Title Page

FINAL CLINICAL STUDY REPORT

Study Title:	Extension of the randomized, double-blind, placebo-controlled single ascending dose study to assess the safety and tolerability of AP30663 in healthy subjects.
Brief Title:	Extension of the single ascending dose study of AP30663.
Study Number:	CHDR number: CHDR2008 Sponsor number: AP30663-1002
Study Phase:	1
Investigational Product:	AP30663
Brief Description:	The study comprised two randomized, double-blind, placebo-controlled, single ascending dose cohorts to assess the safety and tolerability of AP30663 in healthy male participants.
Study Sponsor:	Acesion Pharma ApS Ole Maaløes Vej 3 DK-2200 Copenhagen N Denmark
Study Initiation Date:	04 November 2020 (signed informed consent by first participant)
Study Completion Date:	23 February 2021 (date of last observation from last participant) The analyses presented in this report are based on a database lock date of 23 March 2021.
Regulatory Agency Identifier Number:	EudraCT number: 2020-003116-27 Toetsingonline number: NL74429.056.20 Independent Ethics Committee number: 056
Report Date:	Version 1.0, 16 June 2021
This study was conducted in compliance with the protocol, the principles of the Declaration of Helsinki (www.wma.net), ICH GCP guidelines (http://www.ich.org/products/guidelines.html) and with the laws and regulations of the country in which the clinical research is conducted.	
This report is confidential. Nothing contained within this report may be disclosed in any way without the prior written permission of the study sponsor.	

Synopsis

Study Title:

Extension of the randomized, double-blind, placebo-controlled single ascending dose study to assess the safety and tolerability of AP30663 in healthy subjects.

Brief Title:

Extension of the single ascending dose study of AP30663.

Study Number:

CHDR number: CHDR2008
Sponsor number: AP30663-1002

Study Phase:

Phase 1

Name of Investigational Product:

AP30663

Study Sponsor:

Sponsor
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Regulatory Agency Identifier Number:

EudraCT number: 2020-003116-27
Toetsingonline number: NL74429.056.20

Principal Investigator, Number of Study Centre(s) and Countries:

This study was conducted at a single centre (Centre for Human Drug Research, Zernikedreef 8, 2333 CL Leiden, The Netherlands) that enrolled participants in The Netherlands.

The Principal Investigator was M. Moerland, PhD.
The Medically Responsible was P. Gal, MD, PhD.

Study Period:

04 November 2020 (signed informed consent by first participant) to 23 February 2021 (date of last observation from last participant). The analyses presented in this report are based on a database lock date of 23 March 2021.

Background and Rationale

Atrial fibrillation (AF) can be an invalidating arrhythmia, with frequent recurrences requiring pharmacological or electrical cardioversion. Current medical maintenance or ablative procedures can be hampered by therapy failure. Additionally, pharmacological cardioversion is unsuccessful in many patients, predominantly in patients with persistent AF. AP30663 is a first-in-class compound targeted at cardioversion of both paroxysmal and persistent AF. The compound inhibits the small conductance Ca^{2+} activated K^{+} channels (SK channels). Blocking these channels is associated with a prolongation of the effective refractory period of atrial myocardial cells both in vitro and in vivo.

AP30663 is currently being developed by Acesion Pharma ApS as a new treatment for cardioversion of AF. The compound is designed to be more effective — resulting in a higher proportion of cardioversion from AF to sinus rhythm — compared with drug treatment options in current clinical practice, and AP30663 is also considered to have potential efficacy in patients with persistent AF.

In a randomized, double-blind, placebo-controlled first-in-human study conducted in healthy participants (AP30663-1002), the safety, tolerability, and PK of AP30663 administered at single doses between 1 and 6 mg/kg in 3 different strengths were assessed. The results indicated that AP30663 was safe and well tolerated up to the 6 mg/kg dose level. Systemically, AP30663 induced a concentration-related, reversible prolongation of the QTcF interval. The exact mechanism for this observation could not be deduced from the data obtained, but an impact on SK channels and hERG channels, as suggested by JpTpc/TpTe analysis, could be anticipated. Other systemic effects, including tremors or an effect on QRS duration, were not observed. Analyses of AP30663 PK indicated less than dose proportional increases in C_{max} between the 1 and 6 mg/kg dose levels.

