
	91.1 [78.9, 115.7]	91.2 [79.0, 115.7]	48.3 [22.5, 70.2]	−44.2 [−78.2, −26.7]
Lipoprotein(a), nmol/L	87.3 (103.8)	87.3 (103.8)	67.0 (75.2)	−13.3 (10.2)
	40.9 [12.4, 140.3]	38.8 [12.4, 140.3]	37.4 [11.3, 79.3]	−9.6 [−22.3, −4.8]
Non-HDL-C, mg/dL	123.5 (29)	123.6 (29)	72.4 (32.3)	−41.3 (21.2)
	114.2 [101.4, 138.4]	114.3 [101.4, 138.4]	69.8 [43.3, 92.7]	−36.8 [−64.7, −21.2]
Triglycerides, mg/dL	136.1 (50.5)	136.0 (50.6)	117.7 (43.8)	−12.9 (9)
	133.9 [95.8, 171.6]	133.9 [95.8, 171.6]	118.7 [83.1, 151.2]	−10.5 [−18.4, −5.1]
Apolipoprotein B, mg/dL	84.6 (18.9)	84.6 (18.9)	51.0 (21.2)	−39.4 (20.4)
	79.3 [69.4, 93.7]	79.3 [69.4, 93.7]	49.5 [32.1, 63.9]	−35.5 [−61.1, −20.1]
HDL-C, mg/dL	48.2 (5.9)	48.3 (5.9)	47.0 (6)	−2.7 (3.1)
	47.5 [43.4, 51.7]	47.7 [43.5, 51.7]	45.5 [41.8, 51.5]	−1.1 [−4.4, −0.5]

Baseline, end of follow-up absolute concentrations, and between-arm difference in relative change from baseline to end of follow-up, are displayed as mean (SD) and median [Q1, Q3].
HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 3 Incidence of cardiovascular events simulated in the SIRIUS virtual population

Event	Five-year incidence, %		HR (associated uncertainty)	RRR, %	ARR, %
	Placebo arm	Inclisiran arm			
3-point MACE	14.9	11.3	0.75 (low)	25.2	3.6
Cardiovascular death	4.5	4.2	0.93 (medium)	7.1	0.3
Fatal myocardial infarction	0.5	0.4	0.66 (medium)	34.3	0.2
Fatal ischaemic stroke	0.5	0.4	0.72 (high)	27.7	0.1
Other cause	3.5	3.5	1.00 (medium)	0.0	0.0
Fatal or non-fatal myocardial infarction	8.6	5.7	0.65 (low)	34.8	2.9
Fatal or non-fatal ischaemic stroke	3.4	2.6	0.74 (medium)	26.0	0.9
MALE	0.8	0.5	0.66 (medium)	34.1	0.3

ARR, absolute risk reduction; HR, hazard ratio; MACE, major adverse cardiovascular event; MALE, major adverse limb events; RRR, relative risk reduction.

models based exclusively on data, statistical correlations, and relying only on a probabilistic approach. The use of a knowledge-based mechanistic model allows us to capture the whole dynamic of disease progression, with mechanisms underlying the occurrence of the event, and therefore makes it possible to determine a mechanistic and quantitative causality with regard to whether an event occurs.[33,34] The results of the SIRIUS simulation (*Graphical Abstract*) obtained using a mechanistic model support the evidence that significant and lasting inhibition of PCSK9 and subsequent reduction in LDL-C concentration leads to a reduction in ASCVD events as previously suggested by Mendelian randomization studies and randomized controlled trials.[35]

Nevertheless, this *in-silico* modelling approach involves inherent uncertainties that must be identified and quantified. We acknowledge that in knowledge-based mechanistic modelling, there is currently no established definition of uncertainty nor a pedigree method to quantify prediction uncertainty. Uncertainty can come from the model itself (incorrectly interpreted knowledge, hypothesis-filling knowledge gaps, equations used) and from the parameters used in the model.[12,13] Considering the uncertainty linked to the model, this was assessed and tracked throughout the model-building process, through evaluation of the strength of evidence of each piece of knowledge extracted from the literature to evaluate the level of confidence of the information integrated in the model. Moreover, a multidisciplinary scientific committee comprising six medical experts reviewed and validated the knowledge model and associated modelling hypothesis to ensure credibility.

