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ORIGINAL ARTICLE

A phase 1 trial of AP30663, a $K_{Ca}2$ channel inhibitor in development for conversion of atrial fibrillation

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Aims: AP30663 is a novel compound under development for pharmacological conversion of atrial fibrillation by targeting the small conductance Ca^{2+} activated K^+ ($K_{Ca}2$) channel. The aim of this extension phase 1 study was to test AP30663 at higher single doses compared to the first-in-human trial.

Methods: Sixteen healthy male volunteers were randomized into 2 cohorts: 6- and 8-mg/kg intravenous single-dose administration of AP30663 *vs.* placebo. Safety, pharmacokinetic and pharmacodynamic data were collected.

Results: AP30663 was associated with mild and transient infusion site reactions with no clustering of other adverse events but with an estimated maximum mean QTcF interval prolongation of 45.2 ms (95% confidence interval 31.5–58.9) in the 6 mg/kg dose level and 50.4 ms (95% confidence interval 36.7–64.0) with 8 mg/kg. Pharmacokinetics was dose proportional with terminal half-life of around 3 h.

Conclusion: AP30663 in doses up to 8 mg/kg was associated with mild and transient infusion site reactions and an increase of the QTcF interval. Supporting Information support that the QTc effect may be explained by an off-target inhibition of the I_{Kr} channel.

KEYWORDS
atrial fibrillation, Ca^{2+} activated K^+ ($K_{Ca}2$) channel, QT interval

1 | INTRODUCTION

Atrial fibrillation (Afib) is a common arrhythmia with a prevalence of >37.5 million people worldwide.[1] Both pharmacological and nonpharmacological interventions are used, however with varying success since arrhythmia recurrence frequently occurs in patients, with the underlying atrial cardiomyopathy being considered a progressive disease.[2,3] One of the key issues with currently available pharmacological interventions are ventricular adverse effects that limit the dose of the antiarrhythmic that can be administered safely. As a reflection of this, the guiding principle when choosing between antiarrhythmic drugs to treat Afib, is cardiac safety.[4]

Pim Gal and Anders G. Holst contributed equally to this work.

The authors confirm that the principal investigator for this paper was Dr Matthijs Moerland and the Medical Responsible was Dr Pim Gal. They both had direct responsibility for subjects.

A potential new pharmacological treatment strategy is targeting the inhibition of small conductance Ca^{2+} activated K^+ ($K_{Ca}2$) channels, that, from a functional perspective, may have a more prominent role in atria compared to ventricles. Three subtypes of $K_{Ca}2$ channels exist, 2 of which ($K_{Ca}2.2$ and $K_{Ca}2.3$) have been linked to Afib in genome-wide association studies.[5,6]

Drugs targeting the $K_{Ca}2$ channels have demonstrated efficacy in a range of animal models of Afib as well as functional atrial selectivity in patients in sinus rhythm and in Afib.[7,8] Atrial-selective drug therapy may improve safety due to lack of adverse ventricular effects. This may also allow use of higher doses, and a more effective treatment for both conversion to sinus rhythm as well as maintenance therapy as compared to current therapies.

The novel compound AP30663 is in clinical development as a $K_{Ca}2$ channel inhibitor for conversion of Afib into sinus rhythm. The first-in-human trial with AP30663 was completed, with doses up to 6 mg/kg being administered without encountering dose-limiting toxicity,[9] but with a finding of dose-dependent effect of AP30663 on the QT interval and a finding of infusion site reactions. No further dose cohorts were studied as all protocol-specified cohorts were expended testing different formulations to mitigate infusion site reactions. It is currently being tested in a phase 2 proof-of-concept trial in patients, investigating its efficacy to convert Afib to normal sinus rhythm (NCT04571385).

As animal data[8] indicated potential for additional efficacy at higher doses than tested in the first-in-human trial, and to further characterize the effect on ventricular repolarization, an extension single ascending dose trial was planned.

Therefore, the aim of the present trial was to further characterize the effects of AP30663, in particular the effect on ventricular repolarization. This was done by administering AP30663 to healthy volunteers at similar and a higher dose level to evaluate the safety and tolerability, including the effect of AP30663 on the QT interval.

What is already known about this subject

- AP30663 is a novel compound under development for pharmacological conversion of atrial fibrillation by targeting the $K_{Ca}2$ channel.
- In a first-in-human trial AP30663, in doses up to 6 mg/kg intravenously, was associated with infusion site reactions and QTc increase up to 18.8 ms.

What this study adds

- In this trial, we tested higher doses of AP30663 than obtained in previous clinical testing.
- AP30633, at doses up to 8 mg/kg intravenously, was associated with infusion site reactions and QTc increase up to 50.4 ms
- The QTc effect may be explained by off-target inhibition of the I_{Kr} channel.

