A R T I C L E

An evaluation of a supratherapeutic dose of inclisiran on cardiac repolarization in healthy volunteers: A phase I, randomized study

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Abstract
Inclisiran is a small interfering RNA molecule that has been shown to provide an effective and sustained reduction in low-density lipoprotein cholesterol levels. This study aimed to determine whether a supratherapeutic dose of inclisiran affects cardiac repolarization and conduction in healthy volunteers. A phase I, randomized, double-blind, double-dummy, placebo- and positive-controlled, three-way crossover study was performed in 48 healthy volunteers. Volunteers were assigned to three treatments in a randomized sequence: a supratherapeutic dose of inclisiran sodium (900 mg), placebo, or moxifloxacin 400 mg as a positive control, with a minimum 7-day washout period between treatments. Continuous electrocardiogram monitoring was performed from >60 min before dosing until 48 h after dosing. Pharmacokinetics, pharmacodynamics, and safety were also assessed. Inclisiran, at a supratherapeutic dose, did not show a clinically significant effect on the QT interval (Fridericia correction formula [QTcF]; maximal placebo-and baseline-corrected change: 2.5 ms [90% confidence interval: 0.6, 4.5]) near the maximal plasma concentrations at 4 h. In addition, inclisiran did not show any effects on other electrocardiogram intervals or ST- and T-wave morphology. The positive control, moxifloxacin, demonstrated the expected changes in QTcF interval, validating the adequate sensitivity of the study. A supratherapeutic dose of inclisiran sodium (900 mg) had no effect on the QTcF interval or other electrocardiogram parameters, providing additional insight and reassurance regarding the safety profile of inclisiran.

Study Highlights
WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
Inclisiran is a novel siRNA therapeutic agent that specifically targets the liver and has been shown to effectively, sustainably, and safely lower low-density lipoprotein cholesterol levels, a proven risk factor for atherosclerotic cardiovascular...
INTRODUCTION

Inclisiran is the first and only small interfering RNA (siRNA) that has been shown to effectively and sustainably reduce low-density lipoprotein cholesterol (LDL-C) levels, an established causal and modifiable risk factor for atherosclerotic cardiovascular disease.1–5 An N-acetylgalactosamine moiety enables selective uptake into hepatocytes via asialoglycoprotein receptor (ASGPR)-mediated endocytosis in which inclisiran binds with the RNA-induced silencing complex (RISC) and directs it to mRNA encoding proprotein convertase subtilisin/kexin type 9 (PCSK9), resulting in degradation of PCSK9 mRNA by the RISC. This results in an increased surface concentration of the LDL-C receptor and hepatic uptake of LDL-C, thereby decreasing plasma LDL-C.6 Inclisiran is metabolized by nuclease activity, with the main route of elimination via urinary excretion.7 Inclisiran is undetectable in plasma within 24–48 h after subcutaneous administration, but has a long half-life in the liver, resulting in effective and sustained reduction of plasma LDL-C.7,8 The therapeutic dose of inclisiran and its dosing regimen were established as 300 mg of inclisiran sodium administered subcutaneously on days 1 and 90, and every 6 months thereafter.

Inclisiran is expected to have a minimal presence in the myocardium and has not demonstrated any propensity to prolong the QT interval in preclinical studies (Kv11.1.2 channel inhibition assays and cardiovascular safety studies; unpublished data) or clinical studies.2,3 However, QT interval prolongation can be associated with life-threatening arrhythmias, and its consequences have been documented with a number of drugs.9,10 Therefore, premarketing investigations of the cardiac safety of a new therapeutic agent require rigorous characterization of its effects on the QT/corrected QT (QTc) interval.11

In line with regulatory guidance (International Council for Harmonization [ICH] E14), this study aimed to determine whether a supratherapeutic dose of inclisiran sodium (900 mg), a novel siRNA therapeutic agent, does not affect cardiac repolarization, providing additional reassurance regarding the safety profile of inclisiran. This is the first QT clinical study investigating the effects of a novel siRNA therapeutic agent.

