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Nonclinical Cardiovascular Assessment of the Soluble Guanylate Cyclase Stimulator Vericiguat

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Abbreviations

cGMP, cyclic guanosine monophosphate; CiPA, Comprehensive In Vitro Proarrhythmia Assay; Cmax, maximum drug concentration in plasma; Cmax.u, maximum unbound drug concentration in plasma; ECG, electrocardiogram; EGTA, egtazic acid; GLP, Good Laboratory Practice; HEK, human embryonic kidney; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; hERG, human Ether-a-go-go Gene; HF, heart failure; HR, heart rate; IC20, 20% threshold inhibitory concentration; IC50, half-maximal inhibitory drug concentration; ICH, International Council for Harmonisation; NO, nitric oxide; PEG400, ethanol/polyethylene glycol 400; QTc, corrected QT; QTcF, corrected QT using Fridericia's formula; QTcM, corrected QT using Matsunaga's formula; QTcV, corrected QT using van de Water's formula; SD, standard deviation; SEM, standard error of mean; sGC, soluble guanylate cyclase; Tmax, time at which Cmax was reached.

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Abstract

Vericiguat and its metabolite M-1 were assessed for proarrhythmic risk in nonclinical *in vitro* and *in vivo* studies. *In vitro* manual voltage-clamp recordings at room temperature determined the effect of vericiguat on human Ether-a-go-go Related Gene (hERG) K^+ channels. Effects of vericiguat and M-1 on hERG K^+, Nav1.5, hCav1.2, hKvLQT1/1minK, and hKv4.3 channels were investigated via automated voltage-clamp recordings at ambient temperature. Effects of vericiguat and M-1 on hERG K^+ and Nav1.5 channels at pathophysiological conditions were explored via manual voltage-clamp recordings at physiological temperature. Single oral doses of vericiguat (0.6, 2.0, and 6.0 mg/kg) were assessed for *in vivo* proarrhythmic risk via administration to conscious telemetered dogs; electrocardiogram (ECG) and hemodynamic parameters were monitored. ECG recordings were included in 4- and 39-week dog toxicity studies. In manual voltage-clamp recordings, vericiguat inhibited hERG K^+-mediated tail currents in a concentration-dependent manner (20% threshold inhibitory concentration ~1.9 μM). In automated voltage-clamp recordings, neither vericiguat nor M-1 were associated with biologically relevant inhibition (>20%) of hNav1.5, hCav1.2, hKvLQT1, and hKv4.3. No clinically relevant observations were made for hNav1.5 and hKvLQT1 under simulated pathophysiological conditions. Vericiguat was associated with expected mode-of-action–related dose-dependent changes in systolic arterial blood pressure (up to −20%) and heart rate (up to +53%). At maximum vericiguat dose, corrected QT (QTc) interval changes from baseline varied slightly (−6 to +1%) depending on correction formula. Toxicity studies confirmed absence of significant QTc interval

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changes. There was no evidence of increased proarrhythmic risk from nonclinical studies with vericiguat or M-1.

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Significance Statement

There was no evidence of increased proarrhythmic risk from *in vitro* and *in vivo* nonclinical studies with vericiguat or M-1. The integrated risk assessment of these nonclinical data combined with existing clinical data demonstrate administration of vericiguat 10 mg once daily in patients with heart failure with reduced ejection fraction is not associated with a proarrhythmic risk.

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Introduction

Vericiguat is a soluble guanylate cyclase (sGC) stimulator that was recently approved for the treatment of symptomatic chronic heart failure (HF) following a worsening event in adult patients with reduced ejection fraction (European Medicines Agency, 2021; Food and Drug Administration, 2021; McDonagh et al., 2021). Approval was based on results of the pivotal Phase 3 VICTORIA study, showing a reduction in the composite endpoint of cardiovascular death or HF hospitalization in the vericiguat group relative to the placebo group (Armstrong et al., 2020). Chronic HF is associated with endothelial cell dysfunction and an impaired nitric oxide (NO)–sGC–cyclic guanosine monophosphate (cGMP) signaling pathway (Stasch et al., 2011). The reduced availability of cGMP affects physiological mechanisms including vasorelaxation, platelet aggregation, and myocardial remodeling which, in combination with other pathophysiological pathways, may ultimately result in HF (Stasch et al., 2011).

Symptomatic chronic HF, particularly in conjunction with reduced left ventricular ejection fraction, is a pathophysiological condition with a well-known enhanced risk for potentially lethal cardiac arrhythmias (Grimm et al., 2003; Rashba et al., 2004; Jackson et al., 2012; Halliday et al., 2017). Other HF-associated conditions, such as autonomic dysfunction with sleep apnea (Mehra and Redline, 2014), genetic predisposition to dilated cardiomyopathy (McNally and Mestroni, 2017), and diuretic therapy-mediated electrolyte abnormalities (Laslett et al., 2020), may exacerbate the susceptibility of patients with HF for cardiac arrhythmias.

In this article, we report the results of standard International Council for Harmonisation (ICH) S7B nonclinical studies conducted prior to dosing vericiguat in

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humans: ventricular repolarization *in vitro* (hERG K^+ channel assay) and *in vivo* (electrocardiogram [ECG] in conscious dogs) (European Medicines Agency, 2006; Center for Drug Evaluation and Research, 2021). Following the completion of the VICTORIA trial we conducted a more comprehensive set of *in vitro* voltage-clamp recordings on cardiac ion channel recombinant cell lines with vericiguat and its major metabolite M-1, a *N*-glucuronide. Although no signal for proarrhythmia was observed throughout the vericiguat clinical development program, there was nevertheless recognition of the increased vulnerability to arrhythmia in patients with worsening HF. In light of this and in response to feedback from regulators, we performed these additional electrophysiological studies under conditions consistent with the recently introduced Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) paradigm, as well as those simulating pathophysiological states, including ischemia (at more depolarized potentials) (Shaw and Rudy, 1997; Carmeliet, 1999; Akar and Akar, 2007) and extremes of heart rate to mimic tachy- or bradycardia (using high or low pacing frequencies).

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Materials and Methods

Material and Guidelines

Vericiguat was synthesized and provided by Bayer AG. For *in vitro* experiments, a vericiguat stock solution was prepared (10 mM in dimethyl sulfoxide) and diluted appropriately into various extracellular salt solutions to reach the desired final concentrations. For the *in vivo* study in telemetered dogs, vericiguat was formulated in a vehicle (ethanol/polyethylene glycol 400 [PEG400], 10:90) and administered using gelatin capsules. For the repeat-dose dog toxicity studies, vericiguat was formulated in PEG400 and administered at 1–2 ml/kg.