The credibility of model predictions relies on the processes of calibration, validation, and robustness analysis, which compared the simulated results with more than 40 published studies.[12] These processes assessed the uncertainty of model outputs, considering both the model structure and the values of parameters used in the model.[12,13] The main limitations of the model identified during those steps are related to construction hypotheses made during model development, either because of a lack of knowledge regarding some mechanisms or because of simplification of highly complex mechanisms.

Some of these limitations may have affected the conclusions on the effect of inclisiran on, for instance, cardiovascular death as previously discussed. Some minor discrepancies with the literature were observed on simulated variations in lipoprotein(a) and high-density lipoprotein

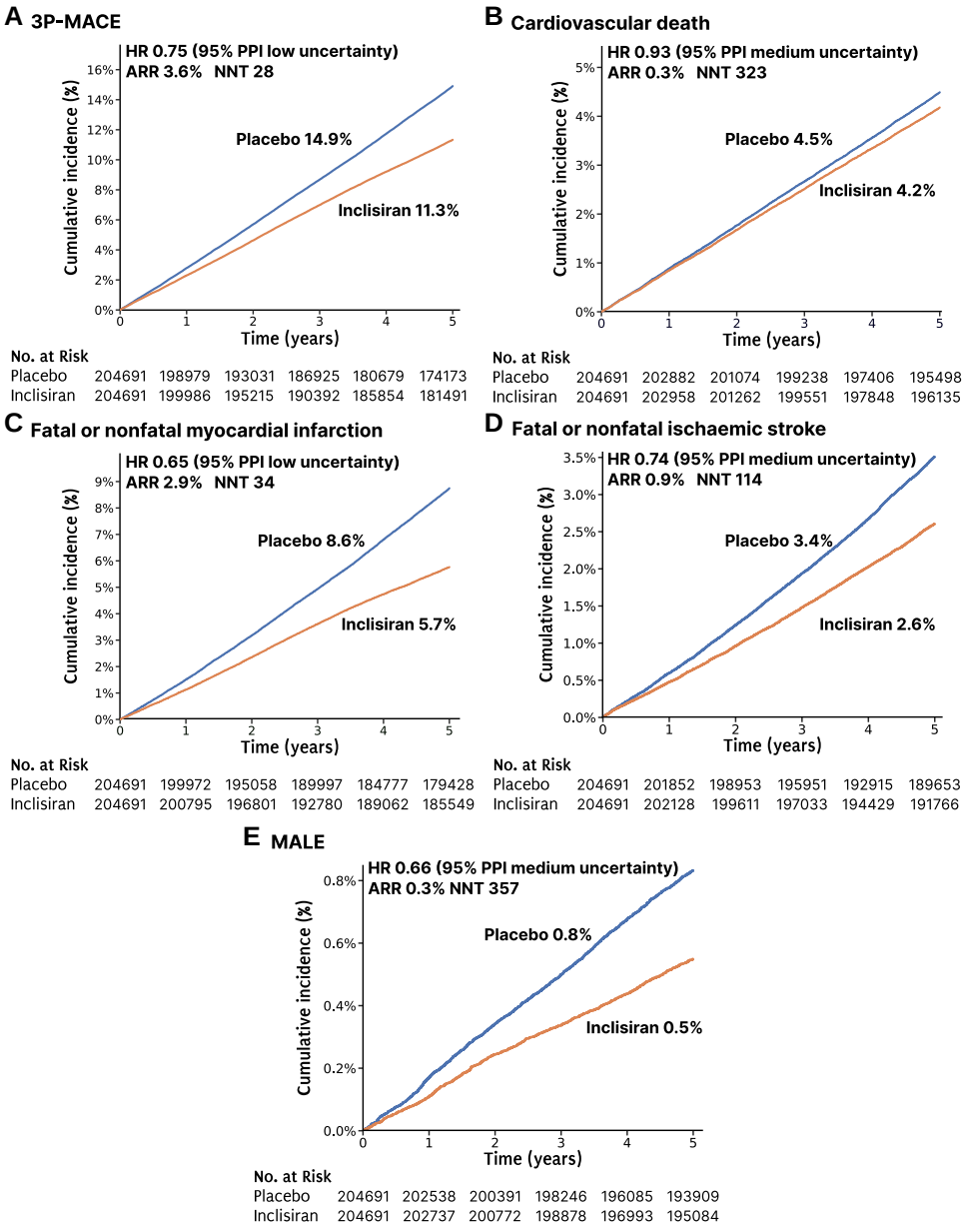


Figure 2 Predicted cumulative incidence of cardiovascular events in the SIRIUS virtual population ($n = 204\,691$). Data are shown for placebo and inclisiran as add-on to high-intensity statin therapy alone or in combination with ezetimibe. 3P-MACE, 3-point major adverse cardiovascular events; ARR, absolute risk reduction; HR, hazard ratio; MALE, major adverse limb event; NNT, number needed to treat; PPI, percentile prediction interval.

