Prediction and modeling of effects on the QTc interval for clinical safety margin assessment, based on Single Ascending Dose study data with AZD3839

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Abbreviations:
Aβ, amyloid beta peptide
Aβ<sub>40</sub>, amyloid-beta peptides of 1-40 amino acids
Aβ<sub>42</sub>, amyloid-beta peptides of 1-42 amino acids
AZD3839, (S)-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1H-isooindol-3-amine hemifumarate
AV, atrioventricular
BACE, β amyloid cleaving enzyme
BSV, between subject variability
Ce, drug concentration in effect delay compartment
Cp, drug concentration in plasma
C.I., confidence interval
Cmax, maximum drug concentration
dECG, 12-lead continuous digital electrocardiogram
DMSO, Dimethylsulfoxide
ECG, electrocardiogram
EC<sub>50</sub>, potency i.e. drug concentration resulting in half of the maximum effect
Emax, maximum drug effect
FOCEI, First order conditional estimation with interaction
FTIM, first time in man
HR, heart rate
hERG, Human Ether-a-go-go-related Gene
IC<sub>50</sub>, drug concentration resulting in half of the maximum inhibition
Ikr, rapid delayed rectifier potassium current
iWRES, individual weighted residuals
ICH, International Conference on Harmonization
MAP, Monophasic Action Potential
MAPD90, Monophasic Action Potential duration at 90% repolarisation
NONMEM, non-linear mixed effects modeling software
PK, pharmacokinetics
PD, pharmacodynamics
PQ interval, distance between the P and Q waves in an ECG
QRS interval, distance between the Q, R and S waves in an ECG
QT interval, distance between the Q and T waves in an ECG
QTc, QT interval corrected
QTcF, QT interval corrected by the Fridericia method
QTcR, heart rate-corrected QT interval
RR interval, distance between two consecutive R waves in an ECG
TQT, Thorough QT study
SAD, Single Ascending Dose
TdP, Torsades de Pointes
CL, drug clearance
Vc, volume of distribution of the central compartment
KA, absorption rate constant
Q, inter-compartmental clearance
Vp, volume of distribution of the peripheral compartment
AMP, amplitude of the cosinus function
PHS, phase shift relative to the time of dose for the cosinus function
Ke0, time delay between drug plasma concentration and QT effect
pcVPC, prediction corrected visual predictive checks
VPC, visual predictive checks

Recommended section: Cardiovascular Pharmacology
ABSTRACT

QTc interval prolongation in humans is usually predictable based on results from preclinical findings. This study confirms the signal from the preclinical cardiac repolarization models (hERG, Guinea pig MAP and dog telemetry) on the clinical effects on the QTc interval. A Thorough QT/QTc (TQT) study is generally required for bioavailable pharmaceutical compounds to determine whether a drug shows a QTc effect above a threshold of regulatory interest or not. However, as demonstrated in this AZD3839 Single Ascending Dose (SAD) study, high resolution digital ECG data in combination with adequate efficacy biomarker and pharmacokinetic data and non-linear mixed effects modeling can provide the basis to safely explore the margins to allow for robust modeling of clinical effect versus the electrophysiological risk marker. We also conclude that a carefully conducted SAD study may provide reliable data for effective early strategic decision making ahead of the TQT study.
Introduction

Blockade of the human cardiac rapid delayed rectifier potassium current ($I_{Kr}$) (also known as the human ether-a-go-go-related gene (hERG) potassium channel) and QT interval prolongation have become surrogate markers for potential pro-arrhythmic effects of pharmaceutical compounds and have received increasing regulatory attention over recent decades (Vik et al., 2008). Drug-induced ion channel mediated prolongation of the heart rate corrected QT interval (QTc) may in particular increase the likelihood of the polymorphous ventricular tachyarrhythmia known as Torsades de Pointes (TdP) (Cavero et al., 2000), that in turn may degenerate into ventricular fibrillation and sudden death (Roden, 2004). After the association between QTc prolongation and risk for TdP/sudden death was established in the mid-1990s, prolongation of the QT interval and/or the development of TdP became the most common reasons for withdrawing or restricting the use of drugs previously approved by the US FDA (Roden, 2004). Regulatory concerns have since been further consolidated because of the enlarging populations at risk, e.g. the elderly who often are more fragile and exposed to polypharmacy with a related increased potential for drug-drug interactions and to patients with underlying cardiac, renal or hepatic impairment that may increase both drug exposure and susceptibility for arrhythmia (Roden, 2004).