WHAT QUESTION DID THIS STUDY ADDRESS?
This study addressed whether or not a supratherapeutic dose of inclisiran had any effects on cardiac repolarization based on the corrected QT interval or other electrocardiogram parameters in healthy participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
A supratherapeutic dose of inclisiran sodium (900mg) was generally safe and well-tolerated and did not show a clinically relevant effect on the QT interval. Other electrocardiogram intervals and ST- and T-wave morphology were also unaltered by a supratherapeutic dose of inclisiran.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
This study demonstrated that a supratherapeutic dose of inclisiran sodium (900 mg), a novel siRNA therapeutic agent, does not affect cardiac repolarization, providing additional reassurance regarding the safety profile of inclisiran. This is the first QT clinical study investigating the effects of a novel siRNA therapeutic agent.

METHODS

Study design

A phase I, randomized, double-blind, double-dummy, placebo- and positive-controlled, three-way crossover cardiac safety study was performed in 48 healthy volunteers who were enrolled at a single center (Spaulding Clinical Research, LLC, West Bend, WI) according to ICH E14 guidance.11 Registration of this phase I trial was not required as it is not an applicable clinical trial according to current US regulatory guidance (Code of Federal Regulations, Title 42, Part 11; www.prisinfo.clinicaltrials.gov). The study protocol and amendments were reviewed
and approved by an independent institutional review board at Advarra, Inc. (Columbia, MD; approval number Pro00029216). The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and in compliance with ICH Good Clinical Practice for protection of participants as required by applicable government regulations, directives, and guidelines in operation at the time of the study. All volunteers provided written, informed consent before enrollment.

The study enrolled healthy male or female volunteers aged 18–60 years with a body mass index of 18–33 kg/m² and body weight of ≥45 kg. The first volunteer was randomized on September 24, 2018, and the last volunteer completed the trial on April 19, 2019. Volunteers must have had no clinically significant findings on their medical history, physical examination, clinical laboratory assessments, or 12-lead ECG at screening, and must not have used nicotine products for at least 90 days before screening. Volunteers were excluded from the study if they had any of the following: a resting heart rate <40 or >100 beats per minute; systolic blood pressure <95 mmHg; diastolic blood pressure <50 mmHg; QTc >450 ms (men) or >470 ms (women); or LDL-C <60 mg/dl. Use of prescription or over-the-counter medications (including antacids) within five elimination half-lives of study initiation was prohibited.

Volunteers were administered three treatments in a randomized, crossover fashion: a supratherapeutic dose of inclisiran sodium (900 mg [equivalent to 852 mg inclisiran] as three subcutaneous injections of 300 mg, 1.5 ml each) plus a placebo tablet (treatment A); placebo solution (three subcutaneous injections of 1.5 ml each) plus a placebo tablet (treatment B); and placebo solution (three subcutaneous injections of 1.5 ml each) plus a moxifloxacin positive control (400 mg oral over-encapsulated tablet; treatment C). Each volunteer was randomized to one of six treatment sequences (ABC, BCA, CAB, ACB, BAC, and CBA), with a minimum 7-day washout period between regimens. The treatment randomization schedule was generated by the statistical department at The Medicines Company and furnished only to the study pharmacist who blinded medication doses to the study team for administration, including placing an over-label on each medication-filled syringe and encapsulating medication tablets using Swedish Orange #00 gelatin. Each treatment was administered on day 1, 9, or 17 after the volunteers had fasted for at least 10 h overnight before dosing.

Samples of blood for pharmacokinetic (PK) analyses were time-matched with ECG recordings and were collected 60, 45, and 30 min before dosing and 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after dosing. A validated liquid chromatography/mass spectrometry/time-of-flight mass spectrometry (LC-TOF-MS) method was used to determine inclisiran plasma concentration by detecting both the antisense strand and the sense strand of inclisiran. Standard curves were prepared with inclisiran concentrations ranging between 10 and 10,000 ng/ml and using a duplex RNA internal standard (IS). Separate standard curves were generated for the sense strand and antisense strand using the peak area ratios of analyte-antisense/IS-antisense and analyte-sense/IS-sense single strands. Plasma samples were spiked with the IS, processed by solid-phase extraction, and analyzed using reversed-phase ultra-high-pressure LC with TurboIonSpray TOF-MS detection (A B Sciex). Analytical precision ranges (percentage coefficient of variation) were 4.2–10.2 (antisense strand) and 5.1–11.2 (sense strand). The analytical accuracy ranges (percentage relative error) were 0.1–5.7 (antisense strand) and 0.5–5.6 (sense strand). The lower limit of quantitation for plasma inclisiran was 10.0 ng/ml. PK parameters were calculated with a noncompartmental approach using Phoenix WinNonlin version 8.0 (Certara, Inc., Princeton, NJ) from the individual plasma concentration profiles of inclisiran.