2 | METHODS

The extension trial was conducted as a randomized, single ascending dose, double blind, placebo-controlled phase 1 trial. The trial was conducted between November 2020 and February 2021 at the Centre for Human Drug Research, Leiden, The Netherlands. The independent Medical Ethics Committee 'Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek' (Assen, the Netherlands) approved the trial prior to any clinical trial activity. All subjects provided written informed consent before participation.

2.1 | Subjects

The main inclusion criteria were male sex, generally healthy, age 18–45 years with a body mass index between 18 and 30 kg/m^2 and absence of clinically significant abnormalities in the 12-lead surface electrocardiogram (ECG). The health status was verified by a detailed medical history, a complete physical examination, vital signs, 12-lead ECG and laboratory testing (including hepatic and renal panels, complete blood count, virology and urinalysis). Subjects were excluded in case of any use of prescription medication within 2 weeks prior to enrolment.

2.2 | Trial design and treatment

Four cohorts of 8 subjects were planned, with increasing dose levels from 6 to 12 mg/kg between cohorts. The trial employed an adaptive design with review of safety and pharmacokinetic (PK) data between each cohort. Within each cohort, subjects were randomized in a 6:2 ratio to 30 min intravenous administration of AP30663 or placebo, respectively. Placebo consisted of 5% glucose.

The Leiden University Medical Center pharmacy performed the reconstitution for all administrations in this trial. The reconstitution consisted of glucose 5% being injected into an empty infusion bag. Subsequently, AP30663 was injected into the infusion bag, which was reconstituted from a vial with a 200 mg/mL concentration. The concentration of AP30663 was 3 mg/mL. The administration was performed with a 16G cannula, and the cannula was flushed with 5 mL 5% glucose solution immediately after completion of the administration. There were 2 venipunctures, 1 in each arm: 1 was used for trial drug infusion and the other for collecting blood samples.

2.3 | Assessments

2.3.1 | Safety and tolerability

Physical examination, vital signs, 24-h Holter ECGs, blood biochemistry, urinalysis and registration of adverse events (AEs) were performed at specific time points during the study for assessment of safety and tolerability. A Safety Review Committee consisting of sponsor representatives and the principal investigator was to review safety data, PK results and pharmacodynamic effects of previous dose levels.

2.3.2 | PK concentrations

Thirteen blood samples for PK measurements (total and free AP30663 concentrations) were collected predose and at 10, 20, 29, 35, 45 min, 1, 1.5, 2.5, 4, 8, 12, 24 and 48 h after dosing. AP30663 PK concentrations were assessed in serum samples using liquid chromatography/mass spectrometry. Analysis was performed by Ardena Biochemical Laboratory (Assen, the Netherlands) using a validated bioanalytical assay and the lower limit of quantification of the method was 5 ng/mL.

2.3.3 | Tremorography

To assess any potential drug-induced tremor, tremorography (ACL300 and DataLINK, Biometrics Ltd) measurements were performed at predefined timepoints pre- (2 h) and postdosing (0.5, 1.5, 2.5, 4, 8 and 24 h). The tremorography measurements were performed as described previously.[9] There was no protocol-mandated neurological examination.

2.3.4 | Telemetry and Holter ECGs

Subjects were monitored via telemetry during the stay at the site, while the Holter recordings covered baseline, infusion and postinfusion for a total of 24 h.

A 12-lead Holter equipment (Mortara Instrument BV) was applied for 24 h in all volunteers, starting just before dose administration. At PK sampling timepoints, subjects were placed in a supine position for at least 5 min. The timepoints for PK and ECG extractions were calculated from the start of the 30-min infusion. In the last 3 min, triplicate 12-lead ECGs were extracted from the Holter ECG by Intermark Technology ECG Research BV (Someren, the Netherlands), and analysis was made from the leads II and V5. The selection of ECG recordings was performed so that an even distribution occurred within the 3-min time frame, and the quality of the ECGs was sufficiently high to perform the measurements. A single certified operator, blinded for treatment and time, measured P wave duration, PR-interval duration, QRS duration, QT interval duration, J-point–T-peak interval, and T-peak–T-end interval (Tp-Tend) and RR interval duration using global median beat analysis in the Mortara E-scribe environment.[10] The QT interval duration was corrected for the RR interval with Fridericia's formula.

The correction of the Jp-Tpc interval was performed using the formula proposed by Johannesen[10] $\mathrm{Jp-Tpc\,interval} = \frac{\mathrm{Jpoint} - \mathrm{Tpeak\,interval}}{\mathrm{RR\,interval}^{0.58}}$. All Holter recordings were analysed for atrial and ventricular arrhythmias.