A single clinical study dedicated to the evaluation of electrocardiographic effects in general and on repolarization in particular, is expected for all bioavailable pharmaceutical compounds during early development. This study, today often referred to as the “Thorough ECG Study”, is expected to test the effects of a supratherapeutic concentration of the drug that with a reasonable margin covers a predicted worst case scenario (Food and Drug Administration, 2005) on the ECG intervals and morphologies. The study is required by the regulatory authorities to determine whether the drug has a threshold effect on cardiac repolarization, primarily as detected by QT/QTc prolongation. The threshold level of regulatory concern is around 5 msec, as evidenced by the upper bound of the 95% one-sided confidence interval around the maximum mean time-matched effect on the QTc...
interval exceeding 10 msec. The International Conference on Harmonisation (2005) Guidance for Industry on QT prolongation further states that “Drugs that prolong the mean QT/QTc interval by around 5 msec or less do not appear to cause TdP.” It also concludes that the data on drugs that prolong the mean QT/QTc interval by more than around 5 and less than 20 msec are “inconclusive, but some of these compounds have been associated with proarrhythmic risk”. 

An alternative evaluation for internal decision making, prior to an evaluation of the outcome of a TQT study, may be to collect thorough data on relevant biomarkers and ECG intervals already in the first time in man (FTIM) study to allow for early assessment of efficacy and ECG safety margins. This approach requires early predictions of the anticipated therapeutic dose range, and the use of one or more robust biomarkers of expected therapeutic effect.

Preclinically, the hERG assay is used to determine the effect of the test compound on $k_r$ conduction, and the guinea pig monophasic action potential (MAP) duration assay is used as early predictor (Kågström et al., 2007) of QT liability in vivo. At a later stage, effects on QT are determined from ECG recordings in dog. These results are used for early estimations of safety margin, although a more precise quantitative prediction of the level of QT prolongation to be expected in a clinical setting will be determined in human studies. A more precise estimate of the safety margin may be obtained by applying population pharmacokinetics/pharmacodynamics (PKPD) modeling techniques thus taking all data into account (Ollerstam et al., 2006; Lalonde et al., 2007).

AZD3839, (S)-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1H-isoindol-3-amine hemifumarate, is a beta secretase inhibitor with a promising preclinical profile for treatment of Alzheimers Disease (Swahn et al., 2012; Jeppsson et al., 2012). Data from the hERG-assay, the Guinea Pig MAP assay, and QTc interval in dogs collectively predicted a potential risk for QT interval prolongation at exposures close to those expected for clinical efficacy. Therefore, a single ascending dose (SAD) study in healthy subjects was
designed to allow for the exploration of pharmacokinetic effects on the QTc interval using high quality digital ECG as a safety marker, and for effects on $\beta_40$ and $\beta_42$ in plasma as efficacy biomarkers, using a cautious approach of small dose increments through a full range of predicted safe doses in humans.
Materials and Methods

In vitro

Effects on Human Ether-a-go-go-related Gene (hERG) Encoded Potassium Channel. hERG-expressing Chinese Hamster Ovary (CHO) cells were recorded at room temperature in the whole cell configuration of the patch clamp technique. AZD3839 was investigated in 5 cells into each of which was added vehicle solution (0.1% Dimethylsulfoxide, DMSO) and AZD3839 at six ascending nominal concentrations (0.3 to 100 μM). Following application of the highest test concentration, vehicle solution was re-applied to test the reversibility of the effect. The positive control (3 μM cisapride) was then applied. For each cell recorded, data obtained in the presence of the test compound were expressed as a percentage of the first application of vehicle solution. The concentration resulting in 50% of the maximum inhibition (IC50) value is based on test concentrations measured using a high pressure liquid chromatography– tandem mass spectroscopy method.

Cardiac liability Ion channels

AZD3839 dissolved in 0.33% DMSO was tested at several concentrations in electrophysiological assays at 7 types of human recombinant voltage-gated cardiac ion channels: hNav1.5, hCav3.2, hKv1.5, hKv4.3/hKChIP2.2, hKv7.1/hKCNE1, hCav1.2/β2/α2δ and hHCN4. Testing was conducted using an automated, 384-well plate-based electrophysiology device (IonWorks™ ) (Schroeder et al., 2003) and based upon the method described by Bridgland-Taylor et al (2006). In brief, for each experimental “Run” of IonWorks™, the device made perforated whole-cell recordings at ~21°C from cells in a 384-well PatchPlate™. Extracellular and pipette solutions of optimal composition and pH were used for each assay; the latter contained 100 μg/ml amphotericin B (Sigma-Aldrich). For hCav1.2 the pipette solution was supplemented with 5 μM Escin, 2 mM Na2ATP and 0.3 mM Na2GTP (Sigma-Aldrich) and pH was adjusted to 7.2. After attainment of the whole-cell
configuration, a pre-compound current was evoked in each cell in the presence of extracellular solution by a voltage pulse specific to each channel type. For hNav1.5 and hKv1.5 assays, the voltage pulse used enabled an assessment of whether any channel block was use-dependent. Test compounds, vehicle or positive control were then taken from a 96-well plate and added to each well and after ~ 3 minutes the voltage pulse was re-applied to generate a post-compound current. In between the pre- and post-compound voltage pulses there was no clamping of the membrane potential.