All *in vivo* studies and the first hERG, human Ether-a-go-go Gene (hERG) K^+ manual voltage-clamp study were conducted in a Good Laboratory Practice (GLP)-compliant manner. Most of the *in vitro* cardiac ion channel studies were not fully GLP compliant. Studies were conducted in compliance with ICH S7A and ICH S7B guidelines or to recent related best practice revisions (draft ICH E14/S7B Q&A) being adopted as a result of the CiPA initiative (European Medicines Agency, 2005; European Medicines Agency, 2020).

All *in vivo* experiments were conducted with local approval and conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, or the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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Study Design

Four separate sets of nonclinical studies were conducted to investigate the effects of vericiguat on blood pressure, heart rate (HR), ECG, and ion channel currents: i) a dog telemetry and pharmacokinetic study, ii) "snapshot" ECG assessments during the 4- and 39-week GLP repeat-dose toxicity studies in dogs, iii) a GLP-compliant hERG K^+ assay, and iv) a series of non–GLP-compliant cardiac ion channel studies.

In Vivo *Assessments of Systemic Exposure, Blood Pressure, HR, and ECG Intervals in Conscious Beagle Dogs*

Systemic exposure was investigated in blood samples collected from nontelemetered male and female beagle dogs (*n* = 3/dose) at 1, 3, 7, and 24 h following single oral administration of 0.6, 2, and 6 mg/kg vericiguat. Blood samples were drawn via the jugular or cephalic vein and centrifuged at 4°C at 3600 rpm for 10 min. The resulting plasma samples were stored at ≤−15°C for the duration of the study. C_{max} was defined as the maximum drug concentration in plasma, and T_{max} as the time at which C_{max} was reached.

Arterial blood pressure (abdominal aorta), ECG (subcutaneous electrodes, standard lead II), and body temperature were continuously monitored over a period of 18 h (2 h 20 mins before and 16 h after dosing) in beagle dogs (two male, two female) that had been surgically implanted with a telemetry system (model TL11M2-D70-PCT; Data Science, Inc., St. Paul, Minnesota). Vericiguat was administered to conscious telemetered beagle dogs at single oral doses of 0 (vehicle), 0.6, 2, and 6 mg/kg body weight following a Latin square study design. Signals were acquired and analyzed with Ponemah P3 Plus, V.4.9 (Data Science, Inc., St. Paul, Minnesota). Data were processed and averaged over a predefined period (logging

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rate) of 5 min. These data were then averaged over intervals of 15 min for each parameter. For calculation of mean maximal changes in cardiovascular parameters, the 15-min bins were collapsed into a superinterval (2–6 h post-treatment) that considers magnitude and duration of the response, and pharmacokinetic properties of the compound encompassing T_{max} and several hours thereafter. Appropriately selected superintervals have been shown to improve statistical sensitivity to detect minor changes (Sivarajah et al., 2010). Systolic, diastolic, and mean arterial blood pressure, and HR were measured using telemetric pressure signals (*n* = 4/dose). PQ and QT intervals and the QRS duration were measured from telemetric ECG signals. QT intervals were corrected for HR by using the formulae of Fridericia (QTcF) (Fridericia, 1920), van de Water (QTcV) (Van de Water et al., 1989), and Matsunaga (QTcM) (Matsunaga et al., 1997).

Dogs did not receive anesthetic agents during the study period as no invasive procedures requiring anesthetics were performed. No dogs were terminated during the conduct of these studies. Beagle dogs employed in the telemetric study had participated in previous experiments (their last treatment was 3–13 weeks before this study). Before the start of the study, dogs received a veterinary health assessment. After surgery, all telemetered dogs were given a recovery period of ≥10 days. At the end of the study and following an appropriate drug washout period, telemetered dogs were assigned to a pool of dogs to be employed in future experiments.

ECG Assessments Following Repeat Dosing in a 4-Week Toxicity Study

Beagle dogs (*n* = 6 per group; three female, three male) initially received a once-daily oral dose of vericiguat of either 0 mg/kg/day, 2.5 mg/kg/day, 7.5 mg/kg/day, or 25 mg/kg/day by gavage. On day 15, the 25 mg/kg dose was reduced to 15 mg/kg

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for the remainder of the study duration, owing to severe gastrointestinal findings (particularly rectum prolapse). Hereafter, this group is described as the 25/15 mg/kg/day group. The vehicle was PEG400 at 2.0 ml/kg. Acute short-term (<60 s) ECG recordings were conducted prior to dosing (baseline) and 2 h post-dose (T_{max}) during week 1 and week 4. Blood pressure was measured using invasive techniques through the femoral artery.

ECG Assessments Following Repeat Dosing in a 39-Week Toxicity Study

Beagle dogs (*n* = 8 per group; four female, four male) received a once-daily oral dose of vericiguat of either 0 mg/kg/day, 0.5 mg/kg/day, 1.5 mg/kg/day, or 5 mg/kg/day by gavage. The vehicle used was PEG400 at 1.0 ml/kg. Acute short-term (<60 s) ECG recordings were conducted prior to dosing (baseline) and 2 h post-dose (T_{max}) during week 13 and week 39. Blood pressure was measured using high-definition oscillometry.

In Vitro *Assessments: Effects of Vericiguat and M-1 on Cardiac Ion Channel Currents*

Cell Lines

Stably transfected human embryonic kidney (HEK) cell lines were used for the hERG K^+, hNav1.5 Na^{2+}, and hKvLQT/minK channel voltage-clamp studies. Stably transfected Chinese hamster ovary cell lines were used for the hCav1.2 Ca^{2+} and hKv4.3 K^+ channel voltage-clamp studies. All cell lines were cultured in a humidified incubator at 37°C and 5% CO_2.

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Manual Voltage-Clamp Technique at Room Temperature (GLP hERG K^+ Assay)

The whole-cell voltage-clamp technique under GLP was used to measure hERG K^+-mediated inward tail currents elicited by hyperpolarizing voltage steps (repeated every 12 s, holding potential −80 mV) from +20 mV to −120 mV (duration 500 ms) at 22°C using standard procedures as previously described (Zhou et al., 1998). Vericiguat delivery to the organ bath was initiated after the initial stabilization period of approximately 6 min. When possible, vericiguat concentrations (0.1, 1, and 10 μM) were applied in a cumulative manner using exposure times of approximately 6 min per concentration, followed by a washout period and subsequent exposure to a high concentration (1 μM) of the selective hERG K^+ channel blocker E-4031.