Continuous ECG monitoring was performed at least 60 min before dosing until 48 h (+30 min) after dosing using a 12-lead ECG (Mortara Surveyor; Mortara Instrument, Inc., Milwaukee, WI). Three recordings (~1 min apart) were extracted from telemetry data during a 5-min window at 60, 45, and 30 min before dosing and at 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h after dosing, with the timing matched PK sampling. Each ECG extraction window was preceded by a supine rest period of ~10 min. ECGs were read centrally using a manually operated high-resolution onscreen caliper method with annotations. The extracted ECG recordings were read by centralized laboratory cardiologists who were blinded to treatment, time, and study day identifiers. Lead II was the lead of choice for interval measurement. Baseline and on-treatment ECGs were extracted on the same lead.

LDL-C and PCSK9 levels were measured during screening and on days 1, 9, 17, 19, 30, 90, and 180. Days after inclisiran dose were calculated as the date of assessment minus the date of inclisiran dose. Volunteers were summarized with their intended assessment date for the visit on day 30 (29 days after inclisiran dose) if the visit occurred within the window of 3 days. Assessments on days 90 and 180 were summarized regardless of days from inclisiran dose. PCSK9 analysis was performed using Quantikine enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions using a Sunrise reader (Tecan, Männedorf, Switzerland) and ethylenediaminetetraacetic acid plasma. Intra-assay and inter-assay coefficients of variation were 4.7% and 5.4%, respectively. Completed volunteer sets were analyzed on
the same plate to eliminate inter-assay variability when comparing results from the same volunteer.

LDL-C values were determined by the Friedewald formula unless volunteers had LDL-C < 40 mg/dl or triglycerides > 400 mg/dl, in which case, LDL-C values were determined by ultracentrifugation. Methods of LDL-C assessment remained the same throughout the trial, including both calibration and reagent systems.

**Data and statistical analysis**

The primary end point was change in the placebo- and baseline-corrected QT interval (ddQTcF), corrected by the Fridericia formula (QTcF) to account for the influence of heart rate (QTc = QT/RR0.33)\(^{12}\). The adequacy of the correction formula was assessed by determining the linear relationship of QTcF to the RR interval, defined as a population of the QTcF RR slope of < 0.045 and a slope of < 0.045 in at least 50% of individual volunteers. These prespecified criteria were met in this study. Secondary end points included other ECG parameters (including heart rate, PR interval, and QRS interval) and the relationship between ddQTcF and the plasma concentrations of inclisiran.

A mixed-effects model was used to evaluate the primary end point and included terms for sequence, treatment (inclisiran, placebo, or moxifloxacin), study period, postdose ECG assessment timepoints, and study drug by postdose ECG timepoint interaction as fixed effects, and volunteers nested within sequence as a random effect.

Baseline QTcF was included in the model as a covariate. The mean and upper one-sided 95% confidence interval (CI; equal to the upper limit of the two-sided 90% CI) of the baseline-adjusted difference between QTcF for the inclisiran and placebo groups (ddQTcF) was determined. If the upper one-sided 95% CI for the mean difference at each postdose timepoint between the inclisiran and placebo groups was < 10 ms, it was concluded that no clinically meaningful QTc interval prolongation occurred. Similar analyses were repeated for the secondary ECG end points using two-sided 95% CIs.

Mixed-effects analyses of change from baseline in QTcF (dQTcF) versus plasma concentrations of inclisiran were performed to evaluate the relationship between dQTcF and plasma concentrations of inclisiran. The mixed-effects model included dQTcF as the dependent variable, and baseline QTcF with random effects on the intercept and slope, treatment, timepoint, and plasma concentration as independent variables. This model was used to estimate the predicted population mean ddQTcF and its corresponding upper one-sided 95% CI (equivalent to the upper two-sided 90% CI) at

The arithmetic mean maximal plasma drug concentration (C\(_\text{max}\)).

The PK, LDL-C, and PCSK9 parameters were summarized descriptively by treatment and timepoint using the absolute and percentage change from baseline.