2.4 | Statistics

Statistics and noncompartmental analysis were performed as defined in Gal *et al.*[9] The statistical methodology and changes in the applied methodology are described in short below.

The PK noncompartmental analysis was performed using Phoenix Winnonlin version 8.3 (Certara USA, Inc., Princeton, NJ, USA). The elimination rate constant (λ_z) and its derived parameters were excluded when they could not be accurately estimated (adjusted R^2 <0.85, nonpositive value for λ_z or <3 data points above the lower limit of quantification available after time of maximum plasma concentration [C_{max}]).

Pharmacodynamic endpoints measured at multiple time points post baseline were analysed with a mixed effect repeated measures model with treatment, time and treatment by time as fixed factors, subject as random factor, and the baseline measurement as covariate. The data from 25 to 60 min after dosing were used in the model as this was expected to coincide with the C_{max} and thereby the maximum pharmacodynamic effect.

A concentration *vs*. QTcF and QT subinterval statistical analysis was performed using the full datasets of both the first-in-human study and the extension study, based on the prespecified model of Garnett *et al.*[11]

Study specific effect parameters were tested for inclusion in the model. Furthermore, besides linear concentration–effect relationships, sigmoidal maximum effect (E_{max}) relationships were tested for inclusion to describe the effects over the full concentration range with model selection based on the objective function value (P < .05) or the Akaike information criterion.

2.5 | Supplementary analyses

See the Supporting Information for supporting genetic analyses and in vitro study methods.

3 | RESULTS

Based on the adaptive study design, it was decided to conclude the trial after enrolling the first 2 cohorts based on concerns with exposing healthy volunteers to increasing QTc effects. In total, 16 healthy male subjects were enrolled in the trial, who were enrolled into 2 cohorts, receiving 6 mg/kg (cohort 1) or 8 mg/kg (cohort 2), or

TABLE 1 Baseline characteristics.

	All participants ($n = 16$)	AP30663 6 mg/kg ($n = 6$)	AP30663 8 mg/kg ($n = 6$)	Placebo ($n = 4$)
Age (years)	22 (18–38)	25 (20–38)	21 (18–34)	22 (21–25)
Height (cm)	179 (170–196)	178 (176–191)	182 (170–196)	185 (176–187)
Weight (kg)	78 (67–95)	78 (72–86)	77 (67–91)	78 (72–95)
BMI (kg/m^2)	24 (21–28)	24 (23–26)	23 (23–24)	24 (21–28)

Note: Values are presented as median (range).
Abbreviation: BMI, body mass index.

placebo. Both cohorts consisted of 8 subjects, 6 of whom received AP30663 and 2 placebo. All enrolled subjects completed the trial and there were no discontinuations. Baseline characteristics are displayed in Table 1.

3.1 | AEs

No serious AEs occurred. Thirty-nine treatment-emergent AEs (TEAEs) occurred. Thirteen of the 16 participants reported TEAEs: 2/4 participants (50%) treated with placebo; 5/6 participants (83%) treated with AP30663 6 mg/kg; and 6/6 participants (100%) treated with AP30663 8 mg/kg. All AEs were mild and all resolved. No arrhythmias were reported.

Eight out of 12 subjects that received AP30663 and 0 out of 4 subjects that received placebo reported TEAEs related to infusion site reactions. No other AE type seemed to be related to treatment (Table 2).

3.2 | Tremorography

There were no signs of tremor and all tremorography recordings were normal and unchanged at all dose levels, with a mean amplitude of 0.2 mG^2/Hz.

3.3 | PK

The PK profiles of AP30663 by dose are displayed in Figure 1. AP30663 was administered as a 30-min linear infusion, reflected in a median time of C_{max} at 30 min. The mean ± standard deviation of clearance was 20.4 ± 4.2 and 15.2 ± 4.0 L/h for the 6 and 8 mg/kg cohorts, respectively. Volume of distribution ranged between 71.6 ± 15.0 L and 64.4 ± 15.1 L for the 6 and 8 mg/kg cohorts, respectively. The mean terminal half-life was 3.0 ± 0.4 and 3.3 ± 0.5 h for the 6 and 8 mg/kg cohorts, respectively. The mean C_{max} of AP30663 increased with increasing doses (11 027 ± 1165 ng/mL and 16 867 ± 2426 ng/mL), as did the AP30663 free compound C_{max} concentration (836.5 ± 64.5 ng/mL and 1070.4 ± 217.7 ng/mL). The AP30663 free compound fraction at C_{max} was 7.6% for the 6-mg/kg dose level and 6.4% for the 8-mg/kg dose level.

3.4 | Holter and ECG parameters

No clinically relevant atrial or ventricular arrhythmias were found in the Holter analyses.