Manual Voltage-Clamp Technique at Physiological Temperature (Non-GLP)

Additional experiments were conducted on hERG K^+, hNav1.5, and hKvLQT1/minK currents using step-ramp voltage protocols repeated at various intervals from 1–30 s (0.033–1 Hz) using manual whole-cell voltage clamp at 37°C as described previously (Crumb et al., 2016). hERG K^+ and KvLQT1/minK were recorded using the following solutions: extracellular NaCl 137 mM, KCl 4 mM, $MgCl_2$ 1.0 mM, $CaCl_2$ 1.8 mM, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) 10 mM, and dextrose 11 mM (pH 7.4 with NaOH); and intracellular KCl 130 mM, $MgCl_2$ 1.0 mM, HEPES 5.0 mM, NaCl 7 mM, egtazic acid (EGTA) 5 mM, and Mg ATP 1.5 mmol/L (pH 7.2 with KOH). hNav1.5 was recorded in (all mM): extracellular NaCl 130, CsCl 4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, HEPES 10, and dextrose 10 (pH 7.4 with NaOH); and intracellular CsCl 130, $MgCl_2$ 1.0, HEPES 5, NaCl 7, EGTA 5, MgATP 5, and TrisGTP 0.4 (pH 7.2 with CsOH). The voltage-clamp protocol for hERG K^+ and KvLQT1/minK was as follows: holding potential −80 mV, step to +40 mV (500 ms), ramp (100 ms) back to −80 mV;

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and a stimulation rate of 1 and 0.033 Hz. The voltage-clamp protocol for Nav1.5 was as follows: holding potential −80 mV, step to −15 mV (20 ms), step back to −80 mV; and a stimulation rate of 3 Hz.

Automated Voltage-Clamp Technique at Room Temperature (Non-GLP)

Automated whole-cell voltage clamp (Patchliner, Nanion, Germany) measured hERG K^+, hNav1.5, hCav1.2, hKvLQT1/minK, and hKv4.3 currents that were stably expressed in HEK293 (hERG K^+, hNav1.5, hKvLQT1/minK) and Chinese hamster ovary (hCav1.2, hKv4.3) cell lines. Current recordings were done using two different planar substrates (Nanion, Munich, Germany): NPC-16 med res 4-hole chips with a pipette resistance of 1–1.2 MΩ for hERG K^+; and hCav1.2 and NPC-16 high res 1-hole chips with a pipette resistance of 3.5–4.5 MΩ for hNav1.5, hKvLQT1, and hKv4.3; and two external EPC −10 quadro amplifiers (HEKA Elektronik GmbH, Rhineland-Palatinate, Germany). The voltage-clamp protocols and solutions used are listed in **Supplemental Tables 1 and 2.** HMR1556 (hKvLQT1 K^+), nifedipine (hCav1.2), and quinidine (hERG K^+, hNav1.5, and hKv4.3 K^+) were used as positive controls to inhibit relevant currents in their respective ion channels.

Owing to excessive current run-down at 37°C, hKvLQT1/minK recordings were done at 21°C using an automated QPatch voltage-clamp system (Sophion, Ballerup, Denmark) to study rate dependence (0.1 vs 0.033 Hz). The extracellular solution was composed of (in mM) NaCl 137, KCl 4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, and dextrose 10 (pH 7.4). The intracellular solution was composed of (in mM) KCl 90, KF 50, $MgCl_2$ 1.6, HEPES 10, EGTA 10, K_2ATP 2.5, and cAMP 0.2 (pH 7.2). The cell membrane was clamped to a holding potential of −50 mV, depolarized to +50 mV (3

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s), and subsequently returned to −50 mV. Data were collated when the peak tail current reached −50 mV.

Voltage-Clamp Data Analysis

When applicable, the concentration dependence of effects was modeled with a standard four-parameter logistic equation:

$$effect = min + (max / (1 + 10^{((logIC_{50} - logX) \times nH)}))$$

with minimal and maximal effects (min, max), half-maximal inhibitory drug concentration (IC_{50}), drug concentration (X), and Hill slope (nH). Minimal and maximal effects were usually treated as constants (max = 100 and min = 0), and IC_{50} and nH as variables. If only one concentration was measured, nH was set to 1.

Exposure Multiple Calculation

Exposure multiples were calculated based on human clinical C_{max} unbound plasma concentrations of 18 nM and 43 nM for vericiguat and M-1, respectively, at the maximum recommended human dose of 10 mg, and in comparison with the IC_{50} or highest concentration tested for each channel (for the *in vitro* studies) or with the maximum unbound drug concentration in plasma ($C_{max.u}$) in the dogs (for the *in vivo* studies).

Statistical Methods

In the blood pressure and HR study in telemetered dogs, the calculations included determination of arithmetical mean and standard deviation (SD) or standard error of mean (SEM). Effects were assessed regarding changes after administration versus

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baseline values compared with changes in the vehicle group; this was done for 15-min bins (results not shown) as well as for the 2–6 h superinterval that was chosen to enhance sensitivity (Sivarajah et al., 2010). The data reported are group mean values and corresponding SD or SEM of *n* experiments. Subsequent data analysis with a one-way ANOVA followed by Dunnett's multiple comparisons test versus vehicle control (differences significant if $p < 0.05$ (multiplicity-adjusted) was done for the 15-min bins (results not shown) as well as for the 2–6 h superinterval. The results of the repeat-dose toxicity studies were not subject to statistical analysis because of the short (<60 s) duration of recording. Calculations and graphical presentation of data were conducted with GraphPad Prism v8.

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Results

In-Vivo Studies in Beagle Dogs

Systemic Exposure Following a Single Oral Dose

Following single oral administration, C_{max} levels for vericiguat were 270 µg/l, 848 µg/L, and 1949 µg/L for the 0.6, 2.0, and 6.0 mg/kg doses, respectively; with calculated T_{max} of 2.1 h (0.6 mg/kg) and 3 h (2 and 6 mg/kg) (**Table 1**). Trough plasma levels were <10% of C_{max} after 24 h. In the corresponding protein-unbound plasma, $C_{max.u}$ levels were 60 nM, 189 nM, and 434 nM for the 0.6, 2.0, and 6.0 mg/kg doses, respectively (**Table 1**). Plasma concentrations of M-1 were neither determined in this single dose study nor in the two repeat-dose toxicity studies mentioned below.