Safety end points included adverse events (AEs), physical examinations, vital signs, laboratory assessments, and ECG parameters (heart rate, PR and RR intervals, QRS duration, ST-, T-, and U-wave morphology, and QT intervals corrected for heart rate using the Bazett correction if QTcF failed to adequately correct). AEs were coded using the Medical Dictionary for Regulatory Activities version 21.1.

The planned study enrollment was 48 volunteers in order to provide at least 90% power for determining whether the upper bound of the one-sided 95% CI for the least-squares mean difference in ddQTcF between a single dose of inclisiran and placebo was < 10 ms. This assumed that the difference in the time-matched change from baseline in QTcF between the inclisiran and placebo groups was < 5 ms, with a common SD of 9 ms for the values of QTcF and a correlation coefficient of 0.9 among the triplicate ECGs extracted at each timepoint. The sample size also provided ~90% power for the lower bound of the one-sided 95% CI for the maximal change from baseline in ddQTcF after moxifloxacin administration was > 5 ms.

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology\(^{11,14}\).

**RESULTS**

All 48 volunteers completed the study to the end-of-study visit at day 30 and were included in their original assigned groups in the PK, ECG, pharmacodynamic (PD), and safety analyses. Four volunteers (8.3%) were lost to follow-up after the end-of-study visit at day 30 before completing the extended follow-up period on day 180 (Figure S1). Volunteers were predominantly men (68.8%) and had a mean age of 36.0 years and a mean body mass index of 26.0 kg/m\(^2\) (Table 1).

**ECG assessments**

The placebo- and baseline-corrected QTcF was significantly increased after the dosing of a positive control, moxifloxacin, as expected (Figure 1). The maximum increase in QTcF after moxifloxacin administration was observed at 4 h after dosing, which was 11.4 ms (90% CI 9.5–13.4). By contrast, the maximum changes in the placebo- and baseline-corrected QTcF observed with inclisiran was 2.5 ms (90% CI 0.6–4.5) and occurred at 4 h after dosing.
near the maximal plasma concentration. The upper bound of the two-sided 90% CI for the placebo- and baseline-corrected QTcF changes was within the predefined limit of 10 ms, as specified in the current ICH E14 guidelines. In addition, the changes observed for QTcF intervals were not influenced by treatment sequence. Thus, inclisiran had no clinically significant effect on QTcF, whereas moxifloxacin had the expected clinical effect as a positive control for this QT study. Categorical analysis of QTcF, PR, QRS, and heart rate is summarized in Table 2. Categorical measurements of QTcF were comparable between inclisiran and placebo, with 6.3% and 4.2% of measurements exceeding 450 ms, respectively. In contrast, 12.5% of volunteers receiving moxifloxacin had a QTcF interval exceeding 450 ms. Moxifloxacin demonstrated a typical percentage of outliers (16.7%) who showed a change in QTcF exceeding 30 ms, whereas only 4.2% of volunteers receiving inclisiran were in this category. Higher relevant changes in QTcF interval, such as exceeding 480 ms or a change in QTcF interval of >60 ms, were not observed in any volunteer. No clinically relevant changes in heart rate, PR interval, or QRS interval were observed (Table 2). In addition, no clinically significant effects on ST- or T-wave morphology were observed (Table S1).

The relationship between the plasma inclisiran concentration and baseline-adjusted QTcF was explored to further support the central tendency analysis. There was no evidence of a relationship, which was further supported by the statistical analysis. The dQTcF versus concentration relationship was not statistically significant (Figure 2). At the mean $C_{\text{max}}$ of inclisiran sodium 900 mg observed in this study (2888 ng/ml), the model-predicted ddQTcF was 1.9 ms (90% CI 0.64–3.17). The range of inclisiran exposures, $C_{\text{max}}$, and area under the curve observed in this study are much higher than the exposures anticipated in participants receiving the proposed therapeutic dose of 300 mg (mean inclisiran $C_{\text{max}}$ 421 ng/ml). In addition, it is also much higher than the exposures in participants with severe renal impairment who received a 300 mg dose in a different study ($C_{\text{max}}$ 1760 ng/ml), at which the model-predicted ddQTcF was 1.1 ms (90% CI 0.34–1.92). The underlying cause that could explain the apparent lack of linearity observed for the 900 mg dose of inclisiran sodium is currently unknown and might be