Based on the statistical model using data from 25 to 60 min after dosing, coinciding with C_{max}, no statistically significant effects of AP30663 were observed on heart rate (AP30663 6 mg/kg $P = .6942$, AP30663 8 mg/kg $P = .2733$), PR-interval (AP30663 6 mg/kg $P = .1606$, AP30663 8 mg/kg $P = .1119$) and P-wave duration (AP30663 6 mg/kg $P = .9656$, AP30663 8 mg/kg $P = .1709$).

A statistically significant, but minor, effect on the QRS interval was found: an estimated mean QRS interval prolongation of 3.4 ms ($P = .0275$) was seen with AP30663 6 mg/kg and 2.8 ms ($P = .0517$) with 8 mg/kg, both *vs.* placebo.

An increase in the QTcF interval was seen for both doses compared to placebo. The maximum effect was seen after 30–60 min (Figure 2) with values decreasing over the course of 1–3 h and with a return to baseline levels after approximately 24 h. Based on the analyses using data from 25 to 60 min after dosing an estimated mean QTcF interval prolongation of 45.2 ms (95% confidence interval [CI] 31.5–58.9, $P < .0001$) was seen with AP30663 6 mg/kg and 50.4 (95% CI 36.7–64.0, $P < .0001$) with 8 mg/kg, both *vs.* placebo. Point estimates indicated a dose–response although CIs are widely overlapping. With AP30663 6 mg/kg individual increases up to 64.3 ms and a QTcF of 463.4 ms were seen. With AP30663 8 mg/kg individual increases up to 64.4 ms and a QTcF of 474.3 ms were seen.

3.5 | Concentration *vs.* QRS, QTcF and QT subinterval analysis

AP30663 concentration *vs.* QTcF and concentration *vs.* QT subinterval analysis showed that AP30663 had an effect on the QTcF, Jp-Tpc and Tp-Tend interval. Significant sigmoidal E_{max} relationships were estimated for the QTcF, Jp-Tpc and Tp-Tend intervals, which provided a better fit to the data compared to linear concentration–effect relationships. A nonsignificant slope of AP30663 ($P = .097$) on the QRS interval was estimated. Model-predicted placebo-corrected change from baseline of QTcF and each of the QT subintervals *vs.* AP30663 concentration are

TABLE 2 Treatment-emergent adverse events by system organ class and preferred term.

System organ class/preferred term	AP30663 6 mg/kg ($n = 6$)			AP30663 8 mg/kg ($n = 6$)			Placebo ($n = 4$)		
	Events, *n*	Participants, *n* (%)		Events, *n*	Participants, *n* (%)		Events, *n*	Participants, *n* (%)	
All	18	5	(83.3)	17	6	(100.0)	4	2	(50.0)
Cardiac disorders	0	0		1	1	(16.7)	0	0	
Dizziness	0	0		1	1	(16.7)	0	0	
General disorders and administration site conditions	12	4	(66.7)	13	5	(83.3)	0	0	
Asthenia	0	0		1	1	(16.7)	0	0	
Fatigue	1	1	(16.7)	0	0		0	0	
Infusion site discomfort*	1	1	(16.7)	2	1	(16.7)	0	0	
Infusion site erythema*	0	0		1	1	(16.7)	0	0	
Infusion site haematoma*	1	1	(16.7)	0	0		0	0	
Infusion site induration*	3	3	(50.0)	2	2	(33.3)	0	0	
Infusion site pain*	1	1	(16.7)	2	2	(33.3)	0	0	
Infusion site reaction*	0	0		1	1	(16.7)	0	0	
Infusion site swelling*	1	1	(16.7)	1	1	(16.7)	0	0	
Infusion site warmth*	0	0		1	1	(16.7)	0	0	
Vessel puncture site erythema*	1	1	(16.7)	2	2	(33.3)	0	0	
Vessel puncture site haematoma*	1	1	(16.7)	0	0		0	0	
Vessel puncture site induration*	1	1	(16.7)	0	0		0	0	
Vessel puncture site swelling*	1	1	(16.7)	0	0		0	0	
Investigations	0	0		1	1	(16.7)	1	1	(25.0)
Blood creatine phosphokinase increased	0	0		0	0		1	1	(25.0)
ECG QT prolonged	0	0		1	1	(16.7)	0	0	
Musculoskeletal and connective tissue disorders	2	2	(33.3)	1	1	(16.7)	1	1	(25.0)
Limb discomfort	1	1	(16.7)	0	0		1	1	(25.0)
Myalgia	1	1	(16.7)	1	1	(16.7)	0	0	
Nervous system disorders	3	3	(50.0)	0	0		1	1	(25.0)
Somnolence	1	1	(16.7)	0	0		0	0	
Tension headache	2	2	(33.3)	0	0		1	1	(25.0)
Psychiatric disorders	1	1	(16.7)	0	0		0	0	
Hallucination | gustatory	1	1	(16.7)	0	0		0	0	
Respiratory, thoracic and mediastinal disorders	0	0		1	1	(16.7)	0	0	
Nasal congestion	0	0		1	1	(16.7)	0	0	
Vascular disorders	0	0		0	0		1	1	(25.0)
Ecchymosis	0	0		0	0		1	1	(25.0)