Blood Pressure, HR, and ECG Intervals Following a Single Oral Dose

Vericiguat was associated with expected pharmacology-mediated dose-dependent changes in cardiovascular function (**Table 2**). The effects of vericiguat on mean arterial blood pressure in conscious telemetered dogs are shown in **Figure 1**. A change was observed in arterial blood pressure, particularly in systolic arterial blood pressure (up to −20%), that was not fully reversible within 16 h. Vericiguat was associated with a dose-dependent increase in HR, with changes from baseline of 17%, 28%, and 53% for 0.6, 2.0, and 6.0 mg/kg doses, respectively. Along with an increased HR (**Table 2** and **Figure 1**), the PQ and QT intervals were shortened (**Table 2**) with administration of vericiguat. PQ interval changed by −7%, −15%, and −17% for 0.6, 2.0, and 6.0 mg/kg doses, respectively. QT interval changed by −5%,

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−7%, and −12% for 0.6, 2.0, and 6.0 mg/kg doses, respectively (**Table 2** and **Figure 2**).

The observed change from baseline of corrected QT (QTc) interval for HR at maximum dose varied slightly between −5.9% and +1.4%, dependent on the formula of correction. The observed change from baseline using the QTcF formula was +0.2%, +0.6%, and +1.4% for 0.6, 2.0, and 6.0 mg/kg doses, respectively (**Table 2** and **Figure 2**). In comparison, the observed change from baseline using the QTcV formula was −1.1%, −1.7%, and −2.8% for 0.6, 2.0, and 6.0 mg/kg doses, respectively. Using QTcM yielded the greatest change from baseline per vericiguat dose, with −2.4%, −3.5%, and −5.9% for 0.6, 2.0, and 6.0 mg/kg doses, respectively. No drug-related or dose-dependent effects on QTc interval using any formulae of correction were observed at tested dosages and at exposure multiples up to 24-fold (**Table 2**).

ECG Intervals and Systemic Exposure Following Repeated Oral Dosing

In the 4-week repeat-dose toxicity study in beagle dogs, QTcF changes from baseline at week 1 were −2%, +2%, and 0% in males for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively, and −5%, +1%, and −11% in females for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively. At week 4, QTcF changes from baseline were −1%, −7%, and −9% in males for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively, and −6%, −3%, and +9% in females for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively (**Supplemental Table 3**).

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In the 4-week toxicity study, mean $C_{max,u}$ of vericiguat at week 1 was 216 nM, 584 nM, and 450 nM for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively. When measured at week 4, $C_{max,u}$ changed from week 1 by −7 nM, −89 nM, and +136 nM for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively (**Supplemental Table 3**).

In the 39-week toxicity study in beagle dogs, QTcF changes from baseline at week 13 were +4%, +11%, and +6% in males for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively, and +9%, +8%, and −7% in females for the 2.5 mg/kg/day, 7.5 mg/kg/day, and 25/15 mg/kg/day dosing regimens, respectively (**Supplemental Table 4**). Cardiac arrhythmias were not observed in this study.

In the 39-week toxicity study, $C_{max,u}$ at week 13 was 59 nM, 192 nM, and 481 nM for the 0.5 mg/kg/day, 1.5 mg/kg/day, and 5 mg/kg/day dosing regimens, respectively. When measured at week 38, $C_{max,u}$ changed from week 13 by +14 nM, +16 nM, and −149 nM for the 0.5 mg/kg/day, 1.5 mg/kg/day, and 5 mg/kg/day dosing regimens, respectively (**Supplemental Table 4**).

In-Vitro Whole-Cell Voltage-Clamp Investigations

Effects of Vericiguat and M-1 on the hERG K^+ Current Channel

In the GLP manual voltage-clamp study at approximately 22°C, vericiguat blocked hERG K^+-mediated tail currents of stably transfected HEK293 cells in a dose-dependent manner. Based on mean values ± SD, curve fitting with a standard four-parameter logistic equation yielded a 20% threshold inhibitory concentration (IC_{20}) of approximately 1.9 µM and IC_{50} of 9.9 µM (**Table 3**).

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In automated voltage-clamp recordings conducted at room temperature, vericiguat inhibited hERG K^+ outward and inward tail currents by 65% at 10 μM at 22°C, with an IC_{50} of 4.4 μM and 4.3 μM, respectively (**Table 3 and Figure 3A and 3B**). Exposure multiples were calculated as 244 and 239 for hERG K^+ outward and inward tail currents, respectively. M-1 inhibited hERG K^+ outward and inward tail currents by 20% and 17%, respectively, at 10 μM at 22°C. Exposure multiples were calculated as >233 (based on an IC_{50} >10 μM) for both outward and inward tail currents.

In manual patch clamp experiments performed to characterize the rate dependence of hERG K^+ current reduction at physiological temperature, vericiguat inhibited hERG K^+ with an IC_{50} of 2.9 μM at 0.03 Hz (73% at 10 μM), compared with 20% at 10 μM at 1 Hz. At 10 μM, inhibition by M-1 was −3.2% at 1 Hz and −0.0% at 0.03 Hz (**Table 3**).

Effects of Vericiguat and M-1 on Other Current Channels

Vericiguat and M-1 did not cause biologically relevant inhibition of hNav1.5, hCav1.2, hKvLQT1, and hKv4.3 at 10 μM, neither at 22°C nor at physiological temperature or in experiments simulating extreme depolarization or pacing rates (**Table 3 and Figures 3C–F**).

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Discussion

The four sets of nonclinical studies demonstrated no evidence of an increased proarrhythmic risk from the *in vitro* and *in vivo* assessment of vericiguat or its major *N*-glucuronide metabolite M-1.

In dogs, administration of vericiguat as single oral doses at a maximum dose of 6.0 mg/kg was associated with dose-dependent decreases in arterial blood pressure and compensatory increases in HR. This is consistent with long-lasting vasodilation attributable to the mode of action of sGC stimulators, which mediate relaxation of smooth muscle cells by increasing cGMP levels (Stasch et al., 2011). QTc intervals using QTcF, QTcV, and QTcM formulae were not prolonged to a meaningful extent in beagle dogs. Recorded exposure multiples of 3.3, 10.5, and 23.6 at 0.6, 2.0, and 6.0 mg/kg, respectively, indicate proarrhythmic risk did not occur at clinically relevant doses.