In vivo

All animal experiments were performed in accordance with the guidelines of The Swedish National Board for Laboratory Animals under protocols approved by the Ethical Committee of Southern Stockholm, Sweden. Studies were carried out in accordance with the Declaration of Helsinki, and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Monophasic action potential (MAP) duration measurements in the guinea pig

Animals

Male Dunkin–Hartley guinea pigs (Lidköpings Kaninfarm, Lidköping, Sweden) were allowed to acclimatize for at least 7 days prior to study in housing conditions according to AstraZeneca internal standard operational procedures. Animals were kept in groups of 5 per cage (1.75m²) at room temperature (20 ± 2°C) and a relative humidity of 40% to 60%, with free access to food (K1, Lactamin, Stockholm, Sweden) and water (municipal tap water, ad libitum). Bedding material consisted of aspen woodcuttings (TAPVEI, Kortteinen, Finland).

Procedures

The surgical procedures have been described in detail previously (Kågström et al., 2007). Guinea pigs were anaesthetized with pentobarbital and mechanically ventilated. A bilateral vagotomy was performed in the neck region to eliminate autonomic influence on the heart,
and beta blockade was achieved by administering 0.5 mg/kg propranolol intravenously 15 min prior to the start of the experiment. Following a subcutaneous dose of Xylocain® the heart was exposed by a left-sided thoracotomy between the 3rd and 4th sternal rib, and a bipolar electrode was clipped to the left atrial appendage for cardiac pacing (1ms duration, double the diastolic threshold) by means of a constant current stimulator (World Precision Instruments, model A385). The pacing rate was set at approximately 20bpm above the spontaneous sinus rate. A custom-designed bipolar suction electrode was placed near the base of the left ventricular epicardium for recording of MAP signals. MAP duration at 90% repolarization (MAPD90), MAP rise time, and AV conduction time were recorded during cardiac pacing. Vehicle or drug (at 6.35 and 8.32 mg/kg) was infused intravenously during 32 min per dose followed by a washout period, and signals were continuously sampled. Blood samples were taken following each recording for determination of the plasma exposure profile. Data are presented as percent changes (mean ±S.E.M.) from the control recordings, and plasma levels as mean (±S.D.).

Cardiovascular measurements in dogs

Animals

Seven male beagle dogs (12–18 kg; Rååhöjden, Örkelljunga, Sweden) were used. All dogs were trained to accept laboratory procedures prior to study. Dogs were studied at two separate occasions 8 months apart. Four animals were used at each occasion, one animal participated at both. Some dogs had participated in similar studies previously and were allowed a wash-out period of at least 3 weeks.

Procedure

At least four weeks prior to study, dogs were chronically instrumented with radiotelemetry probes to measure arterial blood pressure (BP), body temperature and electrocardiogram with subcutaneous electrodes (ECG; Data Sciences International (DSI), Inc, St. Paul, MN, USA.) Surgical procedures have been described in detail elsewhere (Ollerstam et al., 2007).
Telemetry signals were sampled via receivers (RMC-1, DSI) and saved to a PC using Dataquest Open A.R.T.™ with IOX2 software (EMKA Technologies, Strasbourg, France). Data were collected continuously in 20-s segments for approximately 1 hour before dosing and for up to 24 hours post-dose. In study 2, additional recordings were resumed between 27 and 30 hours and between 32 and 48 hours post dose.

Telemetry data were transformed on a PC with ECG-Auto software (EMKA) for analysis. All parameters are reported at nominal time points (±5 min for the 30 min time interval, otherwise ±10 min) of 0, 0.5, 1, 2, 3, 4, 6, 10, 16, 18 and 23.5 (study 1) or 0, 1, 2, 4, 6, 10, 16, 18, 23.5, 29.5, 34, 36, 40 and 47.5 (study 2) hours after dosing. Time 0 is the mean of 4 pre-dose values (-1, -0.75, -0.5, -0.25 hours). All parameters were averaged over 1 minute; or when not allowed due to data quality, over 20 or 40 sec. Selection of data was made based on stable heart rate (Yang et al., 2001). If the initial automated selection was not considered adequate, another part of the recording within the nominal range was selected manually. The ECG signal was checked for correct positioning of ECG-auto markers, and waveform morphology was reviewed for each animal at each time point.

Systolic, diastolic and mean BP, heart rate (HR), ECG parameters (RR, PQ, QRS, QT) and body temperature were measured. Correction of the QT interval for changes in heart rate is required when analyzing for effects on repolarization, pharmacological as well as physiological. Dogs and humans show differences in resting heart rate range and in heart rate variability and different correction formulas will therefore be required in the two species to optimally normalize the QT interval for variations in HR; i.e. to calculate the QTc. As a consequence of the difference in HR range changes in QTc in the two species will not be directly comparable on a msec by msec level unless corrected and transformed as described. Effects on the heart rate-corrected QT interval in dogs (QTcR) was analysed using the individual regression correction formula: QTcR = QT + β*(60-HR) where β was calculated as the slope of QT versus HR, both of which are derived from the data extracted (Ollerstam et al., 2006). Data are presented as percent changes (mean ±S.E.M.) from the time-matched vehicle-values, and plasma levels as mean ±S.D.
Analysis of AZD3839 plasma levels in dog

For the determination of the plasma concentration of AZD3839, blood samples were taken from the jugular vein prior to dosing and at 0.5, 1, 2, 4, 24 and 30 (study 1) or 1, 2, 4, 6, 24, 30 and 48 (study 2) hours post-dose. One sample was taken prior to and one sample 2 hours after administration of the vehicle, and sham blood sampling was performed in the vehicle experiments to replicate the disturbance in the measured parameters from the AZD3839 experiments.