This CSR reports on the results of an extension to Study AP30663-1002, which was planned to investigate the safety, tolerability, PK, and PD effect on ECG parameters of AP30663 administered at single doses from 6 and up to 12 mg/kg. The rationale for this extension was that increasing the exposure had the potential to increase the intended cardiac pharmacodynamic effects and that a higher dose could be needed to reach optimal clinical efficacy.

Objectives and Endpoints

Objectives	Endpoints
Primary Objective	
To evaluate the safety and tolerability of AP30663 in healthy males at doses up to 12 mg/kg.	• Occurrence of treatment-emergent AEs. • Changes in vital signs, temperature, laboratory safety data and ECGs. • Changes in tremorography data. • Changes in physical examination findings. • Administration site reactions.
Secondary Objective	
To evaluate the pharmacokinetic profile of AP30663.	• C_{max} (free and total) • t_{max} (free and total) • AUC_{inf}, AUC_{last}, AUC% extrapolated • CL, V_z, V_{ss}, and $t_{½}$
Exploratory Objective	
To evaluate the effect of AP30663 on electrocardiographical parameters.	• RR interval • PQ, QT/QTcF interval duration • QT subintervals: QRS duration, J-point–T-peak interval, corrected for heart rate (Jp-Tpc) and T-peak-Tend interval (Tp-Tend) • P, P', QRS, QRS', T, T' wave duration, amplitude, and area • R axis • Atrial and ventricular ectopic beats on Holter recording

Abbreviations: AEs: adverse events, AUC_{inf}: area under the concentration-time curve from time 0 to infinity, AUC_{last}: area under the concentration-time curve from time zero to time of last measurable concentration, AUC% extrapolated: area under the concentration-time curve from time t to infinity as percentage of total area under the concentration-time curve, CL: clearance, C_{max}: maximum concentration, ECGs: electrocardiograms, t_{max}: time to maximum concentration, $t_{½}$: half-life, V_z: volume of distribution during terminal phase, V_{ss}: volume of distribution at steady state.

Methodology:

This study comprised up to 4 randomized, double-blind, placebo-controlled, single ascending dose cohorts to assess the safety and tolerability of AP30663 in healthy men.

AP30663 in a concentration of 3 mg/mL was administered as a linear, continuous, intravenous, 30-minute infusion at planned dose levels as follows:

Cohort 1: up to 6 mg/kg
Cohort 2: up to 8 mg/kg
Cohort 3: up to 10 mg/kg
Cohort 4: up to 12 mg/kg

Each cohort was planned with 8 participants: 2 receiving placebo and 6 receiving AP30663 at the dose level indicated.

The total duration of the study for each participant was up to 51 days, divided as follows:

- Screening: up to 42 days before dosing on Day 1
- Treatment and study assessments: Days -1 to 8, including the in-clinic period from Days -1 to 3
- Follow-up visit: Day 8

Participants were admitted to the study unit on Day -1 and were discharged approximately 48 hours after investigational product administration.

The investigator, sponsor team, all site staff, and everyone else with direct involvement in study conduct remained fully blinded throughout the conduct of the study, with the exception of the study pharmacist, statistician, and the laboratory responsible for PK analyses.

The study employed an adaptive design and an interim report containing safety, tolerability, PK, and PD data at previous dose levels was generated and reviewed by the Safety Review Committee (SRC) before proceeding to the next dose level. In addition, the population PK model of the initial part of Study AP30663-1002 was updated with PK data of previous dose levels and was reviewed before proceeding to the next dose level.

Number of Participants (Planned and Analysed):

Number of Participants (Population)					
Randomized (Planned)	**Randomized (Analysed)**	**Completed**	**Safety**	**PK**	**PD**
16	16	16	16	12	16

Abbreviations: PD = pharmacodynamic; PK = pharmacokinetic.

Main Criteria for Inclusion and Exclusion:

Main inclusion criteria
1. Signed informed consent prior to any study-mandated procedure.
2. Healthy male participants, 18 to 45 years of age, inclusive.
3. Body mass index between 18 and 30 kg/m^2, inclusive, and a body weight between 50 and 100 kg, inclusive, at screening.