In several *in vitro* electrophysiological studies, potential effects on cardiac ion channels of vericiguat and M-1 were assessed in a comprehensive manner, covering the most important cardiac ion channels (hERG K^+, hNav1.5, hCav1.2, hKvLQT1/minK, and hKv4.3) at various temperatures (ambient and physiological) and a range of stimulation rates (0.03–3 Hz), simulating extreme pathophysiological conditions that might be observed in patients with HF. Neither vericiguat nor M-1 inhibited cardiac ion channels (hERG K^+, hNav1.5, hCav1.2, hKvLQT1/minK, and hKv4.3) at substantial exposure multiples of therapeutically relevant concentrations. The results for M-1 are consistent with the preponderance of scientific literature that *N*-glucuronide–conjugated drug metabolites are generally unreactive and benign (European Medicines Agency, 2013; Smith et al., 2018). Therefore, these data

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contribute to the current scientific understanding that there is no cause for concern regarding exposure to *N*-glucuronide metabolites. Vericiguat inhibited the hERG K^+ ion channel with an IC_{20} approximately 105-fold higher than the human clinical $C_{max.u}$ of 18 nM at 10 mg at room temperature.

Some technical challenges were encountered during the study of pharmacological effects by vericiguat or M-1 under conditions mimicking pathophysiological conditions. Excessive current rundown was particularly noted in studies measuring KvLQT1/minK currents at physiological temperature at extreme pacing rates, confounding pharmacological assessments. For this reason, a separate assessment was conducted at room temperature, in which little or no effects were noted at the extreme rates that could be tested. Similarly, no assessments of stable hNav1.5 currents were possible from holding potentials depolarized lower than −80 mV, owing to excessive current rundown attributed to the steady state inactivation properties of hNav1.5 currents.

At supratherapeutic concentrations, reverse frequency dependence was observed during the study of hERG K^+ inhibition by vericiguat at extreme pacing rates. The inhibitory potency at the two extremes (1 Hz and 0.033 Hz) was in line with previous assessments at room temperature, and sufficient margins were calculated for the lowest inhibitory concentration. This phenomenon of a so-called “reverse frequency dependent” hERG K^+ inhibition is well known (Weirich and Antoni, 1998) and has been described for many hERG K^+ blockers *in vitro* (Baskin and Lynch, 1994) and *in vivo*, including in humans (Démolis et al., 1996). Although reverse frequency dependence on action potentials has been correlated with drugs potently blocking hERG K^+ current, when frequency dependence of blockade on

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hERG K^+ channels has been systematically studied and modeled, it is clear that the major difference between drugs with high versus low torsadogenic risk is the impact of inward currents mitigating the effect of hERG K^+, and not a systematic difference in the intrinsic frequency dependence of hERG K^+ inhibition (Li et al., 2017). Other important considerations in the overall reverse frequency dependence are the effect of a drug on the KvLQT1/minK current, which contributes to the repolarization reserve (Weirich and Antoni, 1998), and safety margins. Drugs such as loratadine, sold over the counter, have been shown to display rate frequency dependence on hERG K^+ current (Crumb, 2000), but these potential concerns are obviated by the large safety margin to the effective clinical C_{max} (Redfern et al., 2003). Similarly, our study of vericiguat indicates intrinsic blocking properties on the hERG K^+ channel do not translate into adverse effects when sufficient safety margins are established.

Vericiguat had no meaningful effect on QTc interval in dogs when administered at doses ≤7.5 mg/kg/day. This absence of an effect was observed in 4- and 39-week repeat-dose toxicity studies in beagle dogs. It is important to note a positive control was not used in these studies. As *in vitro* hERG K^+ channel studies and *in vivo* telemetered beagle dog assays were deemed negative in terms of meaningful QT prolongation and altered ventricular repolarization at the therapeutic dose, a positive control was waived and is supported by recent recommendations in a cardiac safety regulation protocol (Lester, 2021). Moxifloxacin is often used as a positive control in QT prolongation investigations to determine the sensitivity of an assigned assay owing to its expected prolongation of the QT interval, regardless of therapy area (Carlson et al., 2011; Langenickel et al., 2016; Demmel et al., 2018; Sun et al., 2020). Although a positive control was not per protocol in these experiments, a clinical QTc interval study in patients with chronic coronary heart disease did use

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moxifloxacin as a positive control and similarly concluded that vericiguat 10 mg was not associated with proarrhythmic risk (Boettcher et al., 2021).

In summary, there was no nonclinical evidence of an increased proarrhythmic risk based on comprehensive *in vitro* and *in vivo* assessment of vericiguat or its major *N*-glucuronide metabolite M-1– findings consistent with the lack of any evidence of proarrhythmia seen during the vericiguat clinical development program. The integrated risk assessment of these nonclinical data combined with existing clinical data (Boettcher et al., 2020) demonstrate that administration of vericiguat 10 mg once daily in patients with HF with reduced ejection fraction is not associated with a proarrhythmic risk.

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Authorship Contributions

Participated in research design: Himmel, Imredy, Vömel

Conducted experiments: Himmel, Imredy, Vömel

Contributed new reagents or analytic tools: Himmel, Imredy, Vömel

Performed data analysis: Himmel, Imredy, Vömel

Wrote or contributed to the writing of the manuscript: Amin, Blaustein, Himmel, Imredy, Johnson, Lagrutta, Prescott, Vinzing, Vömel

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Footnotes

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The raw data are available upon reasonable request by an email to the corresponding author.

Herbert Himmel, Matthias Vömel, and Maya Vinzing are employees of Bayer AG, Wuppertal, Germany, and may own stock and/or stock options in the company. Armando Lagrutta, Rupesh P. Amin, John P. Imredy, Timothy Johnson, Judith Prescott, and Robert O. Blaustein are employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, who may own stock and/or stock options in Merck & Co., Inc., Rahway, NJ, USA.

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Figure Legends

Figure 1. Effects of vericiguat on (A) HR and (B) mean arterial blood pressure of conscious telemetered dogs (deviations from baseline)

n = 4/dose; mean values of 15-min bins.

HR, heart rate; MABP, mean arterial blood pressure; po, per oral.

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Figure 2. Effects of vericiguat on (A) QT interval and (B) QTcF interval of conscious telemetered dogs (deviations from baseline)

n = 4/dose; mean values of 15-min bins.

po, per oral; QTcF, corrected QT using Fridericia's formula.

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Figure 3. Effects of vericiguat on (A) outward and (B) inward hERG K^+, (C) hNav1.5, (D) hCav1.2, (E) hKvLQT1, and (F) hKv4.3 currents

Representative tracings from automated voltage-clamp experiments.