### Table 1 Baseline characteristics

| Characteristics | Randomized participants ($N = 48$) |
|-----------------|-----------------------------------|
| Age, years, mean (SD) | 36.0 (10.12) |
| Sex, n (%) | |
| Female | 15 (31.3) |
| Male | 33 (68.8) |
| Race, n (%) | |
| Black or African American | 23 (47.9) |
| White | 25 (52.1) |
| BMI, kg/m², mean (SD) | 26.0 (3.61) |
| SBP, mmHg, mean (SD) | 118.7 (9.47) |
| DBP, mmHg, mean (SD) | 74.0 (8.16) |
| Heart rate, bpm, mean (SD) | 64.2 (6.97) |
| QTcF, ms, median (IQR; min, max) | 407.5 (397–423; 373, 438) |
| RR, ms, median (IQR; min, max) | 928.5 (875–990; 722, 1230) |
| PR, ms, median (IQR; min, max) | 157.0 (140–169; 120, 200) |
| QRS, ms, median (IQR; min, max) | 92.0 (88–98; 76, 110) |
| PCSK9, ng/ml, mean (SD) | 245.18 (64.956) |
| LDL-C, mg/dl, mean (SD) | 100.8 (32.07) |

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; max, maximum; min, minimum; ms, milliseconds; PCSK9, proprotein convertase subtilisin/kexin type 9; QRS, QRS complex; QTcF, QT interval corrected by Fridericia formula; SBP, systolic blood pressure; SD, standard deviation.

### Figure 1

The ddQTcF in healthy volunteers administered a supratherapeutic dose of inclisiran versus moxifloxacin. The dashed line represents the ddQTcF threshold of 10 ms of interest to the US Food and Drug Administration. CI, confidence interval; ddQTcF, time-matched placebo- and baseline-adjusted change in QT interval corrected by the Fridericia formula; LSM, least-squares mean; ms, milliseconds. $N = 48$. 
attributed to relatively high variabilities in plasma concentrations of inclisiran. Because the ASGPR receptor is a very high-capacity receptor and not likely to be saturated, the variability is most likely not attributable to differences in liver uptake. Rather, this observation may be simply a result of the relatively small sample size in this study.

PK results

After the supratherapeutic dose of inclisiran, the peak arithmetic mean (SD) inclisiran $C_{\text{max}}$ was 2888 (1350) ng/ml. The median time to reach $C_{\text{max}}$ occurred at ~4 h after dosing (Figure S2; Table 3). The arithmetic mean (SD) half-life estimate was ~6.09 (19.8) h and the arithmetic mean (SD) clearance over time was 22.39 (5.29) L/h. Concentrations remained quantifiable for all volunteers during the 24 h after dosing and were below the lower limit of quantitation in the majority of subjects (27 of 48 volunteers) at 48 h.

Effect of inclisiran on PCSK9 and LDL-C levels

Mean change from baseline in plasma PCSK9 and LDL-C levels after administration of a single dose of inclisiran

### TABLE 2  Categorical analysis of QTcF, PR, QRS, and heart rate

|                  | Inclisiran (N = 48) | Placebo (N = 48) | Moxifloxacin (N = 48) |
|------------------|---------------------|------------------|-----------------------|
| QTcF, ms         |                     |                  |                       |
| >450             | 3 (6.3)             | 2 (4.2)          | 6 (12.5)              |
| >480             | 0                   | 0                | 0                     |
| >500             | 0                   | 0                | 0                     |
| Increase from baseline >30 | 2 (4.2)             | 0                | 8 (16.7)              |
| Increase from baseline >60 | 0                   | 0                | 0                     |
| PR, ms           |                     |                  |                       |
| >200             | 3 (6.3)             | 3 (6.3)          | 2 (4.2)               |
| >220             | 0                   | 0                | 0                     |
| QRS, ms          |                     |                  |                       |
| >110             | 1 (2.1)             | 2 (4.2)          | 1 (2.1)               |
| Heart rate, bpm  |                     |                  |                       |
| >90              | 0                   | 0                | 0                     |
| >100             | 0                   | 0                | 0                     |
| <40              | 0                   | 0                | 0                     |
| <50              | 10 (20.8)           | 11 (22.9)        | 6 (12.5)              |

Note: Data are presented as the number of participants (%).