Note: All terms are according to Medical Dictionary for Regulatory Activities (MedDRA).
Abbreviation: ECG, electrocardiogram.
*Infusion site refers to the site of treatment infusion. Vessel puncture site refers to the site of drawing the blood sample. These were done in opposite arms.

displayed in Figure 3. In addition, model-predicted placebo-corrected change from baseline of QTcF *vs.* AP30663 from both the first-in-human trial and the current trial (pooled analysis) are provided in Figure 4. The model parameters for the analysis from the first-in-human trial and the current, extension trial are provided in Table 3.

3.6 | Genetic analyses and in vitro study

No genetic variants in the region of *KCNN1–3* genes were found to be associated with the QT interval (Figure S1). Also, no genetic variants in the regions of *KCNN1–3* genes were found to be significantly associated with changes in QT interval in PheWAS databases.

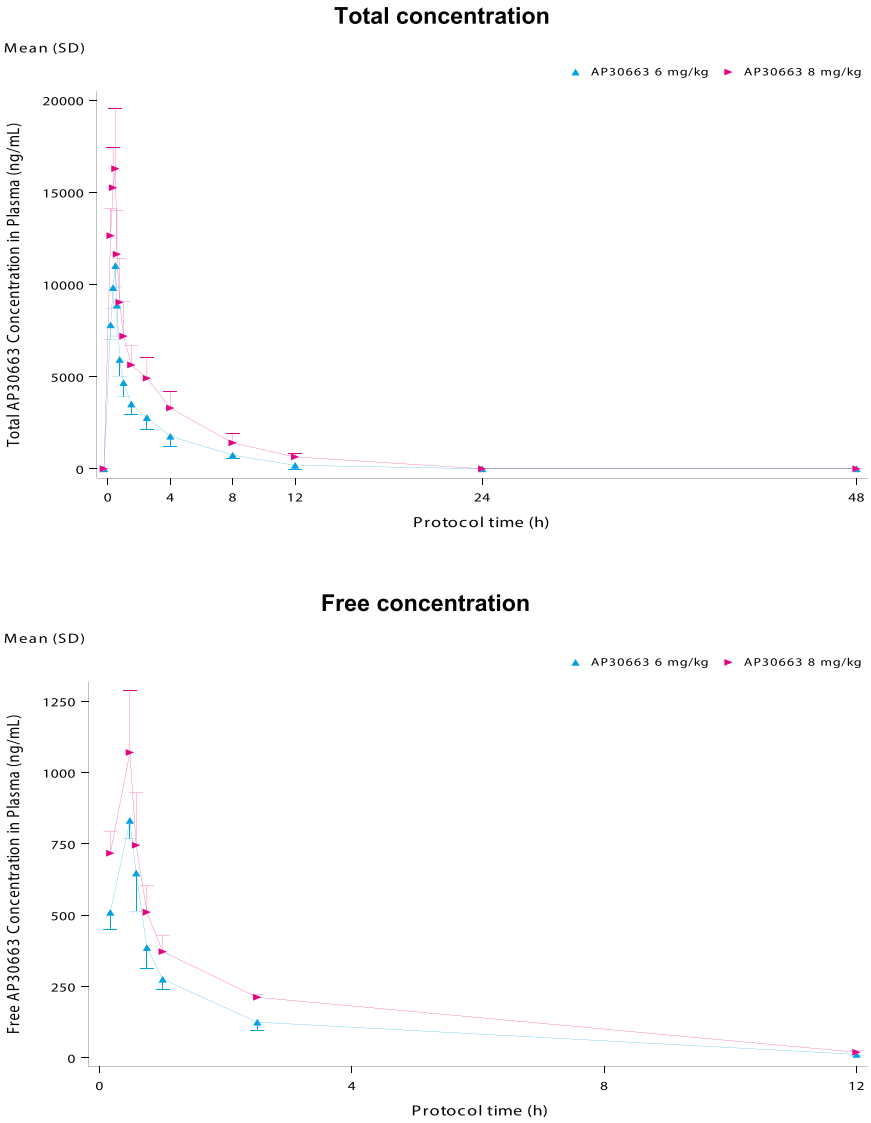


FIGURE 1 This figure displays the total (top panel) and free (bottom panel) concentration of AP30663 after 6 and 8 mg/kg administrations.