Transfected cells were exposed to solvent (0.1% DMSO), vericiguat (1 and/or 10 μM), and the appropriate positive controls (quin, 10 or 300 μM; nif, 1 μM; HMR1556, 10 μM). Voltage-clamp protocols: hERG K^+ (panels A, B), −80 mV (200 ms), +20 mV (1000 ms), −40 mV (outward tail)/−120 mV (inward tail) for 500 ms, −80 mV (200 ms), repeated every 12 s; hNav1.5 (panel C), −80 mV (40 ms), −120 mV (500 ms), −35 mV (20 ms), −120 mV (500 ms), −80 mV (40 ms), repeated every 6 s; hCav1.2 (panel D), −60 mV (100 ms), +5 mV (200 ms), −60 mV (100 ms), repeated every 6 s; hKvLQT1 (panel E), −80 mV (200 ms), +50 mV (2500 ms), −120 mV (500 ms), −80 mV (200 ms), repeated every 12 s; hKv4.3 (panel F), −80 mV (550 ms), +40 mV (200 ms), −80 mV (10 ms), repeated every 12 s. *n* = 3–13.

DMSO, dimethyl sulfoxide; hERG, human Ether-a-go-go Gene; HMR1556, positive control for hKvLQT1 K+ channel; nif, nifedipine; quin, quinidine; veri, vericiguat.

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Tables

Table 1. Plasma concentration and pharmacokinetic parameters of vericiguat in satellite dogs

Parameter	Units	Vericiguat Dose		
		0.6 mg/kg	2.0 mg/kg	6.0 mg/kg
$AUC_{(0–24)}$	μg×h/l	2274	7396	17,964
C_{max}	μg/l	270	848	1949
$C_{max.u}$ [†]	nM	60	189	434
Exposure multiple[‡]	–	3.3	10.5	23.6
T_{max}	h	2.1	3.0	3.0

n = 3/dose. [†]Molecular weight of vericiguat is 426.4 g/mol and the protein-unbound fraction in dogs is 9.5%.

[‡]Exposure multiples calculated based on human clinical C_{max} (unbound) of 18 nM.

$AUC_{(0–24)}$, area under the concentration-time curve from 0–24 h; C_{max}, maximum drug concentration in plasma; $C_{max.u}$, maximum unbound drug concentration in plasma; T_{max}, time at which C_{max} was reached.

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Table 2. Summary of changes in cardiovascular function following a single oral administration of vericiguat in conscious, telemetered dogs

Dose	Cardiovascular Effects (% Change From Baseline)			ECG Effects ((% Change From Baseline)					
(mg/kg)	SABP	DABP	HR	PQ	QRS	QT	QTcF	QTcV	QTcM
0 (control)	+0.3 ± 3.9	−0.5 ± 3.3	−2.7 ± 6.7	−1.5 ± 1.9	−2.6 ± 2.3	−1.2 ± 3.8	−2.4 ± 1.4	−1.9 ± 1.6	−1.7 ± 2.6
0.6	−5.6 ± 2.7	−5.6 ± 3.7	+16.8 ± 8.1	−7.2 ± 3.1	−2.9 ± 2.4	−4.7 ± 0.6	+0.2 ± 1.9	−1.1 ± 0.9	−2.4 ± 1.1
2.0	−11.4 ± 6.4†	−7.7 ± 10.5	+28.1 ± 20.5	−14.6 ± 7.0†	−3.0 ± 4.0	−6.8 ± 7.4	+0.6 ± 3.7	−1.7 ± 4.1	−3.5 ± 5.4
6.0	−20.1 ± 5.6†	−12.2 ± 9.2	+52.7 ± 28.4†	−16.5 ± 10.8†	−7.5 ± 3.1	−12.1 ± 6.1†	+1.4 ± 3.6	−2.8 ± 3.2	−5.9 ± 4.5

n = 4/dose.

Mean values ± SD of N = 4 animals. Values from the original 15-minute time bins were collapsed into a superinterval ranging from 2–6 h post-dose covering maximal effects (compare Figure 1). Statistical analysis (GraphPad Prism 8.0.2): one-way ANOVA followed by Dunnett's multiple comparisons test versus control; †Statistically significant differences versus control if $p < 0.05$ (multiplicity-adjusted). The absolute predrug baseline values of all tabulated parameters did not differ significantly between treatment groups. DABP, diastolic arterial blood pressure; ECG, electrocardiogram; HR, heart rate; PQ, PQ interval in the ECG; QRS, QRS complex in the ECG; QT, QT interval in the ECG; QTcF, corrected QT using Fridericia's formula; QTcM, corrected QT using Matsunaga's formula; QTcV, corrected QT using van de Water's formula; SABP, systolic arterial blood pressure.

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Table 3. *In vitro* electrophysiological assessments of vericiguat and M-1

	Vericiguat					
Ion Channel; Conditions	**IC_{20} (μM)**			**IC_{50} (μM)**		
hERG K^+; ~22°C[†]	1.9 (105-fold higher than the human $C_{max.u}$ 18 nM at 10 mg)			9.9		
	Vericiguat			**M-1**		
	Inhibition at 10 μM	**IC_{50} (μM)**	**Exposure Multiple[§]**	**Inhibition at 10 μM**	**IC_{50} (μM)**	**Exposure Multiple[‖]**
hERG K^+ outward tail (~0.08 Hz); ~22°C[‡]	65%	4.4	244	20%	>10	>233
hERG K^+ inward tail (~0.08 Hz); ~22°C[‡]	65%	4.3	239	17%	>10	>233
hNav1.5 (~0.17 Hz); ~22°C[‡]	2.3%	>10	>556	−0.7%	>10	>233
hCav1.2 (~0.17 Hz); ~22°C[‡]	6.8%	>10	>556	9%	>10	>233
hKvLQT1 (~0.08 Hz); ~22°C[‡]	1.5%	>10	>556	−1.9%	>10	>233
hKv4.3 (~0.08 Hz); ~22°C[‡]	3.4%	>10	>556	−1.6%	>10	>233
hERG K^+ (1 Hz);	19.9%	>10	>556	−3.2%	>10	>233