Blood samples were mixed with K2 EDTA as an anticoagulant and immediately cooled on ice until centrifugation. Plasma was prepared by centrifugation at 4°C for 10 minutes at 1500 g within 30 minutes of blood sampling. Approximately 0.4 ml plasma was transferred to 1.1 mL tubes (Ref. 65-52319BC, caps ref. 65-53101, FluidX Ltd., Nether Alderley, UK) and immediately frozen at or below -70°C. The frozen plasma sample tubes were placed in Roborack (FluidX, Ltd.). The plasma concentration of AZD3839 was determined by ultrafiltration and liquid chromatography followed by mass spectrometric detection (LC-MS/MS). The lower limit of quantification (LLOQ) was set to 0.05 µM. The validated calibration range was 0.05 to 50.0 µM.

Human Study

Single Ascending Dose Study in Healthy Volunteers

The study was performed in accordance with ethical principles based on the Declaration of Helsinki and consistent with the International Conference on Harmonisation (1996) Good Clinical Practice (GCP) guidelines, applicable regulatory requirements, and the AstraZeneca policy on Bioethics and Human Biological Samples. More detailed information regarding the study design PK of AZD3839, effects on the plasma efficacy biomarkers Aβ40 and Aβ42 and PKPD relationship are presented elsewhere (Quartino et al., 2014).

The study was a randomized placebo-controlled SAD study (double-blind within dose
groups) in healthy subjects of 18-55 years of age. In each of the nine dose groups, 6 of the subjects received a single oral dose of AZD3839 and 2 were administered placebo. In each dose group, a sentinel subject was exposed to AZD3839 and one subject was administered placebo on the first day. Provided there were no serious or unexplained safety issues for at least 24 hours post dose as judged by the investigator, the remaining 6 subjects in the dose group were also administered AZD3839 or placebo. Administration of AZD3839 occurred on Day 1 with safety monitoring, including frequent collection of digital and paper ECG and 72 hours telemetry. ECG recordings (RR, PQ, QRS and QT intervals from the primary lead of the digital 12-lead ECG) were performed at the same time-points as AZD3839 plasma concentration sampling (at pre-dose, 20 min, 40 min, and at 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 hours post dose) using a 12-lead continuous digital ECG (dECG) recorder (Schiller Cardiovit CS-200 recorder; Schiller AG, Baar, Switzerland). The QT interval was measured using the tangent method applied on ten 10-sec extractions derived per 5-min recording, at each time point. The pre-dose recording lasted 10 min, and each post-dose recording lasted 5 min. The primary lead chosen for analysis was pre-cordial ventral V2 (with pre-cordial lead ventral V5 as backup in case lead V2 was deemed unsuitable for analysis). The analysis was performed with the internally developed AstraZeneca semiautomatic software application EClysis®, version 3.2 (AstraZeneca, Mölndal, Sweden). In addition to the dECG measurements twelve-lead paper printout ECGs were also recorded at screening, enrollment, at all prescheduled dECG time points for immediate safety assessment during the study day and at follow-up. Effects on the heart rate-corrected QT interval in humans was analyzed using Fridericia’s formula $\text{QTcF} = \frac{\text{QT}}{\sqrt[3]{\text{RR}}}$ (Fridericia, 1920, Roden, 2004, Vik et al., 2008). Serial blood samples for AZD3839 concentrations and efficacy biomarkers (plasma $\beta_1$-40 and plasma $\beta_1$-42) evaluation were drawn up to 72 hours post dose. To be eligible for the study, subjects had to have a QTcF interval of 340-450 msec and have no family history of long QT syndrome. Between each dose group a Safety Review Committee analyzed blinded PK and safety data, including bedside safety ECG's. Digital ECG interval data was also reviewed by a trained cardiologist and were included in the evaluation before
Population Pharmacokinetic/Pharmacodynamic modeling

The PK and QTc data were analyzed using non-linear mixed-effects modeling and the first-order conditional estimation with $\eta$-$\varepsilon$ interaction (FOCEI) using the NONMEM 7.2 software (Icon Development Solutions, Elliot City, MD, USA).

The pharmacokinetics of AZD3839 in dog displayed a two compartment disposition with a first-order absorption and linear elimination. The free fraction in dog was concentration dependent being 6.4% at 0.4 µM, 9% at 10 µM and 15% at 150 µM. Since the highest concentration observed in the dog study was 12.5 µM, an average of 6.5% and 9% equal to 7.75% was used to convert total to free concentration.