In contrast, in the FinnGen study there were highly statistically significant associations (Figure S1) between variants in the region of *KCNN3* and Afib.

AP30663 has previously been found to inhibit heterologously expressed $K_V11.1$ channel current (I_{Kr}) with an IC_{50} value of 4.0 ± 1.5 μM.[12] The measured mean free C_{max} of AP30663 in the 8 mg/kg group was ~1000 ng/mL = 2.5 μM. The % inhibition at 2.5 μM can be calculated to be 39.6%. In silico, an I_{Kr} block of 40% gives rise to a prolongation of the left ventricular human action potential duration of 96 ms. See Supporting Information for detailed results.

4 | DISCUSSION

The present single ascending dose trial found that AP30663 in doses up to 8 mg/kg was associated with mild and transient infusion site reactions and a mean increase in the QTcF interval of 50.4 ms with the highest dose of 8 mg/kg. The Safety Review Committee decided to terminate the study after the 8 mg/kg dose due to increase of the QTcF interval, as it could not be excluded that a further increase of the QTcF induced by higher doses might expose the healthy volunteers to a higher risk than acceptable.

In the first human dose trial,[9] dose levels up to 6 mg/kg were explored. The results from the first human dose trial[9] were overall consistent with the present trial with findings of local infusion site reactions and a mean QTcF increase of 18.8 ms in the 6-mg/kg dose level based on an exposure-response analysis. As such, the estimated QTcF effects were generally lower in the first trial where only 3 subjects were exposed to 6 mg/kg. A likely explanation for the difference in QTcF effect estimates is the sparse number of subjects in the highest dose cohorts in the first human dose trial leading to a large uncertainty in the estimates. The repetition of a 6-mg/kg cohort was expected to increase the robustness of data on this dose and thereby generate additional data to guide the decision to proceed to dose the next cohort with 8 mg/kg. Combining the data from both studies enabled the estimation of an E_{max} relationship compared to linear concentration–effect relationship due to the higher concentrations reached in the current study. Furthermore, the uncertainty in the

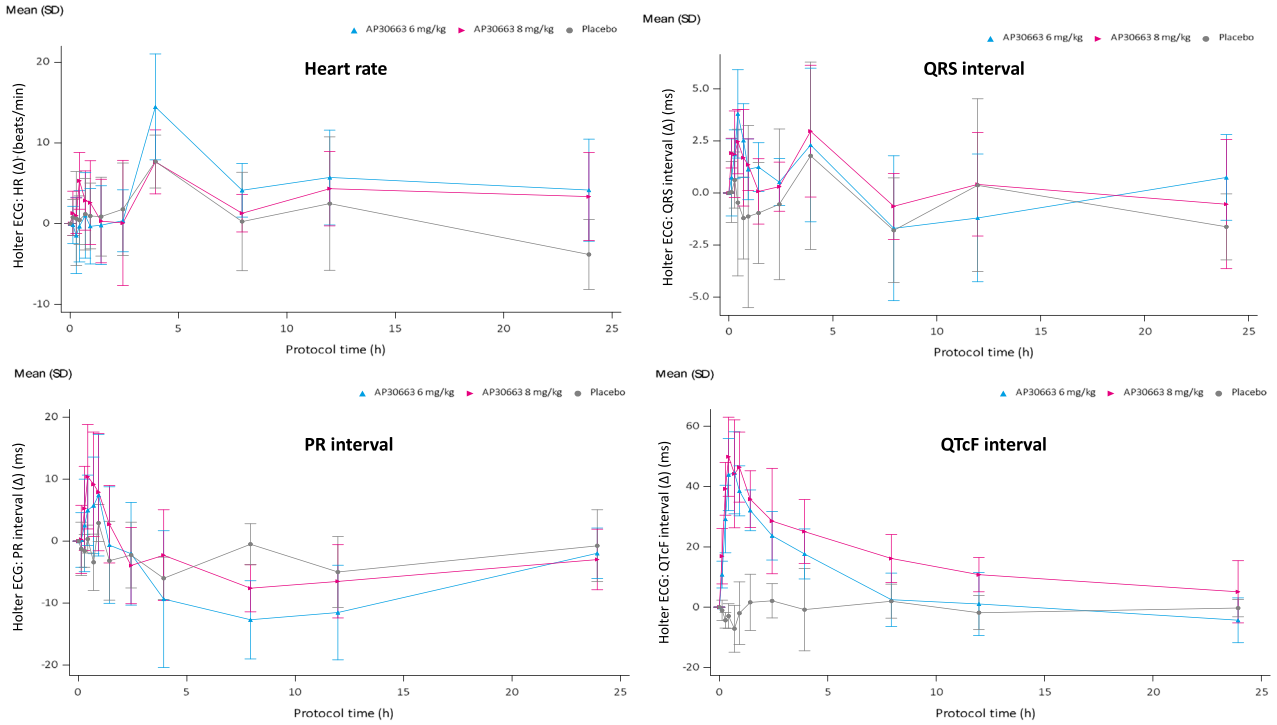
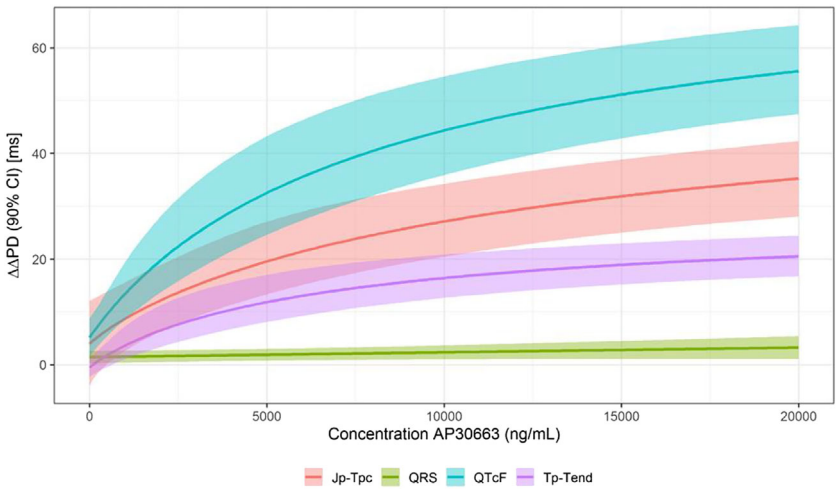