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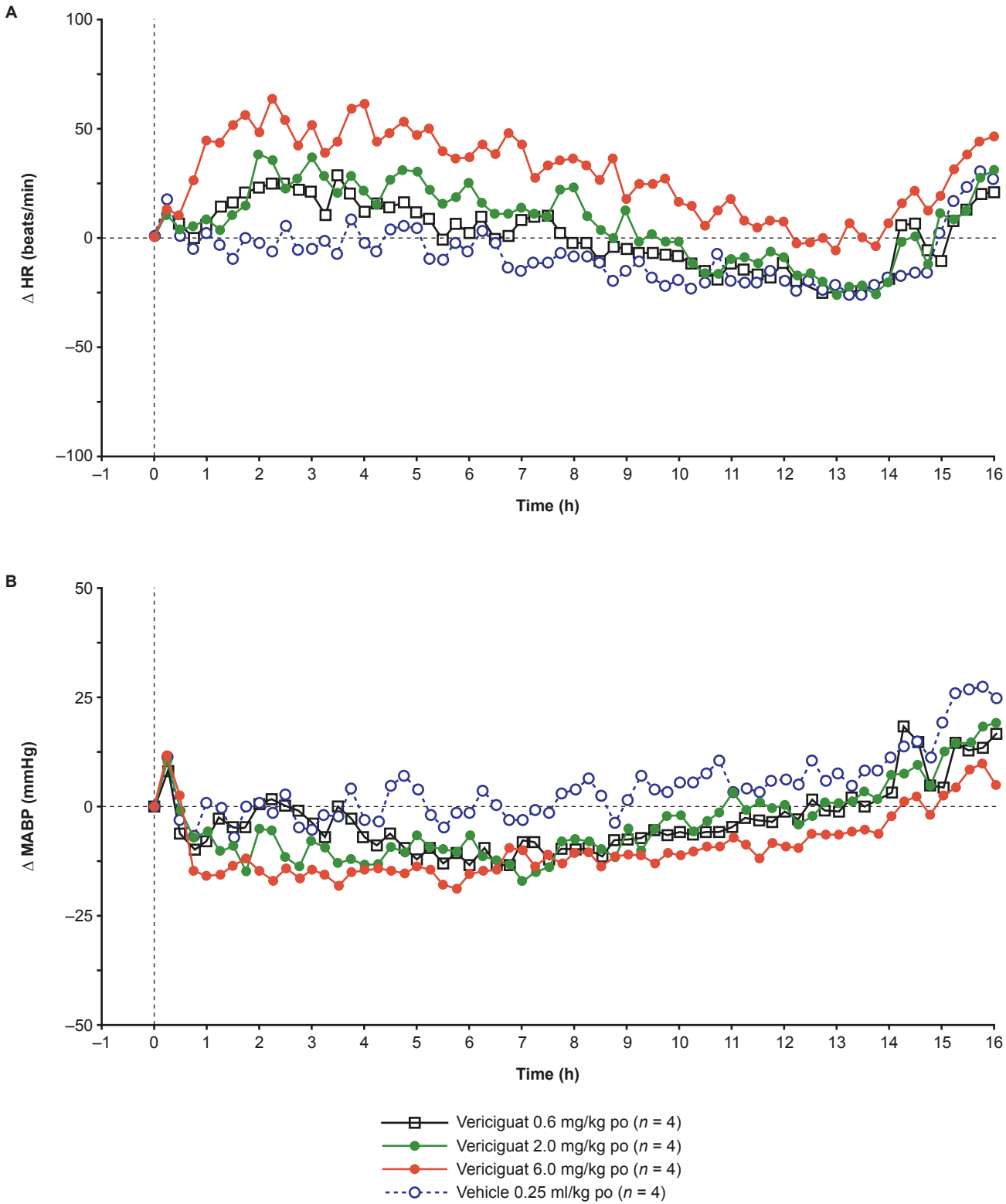
37°C[†]						
hERG K^+ (0.033 Hz); 37°C[†]	73.4%	2.9[#]	161	−0.0%	>10	>233
hNav1.5 (3 Hz); −80 mV; 37°C[†]	−3.3%	>10	>556	−2.3%	>10	>233
hKvLQT1 (0.1 Hz); ~22°C[‡]	−2.9%	>10	>556	−0.1%	>10	>233
hKvLQT1 (0.033 Hz); ~22°C[‡]	−1.3%	>10	>556	−2.7%	>10	>233

[†]Manual voltage clamp. [‡]Automated voltage clamp. [§]Exposure multiples calculated based on human clinical C_{max} (unbound) of 18 nM, compared with the IC_{50} or highest concentration tested for each channel at 10 mg dosage. [‖]Exposure multiples calculated based on human clinical C_{max} (unbound) of 43 nM, compared with IC_{50} or highest concentration tested for each channel at 10 mg dosage. [#]Interpreted as small, reverse frequency dependence of hERG potency, comparing effects at the different stimulation rates tested in the hERG studies listed.

C_{max}, maximum drug concentration in plasma; hERG, human Ether-a-go-go Gene; IC_{20}, 20% threshold inhibitory concentration; IC_{50}, half-maximal inhibitory concentration.

Figure 1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9
10
11
12
13
14
15
16
Time (h)
B
50
25
0
−25
−50
Δ MABP (mmHg)
−1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Time (h)
Vericiguat 0.6 mg/kg po (n = 4)
Vericiguat 2.0 mg/kg po (n = 4)
Vericiguat 6.0 mg/kg po (n = 4)
Vehicle 0.25 ml/kg po (n = 4)