cholesterol under inclisiran (*Table 2*).[5] We considered these limitations resulting from a lack of knowledge on mechanisms of lipoprotein(a) homeostasis and the observed increase in high-density lipoprotein cholesterol after PCSK9-inhibitor treatment to be minor and unlikely to significantly affect the efficacy predictions on clinical outcomes in the SIRIUS population. Indeed, reduction of lipoprotein(a) is believed to be the most impactful on cardiovascular risk for patients with very high baseline lipoprotein(a) levels, which is not the case for most of the SIRIUS virtual population (75% of the virtual patients have a lipoprotein(a) value below 140 nmol/L), and the impact of small variations in high-density lipoprotein cholesterol on atherosclerosis progression is expected to be low.[36,37]

Similarly, the predicted correlation between low baseline eGFR concentrations (i.e. < 60 mL/min/1.73 m^2) and ASCVD risk was low because no direct mechanistic effect of eGFR concentration on atherosclerosis pathophysiology was introduced into the model due to the lack of evidence to complete its architecture (*Figure 3*).

The SIRIUS programme did not incorporate modelling of therapeutic non-adherence or the potential adverse effects associated with inclisiran. Given that the well-tolerated safety profile of inclisiran has been comprehensively established in Phase III trials (ORION-9, ORION-10, and ORION-11) and is supported by real-world evidence, characterized mainly by mild and transient injection-site reactions, modelling adverse events was not within the scope of this project, as

Subgroup		% N	3P-MACE Rate (%) Inclisiran	3P-MACE Rate (%) Placebo	Hazard Ratio	95% PPI Uncertainty
Overall (N = 204691)		100	11.33	14.91	0.748	Low
Age	≤ 65 y	60.8	9.86	13.12	0.740	Low
	65–75 y	31.2	12.82	16.80	0.749	Medium
	> 75 y	8.0	16.79	21.20	0.775	Medium
Sex	Male	75.0	11.83	15.51	0.750	Low
	Female	25.0	9.85	13.12	0.739	Medium
Diabetes	Yes	36.8	14.71	18.93	0.761	Low
	No	63.2	9.37	12.57	0.735	Low
Current smoker	Yes	28.8	12.99	17.03	0.749	Low
	No	71.2	10.66	14.05	0.747	Low
Hypertension*	Yes	33.3	13.35	17.52	0.747	Low
	No	66.7	10.33	13.60	0.748	Low
eGFR	≤ 30	1.0	19.04	22.38	0.835	High
	30–60	20.4	13.13	17.02	0.757	Medium
	> 60	78.6	10.77	14.26	0.743	Low
Medical history	MI	82.4	11.26	14.89	0.744	Low
	IS	19.2	13.44	17.01	0.776	Medium
	PAD	13.7	17.62	22.88	0.750	Medium
Number of affected vascular beds†	1 bed	85.0	10.00	13.27	0.742	Low
	2 beds	14.6	18.54	23.77	0.759	Medium
	3 beds	0.4	32.34	39.67	0.778	High
Number of risk factors‡	0–1	50.9	8.19	10.75	0.753	Low
	2–4	48.9	14.53	19.13	0.743	Low
	≥ 5	0.2	28.29	40.35	0.664	High