In the healthy volunteers study the pharmacokinetics of total plasma concentrations of AZD3839 has been described using a two-compartment PK model with non-linear distribution to a third peripheral compartment, a combined zero and first order absorption process and a linear elimination. The relative bioavailability for AZD3839 was found to be non-linear and resulted in higher than dose-proportional increase in drug exposure with dose. A free fraction of 3.6% was used to convert total to free concentrations in humans (Quartino, et al, 2014).

Between subject variability (BSV) was described by a log-normal variance model and a proportional residual error was applied modeled as additive on log transformed data.

The absolute QTc interval value (QTcR dogs, QTcF healthy volunteers) was related to total plasma concentration of AZD3839 using a sequential modeling approach. First, a placebo model was developed to capture the circadian rhythm seen in QT in the vehicle/placebo group. Secondly, the drug effect model was quantified. During development of the drug effect model, the population parameters of the PK model and the placebo model were fixed.
to the obtained parameter estimates in previous steps and the data was retained in the
dataset (Zhang et al., 2003). The final model was re-estimated and allowed simultaneous
estimation of the placebo and drug effect parameters to obtain the final parameters. For both
species the placebo and drug effect (E) were additive to the estimated QTc baseline value
according to
\[ \text{QTc}(t) = \text{Baseline} + \text{Placebo}(t) + E(C_e) \]  

Eq. 1

The placebo effect was best described by the sum of three cosine functions with time
periods (I) of 24, 8 and 4 hours in dog and 24, 4 and 2 hours in healthy volunteers. Each
cosine function was defined as
\[ \text{AMP} \cdot \cos \left(2\pi \cdot \frac{(t - \text{PHS})}{I}\right) \]  

Eq. 2

where AMP is the amplitude, PHS is the phase shifts relative to the time of dose (t) and I is
the time period. A clear difference in placebo response was seen 10 hours post dose in the
two dog studies. To take this into account, a "study effect" was tested as a categorical
covariate on all parameters in the placebo model and was found significant on PHS for 24
hours.

A delay was seen between the plasma concentration and QTc response in both species and
was captured by an effect compartment model expressed as
\[ \frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \]  

Eq. 3

where the Cp represents the plasma concentration, C_e the concentration in the effect
compartment and ke0 the estimated time delay.

The concentration range was large enough in dogs to quantify both the maximal drug effect
(E_{max}) and the concentration resulting in half of the maximum drug effect (potency, EC_{50})
(Eq 4) while a power model with the estimated parameters slope and the shape-parameter
(\gamma) (Eq 5) was sufficient to describe the concentration-effect relationship in healthy
volunteers.
\[ E(C_e) = \frac{E_{\text{max}} C_e}{(EC_{50}+C_e)} \]  \hspace{1cm} \text{Eq. 4}

\[ E(C_e) = \text{Slope} \cdot C_e \]  \hspace{1cm} \text{Eq. 5}

BSV was described by a log-normal variance model. The covariance matrix for random effects was used to evaluate the correlations between model parameters. In dogs, BSV was found significant on baseline, PHS 24 hour and Emax. In human, BSV was quantified for baseline and residual error magnitude (ETA on Epsilon). The residual variability was described with a proportional residual error model for both species.

Model development and evaluation was based on objective function value (OFV, -2 log-likelihood), precision in parameter estimates and graphical diagnostics. A significance level of p<0.01, corresponding to a decrease of 6.63 in OFV for one degree of freedom, was used through the model development. Graphical visualization of the model fit was performed using the software R with the Xpose 4 library (http://xpose.sourceforge.net/) and for automated estimation and simulation procedures the software perl-speaks-NONMEM (PsN http://psn.sourceforge.net/) was used. The predictive performance of the model was assessed by a prediction corrected visual predictive check, (pcVPC) (Bergstrand et al., 2011) where the observations and predictions (n=2000) were normalized across doses. The robustness of the model and the parameter precision [relative standard error (RSE) %] were assessed by the bootstrap re-sampling method for the clinical data (Lindbom et al., 2005). For each bootstrap, 500 replicated datasets were generated from the original dataset with replacement and stratified by dose and study. For the study in dogs the RSE was calculated based on the SR-matrix obtained in the NONMEM output for the PK model and based on the R-matrix obtained in the NONMEM output for the QTc model.

**Drugs and formulations**

AZD3839 ((S)-1-(2-(difluoromethyl)pyridin-4-yl)-4-fluoro-1-(3-(pyrimidin-5-yl)phenyl)-1H-isooindol-3-amine hemifumarate; MW: 431.4; AstraZeneca R&D, Södertälje, Sweden) and vehicle formulations for oral administration were prepared on the day before the start of the
experiments and were stored refrigerated (+2 to +8°C) in sealed glass bottles, protected from light. AZD3839 was dissolved in 0.3 M Gluconic acid, adjusted to pH 3. Solutions of 0.75, 2.5 and 7.5 mg/ml were prepared and were administered orally by gavage at 2 ml/kg body weight at 1.5, 5 and 15 mg/kg (study 1) and 15 mg/kg (study 2). Guinea-pigs and dogs received vehicle orally 3 days prior to the AZD3839 experiments in both studies.
Results

Preclinical data

The positive control cisapride abolished hERG tail current (data not shown). Normalized hERG tail current plotted against the measured perfusate concentration of AZD3839, and fitted using the non-linear curvefit function of Origin yielded an IC$_{50}$ value (two-sided 95% confidence interval, C.I.) of 4.8 µM (3.8 – 6.2).