Abbreviations: bpm, beats per minute; ms, milliseconds; QRS, QRS complex; QTcF, QT interval corrected for heart rate using the Fridericia correction.

### FIGURE 2  The dQTcF plasma inclisiran concentration regression model.
The shaded areas represent the 90% confidence intervals of the model. dQTcF, baseline-corrected change in QT interval corrected by the Fridericia formula; ms, milliseconds.
INCLISIRAN AND CARDIAC REPOLARIZATION

sodium 900 mg were −77.5% and −42.0%, respectively, at day 30 (29 days after inclisiran dose; Figure 3). Reductions in PCSK9 and LDL-C levels were maintained until day 90 (−79.7% and −47.0%, respectively) and day 180 (−75.7% and −44.8%, respectively; Table S2). The PD findings reported here are consistent with findings from previous studies, which have also shown that administration of a second dose at day 90 lowers LDL-C below the nadir reached after a single dose.2,3,7

Safety

Fifteen of 48 volunteers (31.3%) reported at least one treatment-emergent AE (TEAE), with a higher percentage of volunteers reporting TEAEs after receiving moxifloxacin (14.6%; n = 7) compared with inclisiran (10.4%; n = 5) or placebo (6.3%; n = 3; Table 4). Five volunteers (10.4%; 4 received moxifloxacin and one received inclisiran) reported TEAEs that were considered by the investigator to have a reasonable possibility of being caused by the study drug. Overall, the TEAEs reported by more than one volunteer were diarrhea, feeling hot, headache, nausea, and medical device site reaction. Injection site pain was reported by one volunteer (2.1%) who received inclisiran. One volunteer (2.1%) receiving moxifloxacin reported an event of urticaria, which was moderate in severity. All other TEAEs were mild in severity. There were no severe TEAEs, deaths, treatment-emergent serious AEs, or TEAEs leading to study discontinuation. There were no clinically meaningful changes in clinical laboratory values (including no samples confirmed positive for anti-drug antibodies), vital sign measurements, or ECG parameters.

### Table 3 Plasma inclisiran pharmacokinetic parameters

| Parameter (units) | N | Arithmetic mean | Median | IQR |
|-------------------|---|----------------|--------|-----|
| AUC₀–₂₄ (h·ng/ml) | 48 | 36,300 (SD: 10,623) | 35,290 | 30,200–40,900 |
| AUC₀–∞ (h·ng/ml) | 42 | 40,260 (SD: 10,249) | 38,910 | 33,700–44,700 |
| Cmax (ng/ml)      | 48 | 2888 (SD: 1350) | 2665 | 2030–3300 |
| Tmax (h)          | 48 | 4.95 (min, max: 0.5, 12.0) | 4.00 | 4.00–6.00 |
| t½ (h)            | 42 | 6.09 (SD: 1.98) | 5.62 | 4.87–6.77 |
| CL/F (L/h)        | 42 | 22.39 (SD: 5.29) | 21.90 | 19.0–25.3 |

Abbreviations: AUC₀–∞, area under the plasma drug concentration–time curve to infinity; AUC₀–₂₄, area under the plasma concentration–time curve over the last 24 hours; CL/F, clearance over time; Cmax, maximal plasma drug concentration; h, hours; max, maximum; min, minimum; SD, standard deviation; t½, half-life; Tmax, time to maximum plasma concentration.

*Parameters for inclisiran were not calculable for six volunteers because the terminal elimination phase was not apparent (Cmax was one of the last three available data points).

**Figure 3** Changes in PCSK9 and LDL-C levels following inclisiran administration. Days after inclisiran dose were calculated as the date of assessment minus the date of inclisiran dose. Volunteers are summarized with their intended assessment date for the visit at day 30 (29 days after inclisiran dose) if the visit occurred within the window of 3 days. Assessments on days 90 and 180 were summarized regardless of days from inclisiran dose. LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; SD, standard deviation. N = 48.
DISCUSSION

The prolongation of cardiac repolarization can increase the probability of cardiac arrhythmia; therefore, it is paramount to determine whether a novel therapeutic agent, such as the siRNA molecule inclisiran, has a threshold pharmacologic effect on cardiac repolarization. In this thorough QT study, we demonstrated that inclisiran does not have an effect on QTc intervals or other ECG parameters. Furthermore, the exposure–response analysis confirmed the absence of a relationship between inclisiran concentration and the baseline-adjusted QTcF. Therefore, we conclude that inclisiran had no clinically significant effect on cardiac repolarization at a supratherapeutic dose three-fold higher than the proposed therapeutic dose. The current study also provides valuable insight into the PK and PD of a supratherapeutic dose of a novel class of therapies (siRNA).