FIGURE 2 This figure displays the effect of 6 and 8 mg/kg AP30663 administration on the heart rate (top left panel), QRS interval (top right panel), PR-interval (bottom left panel) and QTcF interval (bottom right panel). Δ: change from baseline.

FIGURE 3 This figure displays the model-predicted association between AP30663 concentration and the QTcF interval, including the subintervals; QRS interval, Jp-Tpc interval and Tp-tend interval. Solid line = mean, shaded area = 90% confidence interval. ΔΔPD: baseline- and placebo-corrected electrocardiographic measure.



model parameters was reduced after combining the data from both studies.

A previous study in pigs with AP30663 found limited effects on the QTc interval at high doses (20 mg/kg)/plasma concentrations (4532 ± 844 ng/mL)[8] and, taken together, the magnitude of QTcF effects found in the present trial was unanticipated. Data from our studies on I_{Kr} inhibition in vitro and in silico, and genetic studies give some support to the view, that the observed increase in QTcF may be caused by I_{Kr} inhibition rather than by $K_{Ca}2$ inhibition. This indicates an opportunity to identify future $K_{Ca}2$ inhibitors without QTc effects.

One of the objectives with the current trial was to collect enough information in phase 1 studies in order to mitigate the need for a *thorough QT* (TQT) study. Based on the generated QTc results, it is certain that a TQT study would be positive (by not being able to rule out a 10-ms increase in QTcF). Since the main objective with a TQT study is to establish whether there is a risk of clinically relevant QTc increases, and we now know that this is the case with AP30663, a TQT study is probably not needed. We will, however, need to follow the regulatory guidance for a positive TQT study in any subsequent clinical development phases where a focus will be to assess the risk of proarrhythmia in the Afib patient population. Currently, the therapeutic doses of AP30663 have not been established and the QTcF effect at these is therefore unknown. Based on a study in pigs that measured the effect on the pharmacodynamic