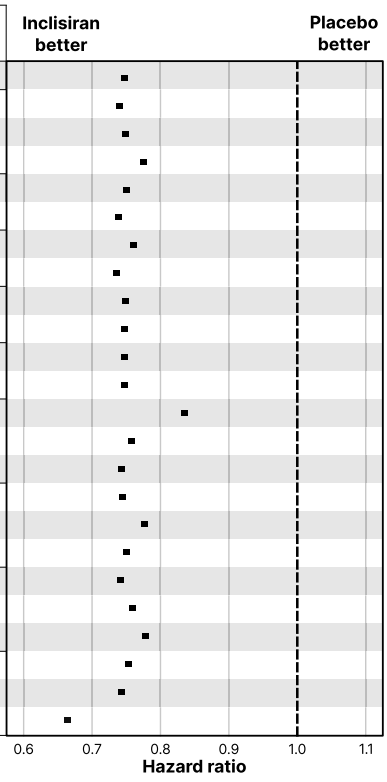


Figure 3 Predicted three-point MACE incidence rates and hazard ratio forest plot for subgroups in the SIRIUS virtual population. *Hypertension was defined as uncontrolled high blood pressure and corresponds to sitting systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. †Number of affected vascular beds that led to a clinical event was computed as the sum of previous myocardial infarction, previous ischaemic stroke, and previous lower extremity symptomatic peripheral artery disease. ‡Number of risk factors was computed as the sum of diabetes, current smoker, multivascular bed lesions, uncontrolled high blood pressure, and baseline low-density lipoprotein cholesterol >100 mg/dL. 3P-MACE, 3-point major adverse cardiovascular event; eGFR, estimated glomerular filtration rate (mL/min/1.73 m^2); PPI, percentile prediction interval.

it would require dedicated mechanistic sub-models for each event of interest.[38] Regarding LLT adherence, the SIRIUS assumption represents 'ideal-use' conditions. Modelling non-adherence to background oral therapies (statins, ezetimibe) would mainly affect absolute event rates but have little impact on the relative comparison between inclisiran and placebo, since these treatments were equally used in both arms.

While there are several sources of prediction uncertainty (model structure, parameters, inter-patient variability) for outputs measured at the population level (e.g. HR), we could consider that prediction uncertainty is primarily driven by inter-subject variability.[11] To date, there is no standard quantitative marker that allows to reflect all the sources of prediction uncertainties generated by the model. We therefore proposed to use the 95% PPI, which by construction intrinsically includes both the uncertainty on the model and on the parameters as well as the inter-patient variability. While, due to its method of calculation, we acknowledge that the 95% PPI can be considered as reflecting more the inter-patient variability than the uncertainty linked to the model and its parameters.

In the SIRIUS *in-silico* modelling programme, we assume that since we are not working on a sample of the population of interest but a representation of the entire ASCVD population, the sampling-theory-based statistical tests cannot apply.[26,27] Moreover, an *in-silico* model allows evaluation of a treatment efficacy that is not impacted by potential imbalance of the randomization process and patient-to-patient variability between the two treatment arms, because each virtual patient is used as its own control. Thus, efficacy results are expressed as HR or incidence curves over time without 95% CI or *P*-values. The 95% PPI should not be confused with 95% CI, the former measuring degree of uncertainty in model predictions, the latter measuring, in a clinical trial context, the uncertainty linked to sampling (see Supplementary material online, *Figure S2*).

The 95% PPI is not a measure of the significance of results. When the PPI is large (high uncertainty), this does not call into question the reliability of the prediction obtained from the validated model. A large PPI means a high variability of the measure between samples extracted from the large virtual population (see Supplementary material online, *Figure S5*). The width of the PPI represents the variability of the efficacy results when measured at the level of samples drawn in the entire large virtual population.

The SIRIUS programme's prediction of a 25% reduction in 3P-MACE over 5 years with the addition of inclisiran to high-intensity statin therapy, alone or combined with ezetimibe, in patients with ASCVD should be regarded as hypothesis-generating. The SIRIUS simulations are designed to inform hypothesis generation and trial design rather than to guide therapeutic decisions directly. The mechanistic model provides causal insight into the expected magnitude and direction of treatment effects under controlled assumptions. Ultimately, the predictive validity of the model will be evaluated against Phase 3 trial outcomes, in line with the model-informed drug development framework endorsed by the EMA and FDA.

SIRIUS differs from previous cardiovascular *in-silico* efforts by mechanistically linking lipid-lowering drug mechanisms to plaque biology and hard outcomes across coronary, cerebrovascular, and peripheral beds, and by validating against multiple external trials. By using each virtual patient as their own control and modelling specific event risks within a validated framework, SIRIUS moves beyond previous large-scale models to provide biological predictions.

In conclusion, the SIRIUS predictions provide early insights into the potential effect of inclisiran on cardiovascular events, suggesting a predicted reduction in 3-point MACE, a couple of years before the results of ongoing Phase 3 trials (ORION-4 and VICTORION-2-Prevent) become available. Whether the predicted SIRIUS results align with the outcomes of the ORION-4 and VICTORION-2-Prevent trials remains to be confirmed.