Main exclusion criteria
1. Evidence (following a detailed medical history, physical examination, vital signs, 12-lead ECG and clinical laboratory parameters) of any active or chronic disease or condition that could have interfered with, or for which the treatment might have interfered with, the conduct of the study, or that would have posed an unacceptable risk to the participant in the opinion of

the investigator.
2. Clinically significant abnormalities, as judged by the investigator, in laboratory test results (including hepatic and renal panels, complete blood count, chemistry panel and urinalysis). Minor deviations of laboratory values from the normal range were accepted, if judged by the investigator or medically qualified designee as not clinically significant. In the case of uncertain or questionable results, tests performed during screening may have been repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy participants.
3. Positive hepatitis B surface antigen, hepatitis B antibody, hepatitis C antibody, or human immunodeficiency virus antibody at screening.
4. Systolic blood pressure > 140 or < 90 mmHg, and diastolic blood pressure > 90 or < 50 mmHg at screening.
5. Abnormal findings in the resting ECG at screening defined as:
- QTcF > 450 or < 300 msec;
- Notable resting bradycardia (HR < 45 bpm);
- Notable resting tachycardia (HR > 100 bpm);
- Personal or family history of congenital long QT syndrome or sudden death;
- ECG with QRS and/or T wave judged unfavorable for a consistently accurate QT measurement (e.g., neuromuscular artefact that cannot be readily eliminated, arrhythmias, indistinct QRS onset, low amplitude T wave, merged T- and U-waves, prominent U waves);
- Evidence of a sustained atrial or ventricular arrhythmia, either by anamnesis or by Holter or telemetric observation.
- Pre-excitation (Wolff-Parkinson-White syndrome)
- PR interval > 220 msec
6. Routine smoker or history of nicotine abuse (average of > 5 cigarettes per day for > 3 months).

Study Treatments:

Investigational product: AP30663 concentrate for solution for infusion was supplied in a strength of 200 mg/mL, with 5 mL of concentrate in a 10 mL vial. The investigational product was diluted with sterile 5% glucose to a concentration of 3 mg/mL for intravenous administration in the current study.

Placebo: 5% glucose (Manufacturer: B Braun, Melsungen, Germany) solution for intravenous administration.

All study treatment was prepared by the pharmacy at the Leiden University Medical Centre, Leiden, The Netherlands.

Duration of Study Treatment:

Per cohort of 8 participants, 6 were treated with AP30663 and 2 were treated with visually indistinguishable placebo formulations. The study treatments were prepared so that the participants and CHDR staff remained blinded for treatment allocation. AP30663 and placebo were administered as a 30-minute, linear, continuous, intravenous infusion.

Dose levels in the study could be adapted with ongoing assessment of safety, tolerability, and systemic exposure prior to initiation of the next dose level. AP30663 or placebo was administered following an overnight fast.

The following dose levels were planned to be administered:

- Cohort 1: up to 6 mg/kg AP30663 or matching placebo
- Cohort 2: up to 8 mg/kg AP30663 or matching placebo
- Cohort 3: up to 10 mg/kg AP30663 or matching placebo
- Cohort 4: up to 12 mg/kg AP30663 or matching placebo

In practice, the study was halted after administration of the 6 mg/kg (Cohort 1) and 8 mg/kg (Cohort 2) dose levels.

Statistical Methods:

The individual plasma AP30663 concentrations (total concentration and free fraction) are listed by treatment, participant and time. Individual plasma AP30663 concentrations versus time are plotted in panel plots for each treatment using both a linear and log y-axis. The individual plasma AP30663 concentrations (total concentration and free fraction) are summarised (number of samples [n], mean, standard deviation [SD], % coefficient of variation [%CV], median, minimum [min] and maximum [max] values) by treatment and time, and are also presented graphically as mean over time, with standard deviation as error bars. The individual PK parameters (except t_{max}) are summarized (n, mean, SD, %CV, geometric mean, geometric %CV, median, min and max) per treatment group and are presented graphically as boxplots. For t_{max}, the n, median, Min and Max statistics are reported. To establish whether significant treatment effects can be detected on the repeatedly measured PD parameters, each parameter is analyzed with a mixed model analysis of covariance (ANCOVA) with treatment, time and treatment by time as fixed factors and participant as random factor and the (average) baseline measurement as covariate. This model only included data from 25 to 60 minutes after dosing based on the anticipated t_{max} of AP30663.

Participant Disposition:

A total of 49 participants were screened, and 16 participants were enrolled. Treatment compliance was 100%. In total, 16 participants completed the study.