The absence of any effect of inclisiran on QTc interval was expected based on the relatively large molecular size (inclisiran sodium, 17,284.72 g/mol), lack of in vitro effects on human Ether-a-go-go-related gene assay, low and short-lived systemic exposure,15 minimal extrahepatic distribution, and lack of accumulation in cardiac tissues. This result is also consistent with previous clinical findings in patients with hypercholesterolemia who have received inclisiran.2

In addition to providing reassuring evidence regarding the absence of an effect of inclisiran on ECG parameters despite the supratherapeutic dose, the current study also provides additional insight into the PK and PD of a supratherapeutic dose of a novel class of therapies (siRNA).

The change in LDL-C levels following inclisiran dosing appeared to peak before day 180; however, this estimate was limited by the lack of sample collection between 29 days after inclisiran administration and day 180, preventing precise peak determination.

From a clinical perspective, the observation in the current study that inclisiran (at a supratherapeutic dose) does not affect cardiac repolarization is reassuring. A delay in cardiac repolarization is a potential concern for any drug with systemic exposure because it creates an electrophysiologic environment that favors cardiac arrhythmias, including torsade de pointes, which can lead to cardiac arrest.17 Hence, ICH E14 guidance recommends rigorous evaluation of a drug's effect on the QT interval for new drugs with systemic bioavailability.11 To our knowledge, this is the very first clinically thorough QT study that has been conducted with an siRNA therapeutic agent for the treatment of chronic diseases.

Another important finding from this study is that a supratherapeutic dose of inclisiran sodium 900 mg was generally safe and well-tolerated. The incidence of TEAEs remained low and was comparable with placebo despite the higher plasma concentrations observed. This information is relevant because higher circulating levels of inclisiran can occur in the setting of renal impairment. The exposure (C_{max} and area under the curve) observed in this study at the supratherapeutic dose of inclisiran fully covers the potentially enhanced exposures observed in a previous study in individuals with
renal impairment who had received a 300 mg dose of inclisiran. The low incidence of TEAEs is also consistent with the recently published clinical safety profile of inclisiran from two phase III studies in patients with elevated LDL-C in which rates of TEAEs were comparable with those observed with placebo. 18

The design, conduct, and interpretation of this study followed the recommendations provided by the ICH E14 guidance for the assessment of a drug’s potential to delay cardiac repolarization. 11

In this study, inclisiran had no clinically significant effect on cardiac repolarization assessed by the QTc interval or other ECG parameters, even at a supratherapeutic dose in healthy participants. Inclisiran demonstrated a safety profile comparable with that of placebo and exhibited a PK and PD profile consistent with previous reports. Inclisiran is a novel and promising siRNA therapeutic agent that has been shown to potently lower LDL-C levels with a twice-yearly dosing regimen following loading doses on days 1 and 90.

AUTHOR CONTRIBUTIONS
D.K., J.M., P.F.S., M.J.K., R.S., Y.L.H., and P.W. wrote the manuscript. D.K., J.M., P.F.S., and P.W. designed the research. D.K., J.M., and P.F.S. performed the research. D.K., J.M., P.F.S., M.J.K., R.S., Y.L.H., and P.W. analyzed the data.

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CONFLICT OF INTEREST
D.K., R.S., and P.W. were employees of The Medicines Company, receiving a salary and stock options. P.W. was an employee of Novartis receiving a salary. Y.L.H. is an employee of Novartis, receiving a salary and/or stock options. D.K. is an employee of DalCor Pharmaceuticals. M.J.K. is an employee of Jacksonville Center for Clinical Research, which receives study grants and modest consulting fees from multiple manufacturers of lipid therapies. P.F.S. is an employee of a company that received consulting fees from The Medicines Company. J.M. declared no competing interests for this work.