At other cardiac ion channels AZD3839 caused IC$_{50}$'s (µM) of 23.6 at hNav1.5 and 62.3 at hCav3.2. Maximum percentage inhibition at 100 µM was 43.1 at hKv4.3 / hKChIP2.2 and 31.4 at hKv 1.5. AZD3839 was inactive (<25 µM) at hCav 1.2/β2/α2δ, hKv7.1 / hKCNE1 and hHCN4.

In the Guinea Pig MAP assay (n=4), AZD3839 caused a maximum mean prolongation (± S.E.M.) of MAPD90 of 10.7% (±1.2) at 37 min after start of infusion resulting in a maximum mean difference from vehicle of 8.6 % ±6.1 (95% C.I.). AZD3839 had no significant effect on other MAPD parameters compared to vehicle treatment. A maximum mean (µM ±S.D.) AZD3839 plasma concentrations (C$_{max}$) of 10.1 ± 3.5 (2.02 ± 0.4, free) was obtained 57 min after start of infusion.

AZD3839 in the dog telemetry study induced a dose dependent increase in plasma concentration (fig. 1a) and dose related increase in heart rate-corrected QT interval (QTcR) (fig. 1b). A maximum mean QTcR prolongation of 29 msec (12 %) was observed at 4 hours following a dose of 15 mg/kg. The C$_{max}$ (µM ±S.D.) of AZD3839 at this dose was 9.22 ± 0.88 (0.71 ± 0.07 free). The no effect dose in this study was 5 mg/kg (4 µM total C$_{max}$ free C$_{max}$ 0.31 µM. The average fraction unbound used for conversion of total to free drug concentration in dog was 7.75%. No arrhythmia was observed and there were no significant changes in heart rate, PQ or QRS intervals.

Clinical data
The SAD study comprised nine dose groups in the dose range 1 to 300 mg. Digital ECG, bedside safety paper ECG and telemetry was performed, and a safety analysis blinded to treatment was done before each dose escalation. A dose dependent increase in plasma concentrations (fig. 1c) and a corresponding QTcF prolongation (fig. 1d) was demonstrated following single oral doses of AZD3839. The mean (range) individual maximum QTcF prolongation was 21 (17-26) msec in the highest dose group (300 mg). The QRS, RR, and PQ intervals showed no significant changes and no arrhythmia was observed. All doses were well tolerated and dose escalation was stopped due to reaching the predefined exposure limit for $C_{\text{max}}$. The pharmacokinetics of AZD3839 in healthy humans was highly variable between subjects, in particular the $C_{\text{max}}$ (mean 0.70 [range 0.34-1.42 for 1 mg] and 3400 [2500-7200] nM for 300 mg). ECGs were closely monitored and no subject had a QTc interval exceeding 450 msec or a change from baseline >30 msec at any measurement, suggesting that the precautions taken (e.g. eligibility criteria, restrictions, rate of dose escalation) and the level of monitoring was appropriate and that the individual risk for proarrhythmia was minimized in this study, i.e. no subject showing a change from baseline >60 msec, or an absolute QTcF exceeding 500 msec.
Concentration-response relationship in dog and human

In order to quantify the concentration-response relationship in dog and healthy subjects, a population modeling approach was used. The population PK model describing the total plasma concentrations of AZD3839 in humans is presented elsewhere (Quartino et al., 2014). In the dog, the total concentrations of AZD3839 were well characterized by a two compartment model with first order absorption and linear elimination. The effects on QTc seen in dog and human were each quantified using a PKPD model where the observed QTc response was modeled as the sum of the vehicle/placebo response and the drug effect. A time delay between peak drug concentration and maximal effect on QTc was seen for both species (fig. 2) and was handled by including an effect delay compartment model.

The estimated population parameters and their precision for the final PK and PKPD models are presented in Tables 1 and 2. The epsilon-shrinkage was low (3.6%, 2.6% and 5.5% for the PK model in dog, QTc model in dog and QTc model in healthy subjects, respectively), indicating that the model accurately can predict individual time-course data for the dogs and healthy subjects in these studies, confirming the appropriateness of the sequential PK and PKPD modeling approach and use of goodness-of-fit plots for model evaluation. The models were evaluated using graphical presentation and internal validation methods, and goodness-of-fit plots showed no trends, confirming that the models satisfactorily can describe and predict real data (fig 2 a, c, e). Further, the pcVPCs showed that the simulated and observed 95% C.I. of the median overlapped (fig 3b,d,f).