Demographic and Other Baseline Characteristics:

Participants had a mean age of 23.9 years (range: 18 to 38), all 16 participants were white males. Demographics and other baseline characteristics were generally similar among treatment arms.

Exposure:

Treatments were administered to the participants under supervision at the study centre. Treatment compliance was 100%.

Safety Results:

No serious AEs were reported, and all AEs were of mild intensity.

The most common AEs reported during the study were AEs related to infusion site reactions, and all other types of AEs occurred one or two times per treatment group. Infusion site reactions were reported in 7/12 of those treated with AP30663 and in 0/4 of those treated with placebo. There was no clustering of systemic AEs.

Slight and transient increases in CRP values were observed with both doses of AP30663.

Tremorography measurements
No indications of treatment effect on tremorography parameters were observed.

Holter-ECG
An acute increase in the QTcF interval was seen for both doses compared to placebo. The maximum effect was seen after 30-60 minutes with values rapidly decreasing over the course of 1-3 hours and with a return to baseline levels after 24 hours. This time course was anticipated and statistical analyses were prespecified to assess maximum PD effects using data from 25 to 60 minutes after dosing. Based on these analyses an estimated mean QTcF interval prolongation of 45.2 msec (95% CI 31.5 - 58.9) was seen with AP30663 6 mg/kg and 50.4 (95% CI 36.7 - 64.0) with 8 mg/kg, both versus placebo. Point estimates indicate a dose-response although confidence intervals are widely overlapping.

With AP30663 6 mg/kg individual increases up to 64.3 msec (participant 17004) and a QTcF of 463.4 msec (participant 17003) were seen.

With AP30663 8 mg/kg individual increases up to 64.4 msec (participant 18008) and a QTcF of 474.3 msec (participant 18007) were seen.

The majority of the treatment effect on the QTc interval was explained by an effect on the Jp-Tpc interval compared to the Tp-Tend interval.

No statistically significant effects of AP30663 were observed on the HR (AP30663 6mg/kg P=0.6942, AP30663 8 mg/kg P=0.2733), PR-interval (AP30663 6 mg/kg P=0.1606, AP30663 8 mg/kg P=0.1119) and P width (AP30663 6 mg/kg P=0.9656, AP30663 8 mg/kg P=0.1709). The P-value for QRS interval (AP30663 6 mg/kg P=0.0275, AP30663 8 mg/kg P=0.0517) was borderline significant. However, point estimates for treatment effect versus placebo was 3.4 msec for 6 mg/kg and 2.8 for 8 mg/kg, indicating no clinically meaningful effect.

Pharmacokinetic Results:

A pharmacokinetic profile of AP30663 with a moderate level of variability was observed. The mean peak concentrations of free AP30663 for both the 6 and 8 mg/kg dose levels were well beneath the predetermined maximum target free concentration of 2000 ng/mL. Based on C_{max} and AUC parameters, the increase in total AP30663 exposure was more than dose-proportional between the 6 and 8 mg/kg dose levels. There was no indication of a saturable plasma protein binding of AP30663 at either dose level.

Pharmacodynamic Results:

The tremorography and Holter ECG data were analysed as safety as well as PD data and are summarized in the safety results section above.

Conclusions:

No serious AEs were reported, and all AEs were of mild intensity.

The most common AEs reported during the study were AEs related to infusion site reactions, and all other types of AEs occurred one or two times per treatment group. Infusion site reactions were reported in 7/12 of those treated with AP30663 and in 0/4 of those treated with placebo. There was no clustering of systemic AEs.

An acute and transient increase of the Holter QTcF interval was observed with treatment: an estimated mean QTcF interval prolongation of 45.2 msec (95% CI 31.5 - 58.9) was seen with AP30663 6 mg/kg and 50.4 (95% CI 36.7 - 64.0) with 8 mg/kg, both versus placebo. No cardiac arrhythmias were observed. Due to the QTcF effect, no further doses were tested.

A pharmacokinetic profile of AP30663 with moderate levels of variability was observed. The mean peak concentrations of free AP30663 for both the 6 as well as the 8 mg/kg dose levels were well beneath the predetermined maximum target free concentration of 2000 ng/mL. Based on C_{max} and AUC parameters, the increase in total AP30663 exposure was more than dose-proportional between the 6 and 8 mg/kg dose levels. There was no indication of a saturable plasma protein binding of AP30663 for both dose levels.

Date and Version of This Report:

Version 1.0, 07 June 2021