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REFERENCES
1. Cholesterol ‘Treatment Trialists’ (CTT) Collaborators. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. Lancet. 2012;380(9841):581-590. doi:10.1016/S0140-6736(12)60367-5
2. Ray KK, Landmesser U, Leiter LA, et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. N Engl J Med. 2017;376:1430-1440. doi:10.1056/NEJMoa1615758
3. Ray KK, Stoenkbroek RM, Kallend D, et al. Effect of 1 or 2 doses of inclisiran on low-density lipoprotein cholesterol levels: one-year follow-up of the ORION-1 randomized clinical trial. JAMA Cardiol. 2019;4(11):1067-1075. doi:10.1001/jamacardio.2019.3502
4. Raal FJ, Ray KK, Kallend D, et al. Safety and efficacy of inclisiran in patients with heterozygous familial hypercholesterolemia – results from the phase 3 ORION-9 trial. Circulation. 2019;140:e987.
5. Wright RS, Ray KK, Kallend D, et al. Safety and efficacy of inclisiran in patients with ASCVD and elevated LDL cholesterol – results from the phase 3 ORION-10 trial. Circulation. 2019;140(25):e965-e1011.
6. Stoenkbroek RM, Kallend D, Wijngaard PLJ, Kastelein JJP. Inclisiran for the treatment of cardiovascular disease: the ORION clinical development program. Future Cardiol. 2018;14(6):433-442. doi:10.2217/fca-2018-0067
7. Wright RS, Collins MG, Stoekenbroek RM, et al. Effects of renal impairment on the pharmacokinetics, efficacy, and safety of inclisiran: an analysis of the ORION-7 and ORION-1 studies. Mayo Clin Proc. 2020;95(1):77-89. doi:10.1016/j.mayocp.2019.08.021
8. Ray KK, Stoenkbroek RM, Kallend D, et al. Effect of an siRNA therapeutic targeting PCSK9 on atherogenic lipoproteins: prespecified secondary end points in ORION 1. Circulation. 2018;138(13):1304-1316. doi:10.1161/CIRCULATIONAHA.118.034710
9. Cubeddu LX. QT prolongation and fatal arrhythmias: a review of clinical implications and effects of drugs. Am J Ther. 2003;10(6):452-457. doi:10.1097/00045391-200311000-00013
10. Van Noord C, Eijgelsheim M, Stricker BHC. Drug- and non-drug-associated QT interval prolongation. Br J Clin Pharmacol. 2010;70(1):16-23. doi:10.1111/j.1365-2125.2010.03660.x
11. US Food and Drug Administration. Guidance for industry. E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. 2005. Available from: https://www.fda.gov/media/71372/download. Accessed March 15, 2022.
12. Pater C. Methodological considerations in the design of trials for safety assessment of new drugs and chemical entities. Curr Controlled Trials Cardiovasc Med. 2005;6(1):1. doi:10.1186/1468-6708-6-1
13. Taubel J, Ferber G, Lorch U, Batchvarov V, Savelieva I, Camm AJ. Thorous QT study of the effect of oral moxifloxacin on QTc interval in the fed and fasted state in healthy Japanese and Caucasian subjects. Br J Clin Pharmacol. 2014;77(1):170-179. doi:10.1111/bcp.12168
14. Curtis MJ, Alexander S, Cirino G, et al. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br J Pharmacol. 2018;175(7):987-993. doi:10.1111/bph.14153
15. Morganroth J, Wang Y, Thorn M, et al. Moxifloxacin-induced QTc interval prolongations in healthy male Japanese and Caucasian volunteers: a direct comparison in a thorough QT study. Br J Clin Pharmacol. 2015;80(3):446-459. doi:10.1111/bcp.12684

16. Fitzgerald K, Kallend D, Simon A. A highly durable RNAi therapeutic inhibitor of PCSK9. N Engl J Med. 2017;376(18):e38. doi:10.1056/NEJMoa1609243

17. Shah RR. The significance of QT interval in drug development. Br J Clin Pharmacol. 2002;54(2):188-202. doi:10.1046/j.1365-2125.2002.01627.x

18. Ray KK, Wright RS, Kallend D, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med. 2020;382(16):1507-1519. doi:10.1056/NEJMoa1912387

**SUPPORTING INFORMATION**

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

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