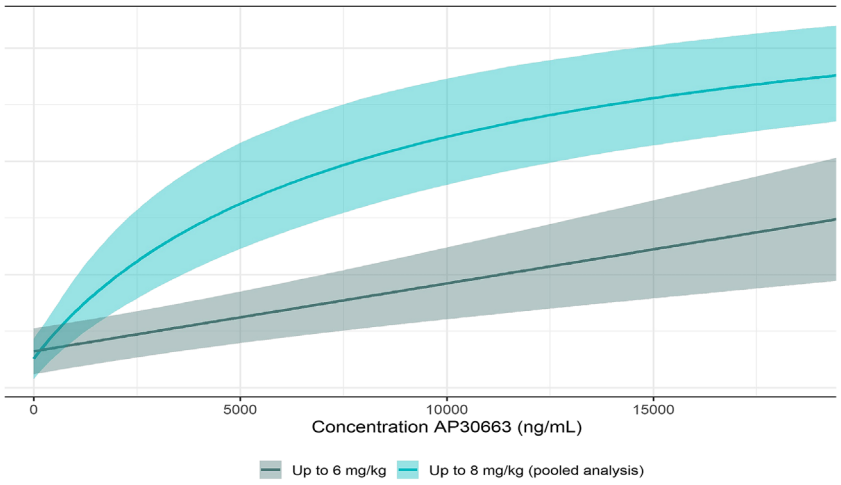


FIGURE 4 Model-predicted association between AP30663 concentration and the QTcF interval for the first-in-human (initial; up to 6 mg/kg) trial and the follow-up (up to 8 mg/kg) trial. Solid line = mean, shaded area = 90% confidence interval. ΔΔQTcF: baseline- and placebo-corrected measure.

TABLE 3 Concentration-QTcF model parameters for both the first-in-human (initial) trial and the current (follow-up) trial.

	First-in-human trial					Follow-up trial				
Parameter	Estimate	SE	RSE %	CI05	CI95	Estimate	SE	RSE %	CI05	CI95
Intercept (ms)	−2.68	2.918	108.9	−8.39928	3.03928	−4.71	2.64	55.99	−9.89	0.459
Slope (ms/[ng/mL])	0.001201	0.000431	35.9	0.00035624	0.00204576					
EC50 current study (ng/mL)	NA	NA	NA	NA	NA	8660	2940	33.94	2900	14 400
EC50 first-in-human study (ng/mL)	NA	NA	NA	NA	NA	35 400	9300	26.24	17 200	53 700
Emax (ms)	NA	NA	NA	NA	NA	71.9	6.58	9.15	59	84.8

Abbreviations: CI05, confidence interval 5% percentile; CI95, confidence interval 95% percentile; EC50, concentration at which 50% of the max effect has been achieved; Emax, max effect; NA, values not available as no Emax was fitted in the initial trial; RSE, relative standard error; SE, standard error.

marker atrial effective refractory period, it is likely that initial efficacy can be seen at a dose of 3 mg/kg, however, as noted in the Introduction, there is potential for increased efficacy up to 8 mg/kg and beyond.[8] All existing antiarrhythmic drugs are associated with a risk of proarrhythmia[13] and future clinical trials in AFib patients with AP30663 will generate data on how AP30663 compares with regard to proarrhythmic risk.

A small increase of the QRS interval with AP30663 was found. This may be due to a slight change in the resting membrane potential through the potassium channel inhibition, causing a secondary inhibitory effect on the fast sodium current.

In the preclinical safety–toxicology studies, AP30663 induced tremor in minipigs and rats at high dose levels and a sensitive assessment with tremorography was included in the present trial. Even though the plasma concentrations in the current trial exceeded those where tremors were observed in preclinical toxicology studies based on AUC in both minipigs and rats and based on C_{max} in rats, all tremorography recordings in the healthy volunteers were normal and unchanged at all dose levels, with a mean amplitude of 0.2 mG^2/Hz, which is nearly 200-fold lower than in Parkinson's disease patients.[14] The results from preclinical animal studies thus did not translate well to humans, since tremor was observed in pigs and rats but not in humans, and the magnitude of QTcF increase was not well predicted.

The present study has the limitation of a small sample size, which constitutes it as an explorative study. Therefore, even though adverse drug reactions were noted, it is not possible from this study to provide definitive answers with regards to safety. In line with this, we were mainly able to assess ECG surrogate markers for proarrhythmia and not proarrhythmia risk itself due to the limited sample size. Also, markers of efficacy were not assessed in the trial.

Only single intravenous doses of AP30633 were tested and results should not be inferred to chronic dosing, where there is a major unmet need for new Afib therapies for maintenance of sinus rhythm. Finally, healthy volunteers constitute a different population than patients with Afib. Risks, including those of an increased QTcF interval, may be expected to be higher in patients than in healthy volunteers.

In conclusion, the present single ascending dose trial found that AP30663 in doses up to 8 mg/kg was associated with mild and transient infusion site reactions and a mean increase in the QTcF interval of 50.4 ms with 8 mg/kg, which was the highest dose tested. No higher doses were tested due to the effects on QTcF.

AUTHOR CONTRIBUTIONS

All authors designed the research. Birgitte Vestbjerg, Juliette van't Westende, Nils Edvardsson, Boukje C. Eveleens Maarse, Michiel J.B. Kemme, Matthijs Moerland, Pim Gal and Anders G. Holst executed the study. Michiel J. van Esdonk and Erica S. Klaassen performed the statistical analyses. Christina Yfanti, Pim Gal and Anders G. Holst drafted the paper. All authors reviewed and approved the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Embargo on data due to commercial restrictions.

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SUPPORTING INFORMATION

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

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