The maximal QTc prolongation in dog was estimated to 33 msec (14% at a baseline QTcR of 255 msec) while it could not be quantified in humans in the dose range used in the SAD study. The model-predicted time-course of the QTc changes caused by vehicle/placebo, the true drug effect and the vehicle/placebo plus drug effects (i.e. what is observed in the treated dogs/healthy subjects) following 15 mg/kg in dog and a 300 mg dose in healthy humans are shown in fig. 3. The concentration-effect relationship was very similar for dog and healthy humans when the uncertainty in estimated population parameters were taken into account (fig. 4) In healthy subjects the model estimated that a QTcF prolongation of 5
msec at 131 nM (free 4.7 nM) and 10 msec at 806 nM (free 29 nM) of AZD3839 in plasma. Given the estimated population baseline value for QTcF in humans (363 msec), 5 and 10 msec corresponds to 1.4% and 2.8%, respectively.

**Translational QT diagram**

A graphic translational comparison between hERG, guinea pig MAP, and dog and human QT prolongation was constructed, comparing the percentage change in QT related measures against the free concentrations of AZD3839 (fig. 5). A clear leftward shift of the concentration-effects curves from in vitro (hERG) via guinea-pig, to dog and to human can be seen. Although the expected margin in humans between the predicted minimal therapeutic effect level of at least 20% reduction of $\text{A}_\beta_{42}$ in plasma and QT prolongation based on preclinical data was predicted to be narrow, the SAD study was conducted to determine the magnitude of the expected QT prolongation, to better understand the PK/PD relationship in humans and to confirm or refute a possible therapeutic window. The diagram shows that the preclinical models were concordant and did well predict the QT findings in the SAD study.
Discussion

The main objective of the SAD study was to evaluate safety and tolerability in general, and the PKPD relationship between dose, plasma concentration and effects of AZD3839 on plasma biomarkers (Aβ$_{40}$ and Aβ$_{42}$) and on QTcF. In this paper, we focus on describing the translation of non-clinical to clinical QT-related effects and on presenting the QTcF data from the SAD study.

The preclinical findings of hERG blockade, Guinea Pig MAPD90, and QTc interval prolongation in dog were considered when selecting the starting dose, rate of dose escalation and exposure limit for the SAD study. In spite of a high degree of between subject variability in pharmacokinetics in humans (Quartino et al., 2014), a clear dose-dependent increase in QTc interval prolongation was demonstrated in the SAD study.

The concentration-effect predictions of human QTc prolongation, based on the preclinical findings, were well confirmed in the human study, showing an overlap of the 95% confidence intervals across the entire concentration effect curve for the dog and human data. Further, the hERG and Guinea Pig MAP data served as good predictors of QT prolongation in the dog as well as in humans.

Given the strength of the mechanistic link between hERG block and QTc prolongation, it would be tempting to expect a quantitative relationship between hERG potency and free plasma levels at which QTc increases are seen in man. Whilst there is evidence of a quantitative link between the two (Gintant, 2011, Wallis, 2010), it would be surprising if this was the case for all compounds. It is, after all, a comparison between hERG potency measured in a cell line in a way that cannot completely replicate the channel's behavior in vivo, and a QTc effect in dogs/man that is plotted relative to free plasma levels (as opposed to intracellular ventricular myocyte concentration, which is the effect compartment). Although there is no detailed quantitative comparison of guinea-pig to man/dog QT data, it is unlikely that an increase in MAP duration in an anaesthetized, vagotomized, beta-blocked guinea-pig would necessarily overlay QTc data in conscious dogs/humans; dogs and guinea-pigs have
a species homologue of hERG, but the overall molecular physiology of repolarization is not
the same across the species (Nerbonne and Kass, 2005). Hence, despite the difference in
the hERG/guinea-pig data relative to that of dog/man, it is unlikely, in our opinion, that the
effect is mediated via a non-hERG block mechanism.

It has previously been reported that drugs without reports of TdP in humans have a >30-fold
separation between hERG IC\textsubscript{50} and free plasma concentrations at therapeutic levels
(Redfern et al., 2003). However, drugs with high hERG affinity do not necessarily produce
QTc prolongation in the clinic. A well known exception to this correlation is verapamil, a
potent blocker of the hERG channel but without associated with QTc prolongation or TdP
arrhythmia (Zhang et al., 1999; Yang et al., 2001). Another example is the muscarinic
antagonist tolterodine with a hERG IC\textsubscript{50} of 11 nM and a prolonged action potential duration in
guinea pig cardiomyocytes, but with no QTc prolongation in man (Kang et al., 2004). These
apparent discrepancies are probably due to a concurrent blockade of cardiac calcium
channels by these compounds that may attenuate the QT prolonging effects. In contrast,
AZD3839 did not display significant effects on other cardiac ion channels at therapeutically
relevant concentrations.