Supplementary material

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

Acknowledgements

Sophie Rushton-Smith (MedLink Healthcare Communications) provided editorial assistance, limited to editing for style, and was funded by Novartis Pharma SAS.

Author contribution

D.A., P.A., A.B., A.F., F.B., B.C., G.M., L.P., P.G.S., E.B., S.-G.N., and J.P.-B. contributed to the conception and design of the study. E.P., E.C., A.D., S.P., and Y.W. built the *in-silico* model. E.P., E.C., and Y.W. performed the SIRIUS *in-silico* trial. D.A., P.A., A.B., F.B., B.C., G.M., L.P., P.G.S., E.B., S.-G.N., J.P.-B., D.A., L.P., E.B., E.P., E.C., A.F., R.K., and Y.W. analysed the data. D.A., E.B., L.P., and S.-G.N. drafted the manuscript. P.A., A.B., F.B., B.C., G.M., P.G.S., J.P.-B., and A.F. critically revised the manuscript. All gave final approval and agreed to be accountable for all aspects of work, ensuring integrity and accuracy.

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Funding

The SIRIUS *in-silico* programme (Clinicaltrials.gov identifier: NCT05974345) is funded by Novartis Pharma SAS.

Conflict of interest: D.A. reports having received payment or honoraria for lectures, presentations, speakers' bureaus, or educational events from Amgen, Alnylam, Amarin, AstraZeneca, Boehringer, BMS, Bouchara Recordati, Pfizer, Novartis, Novo Nordisk, Organon, Sanofi, Servier, and Vifor in the last three years.

Emmanuel Peyronnet, Jean-Pierre Boissel, Eulalie Courcelles, Alizée Diatchenko, Solène Granjeon-Noriot, Riad Kahoul, Solène Porte, Yishu Wang, and Emmanuelle Bechet are employees of Nova In Silico. B.C. reports having received payments for consulting, speaking, or educational events from Amgen, AstraZeneca, BMS, Eli Lilly, Novartis, Novo Nordisk, Sanofi, and Ultragenyx. P.A. reports having received grants from: the French government (PHRC: RIISC-THETIS, TST-40, SPICAF trials), Pfizer, and AstraZeneca (TST trial); speaker fees from Novartis, Viatris, and Sanofi; is on advisory boards for Novartis and Neuraltide; and is on a steering committee for Bayer. F.B. has received payments for consulting, speaking, or educational events from Amgen, Amarin, Novartis, Novo Nordisk, Boehringer, Servier, ViiV Healthcare, Gilead, and Sanofi. A.B., A.F., and L.P. are employees of Novartis. G.A. reports having received payments for consulting, speaking, or educational events from Amgen, Amarin, Bayer Healthcare, BMS, Leo Pharma, Novartis, Novo Nordisk, Pfizer, and Sanofi. P.G.S. reports having received research grants from Amarin and Sanofi; involvement in clinical trials (steering committees, clinical events committees, data and safety monitoring boards) for Amarin, Amgen, AstraZeneca, Bayer, Bristol-Myers Squibb, Idorsia, Janssen, Novartis, Novo-Nordisk, Pfizer, and Sanofi; consulting or speaking for Amarin, Amgen, BMS, and Novo-Nordisk; is Senior Associate Editor at Circulation; and Chief Medical Officer at Bioquantis.

Data availability

Novartis is committed to sharing access to virtual patient-level data and clinical study reports from eligible studies with qualified external researchers upon positive evaluation of a formal data-sharing request and related requirements.

Access to csv files containing extracted simulation outputs, which were used to generate the figures presented in the article, is shared via GitHub (link).

The entire datasets generated during the current study by computational simulations (virtual patient level data) are available from the corresponding author upon positive evaluation of a formal data sharing request and related requirements.

Code availability

The access to the model will be provided upon request on Jinko only (link). The access will be granted to anyone who asks for it, at any time, as

long as their use is not for commercial purposes. The use of the model is subject to a custom licence based on the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0) with additional restrictions (available in JINKO). Note that in Jinko the model will not be modifiable, nor downloadable. However, the user will be able to run simulations to explore the behaviour of the model and to reproduce the results presented in the manuscript.

For replication purposes, access to raw data (parameter value distributions) of the SIRIUS Vop generated for this study and simulation protocol used are provided on Jinkō upon request (link).

Access to the entire set of Python scripts required to reproduce results presented in the manuscript is shared via GitHub (link).

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