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Figure 2

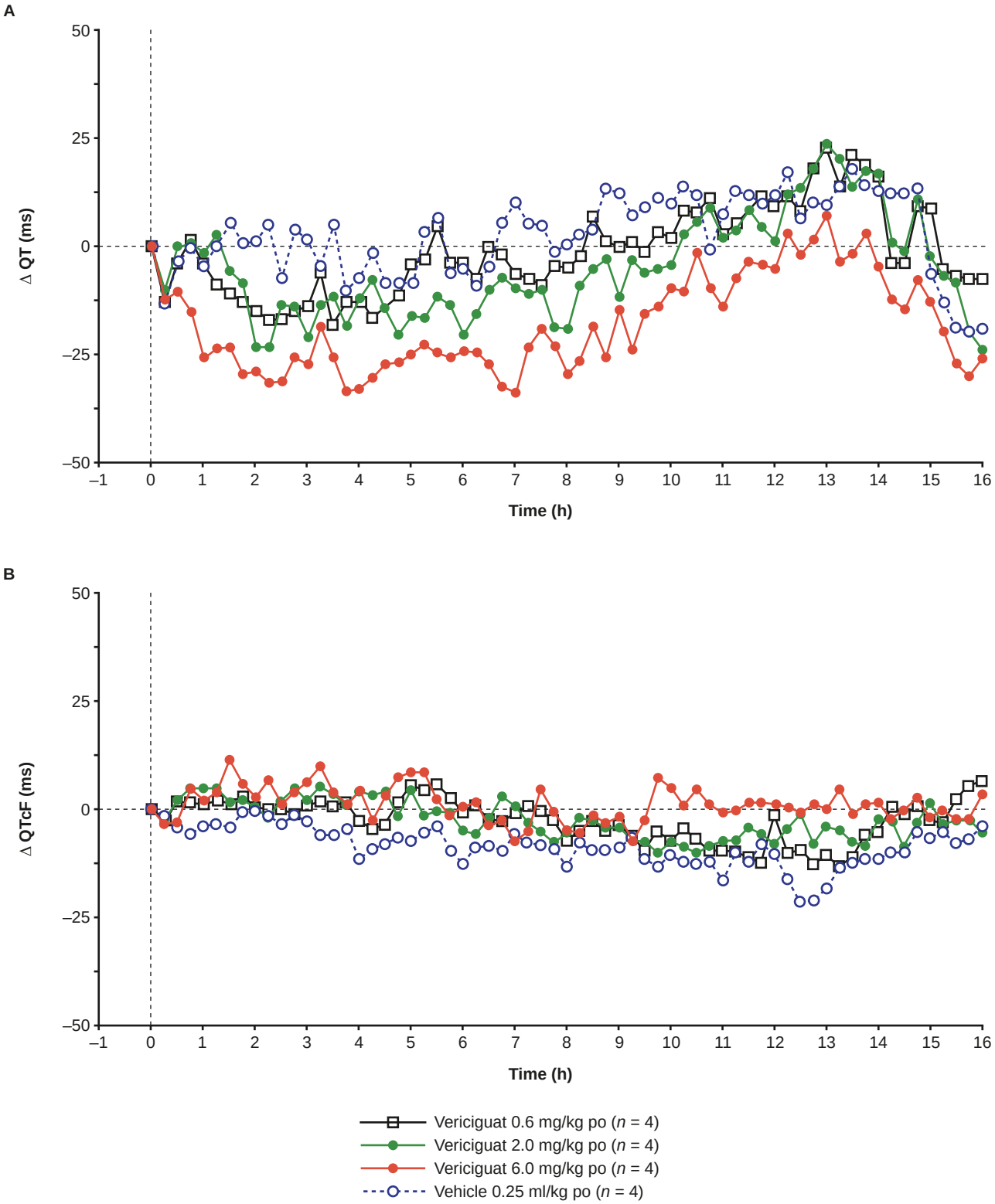
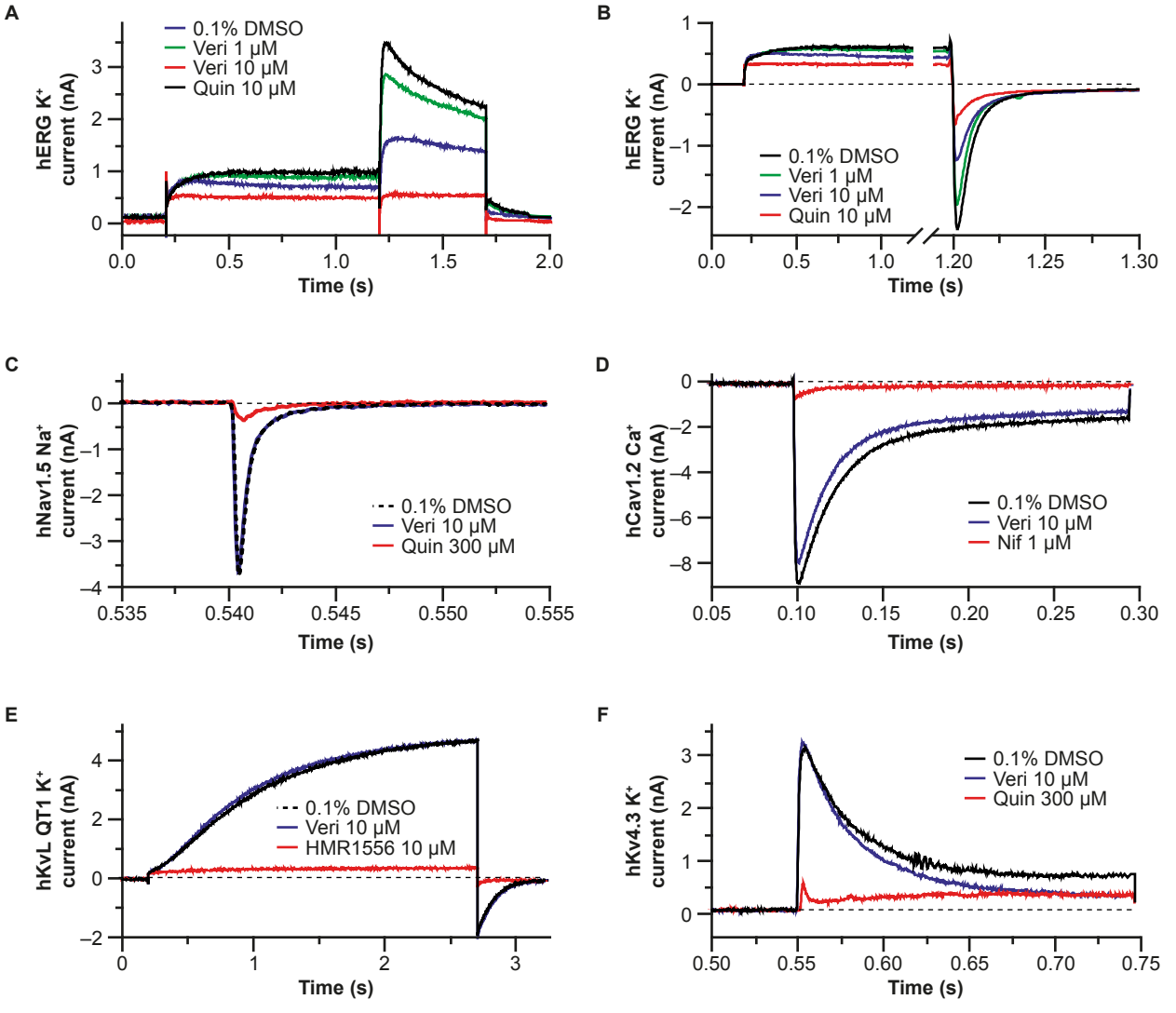


Figure 3


A
hERG K+ current (nA)
0.1% DMSO
Veri 1 μM
Veri 10 μM
Quin 10 μM
Time (s)
B
hERG K+ current (nA)
0.1% DMSO
Veri 1 μM
Veri 10 μM
Quin 10 μM
Time (s)
C
hNav1.5 Na+ current (nA)
0.1% DMSO
Veri 10 μM
Quin 300 μM
Time (s)
D
hCav1.2 Ca+ current (nA)
0.1% DMSO
Veri 10 μM
Nif 1 μM
Time (s)
E
hKvL QT1 K+ current (nA)
0.1% DMSO
Veri 10 μM
HMR1556 10 μM
Time (s)
F
hKv4.3 K+ current (nA)
0.1% DMSO
Veri 10 μM
Quin 300 μM
Time (s)