Recently, the BACE inhibitor MK-8931 was reported to induce 6-15 msec increases in the
QTc interval following single doses of 300 mg or higher to healthy volunteers (Tseng et al.,
2012). On the other hand, although no numerical values were provided, the discontinued Eli
Lilly BACE inhibitor LY2811376 was reported not to induce any clinically significant
alterations in the ECG in a single-dose study in man (May et al., 2011). The QTc
assessments performed in the AZD3839 SAD-study through the use of high resolution digital
dECG data allowed for detecting modest QTc prolongations of the magnitude of regulatory
concern that are usually not investigated prior to the TQT study.

Using the PKPD model, the safety margin between an anticipated therapeutic effect level
(minimally an average of 20% reduction of A\textsubscript{B42} in plasma) and QTcF prolongation of
regulatory concern (around 5 msec) was also predicted if given twice daily and every hour. A
20% average reduction in plasma Aβ42 was achieved by doses of 110 mg twice daily or 8 mg every hour, yielding steady-state maximum concentrations (Cmax) of 400 nM and 45 nM, respectively (Quartino et al., 2014). At these concentrations a 7 msec and 3.3 msec QTcF prolongation are expected based on the presented PKPD model herein. Thus the model predictions showed that even if the drug was to be administered every hour, mimicking an extended release formulation, an acceptable safety margin would not be obtained. The between and within-subjects variability in PK and PD data further contribute to the narrowing of the safety margin. Moreover, preclinical data suggested both potential induction and inhibition of CYP3A4, which was also shown to be the main route of elimination (data not shown). Therefore, kinetics in repeated dosing are difficult to predict and potential concomitant administration of a strong CYP3A4 inhibitor, such as ketoconazole, would decrease margins even further.

Thus, by using population modeling in combination with careful collection and analysis of multiple replicate digital ECG data, this study established an exposure-response relationship for QTcF within a modest range of QT prolongation, demonstrating the benefits of high quality dECG data generated in the SAD study. The current data confirm the predictability of the nonclinical models to humans, and show that preclinical data together with a carefully designed and monitored SAD study may provide reliable information for an early and science-based development decision.
Acknowledgements

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Authorship Contributions

Participated in research design: Bridgland-Taylor, Bulgak, Kågström, Sjödin, Al-Saffar, Teiling-Gårdlund, Swedberg, Sparve, Paulsson

Conducted experiments: Bridgland-Taylor, Kågström, Sjödin, Bulgak,

Performed data analysis: Bridgland-Taylor, Kågström, Sjödin, Bulgak, Quartino, Tunblad, Sparve, Paulsson, Alexander

Wrote or contributed to the writing of the manuscript: Sparve, Quartino, Swedberg, Lüttgen, Tunblad, Vik, Paulsson, Fältting, Pollard.
References


JPET/2014/215202


Footnotes

Authors were employees of AstraZeneca at the time of the studies.

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Figure legends

Figure 1. Observed geometric mean plasma concentrations of AZD3839 vs. time after a single oral dose of AZD3839 in dog (a) and in human (c). Observed mean QTc change from baseline (msec) vs time in dog (b) and in human (d).

Figure 2. Graphical evaluation of the model fit. The goodness-of-fit plots for (a) PK in dog, (b) QTcR in dog and (c) QTcF in human shows no trends for observed data vs population model predictions (upper left); for observed data vs individual model predictions (upper right); for individual weighted residuals vs individual model predictions (lower left); for conditional weighted residuals vs time after dose (lower right). The prediction corrected visual predictive check for (d) PK and (e) QTcR in dog and (f) QTcF in human are normalized for dose and study differences. Observed data (dots) and the median of the observed data (solid lines) are shown together with the model-simulated median (dashed lines) and the 95% confidence intervals around the model-simulated median (shaded area). For QTcF in humans also the 10th and 90th percentile of the observed and simulated data. Solid lines (black) are the corresponding percentiles of the observed data are shown. The pcVPCs demonstrate that the model can capture the central tendency and variability of the observed data.

Figure 3. The model predicted plasma concentration of AZD3839 (dashed line) and QTc change due to vehicle/placebo effect (dotted line), drug effect (dashed-dotted line) and the vehicle/placebo effects plus drug effect (solid line) for dogs study 1 following a single oral dose of 15 mg/kg (left) and in human in SAD study following a single oral dose of 300 mg (right).

Figure 4. Model predicted concentration-effect relationship for change in QTcR in dog (dashed black line) and change in QTcF in humans (solid grey line) with the corresponding 95% parametric C.I. (shaded areas) accounting for the uncertainty in model parameter
estimates (calculated using the covariance matrix in dog and the bootstrap in humans. A clear overlap can be seen in QTc response between the dog and human indicating the dog being a predictive model for QTc in humans.

Figure 5. Concentration-effect relationship across studies. Percentage inhibition of the hERG channel and percent change on the action potential duration at 90% repolarization in guinea pig plotted as mean (±S.E.M.) based on observed data. Percent changes from baseline in QTcR in dog and in QTcF in human are based on the developed PK/PD models at a QTc baseline of 244 msec in dog and 363 msec in humans. The estimated minimum therapeutic concentration (40 nM, free 1.45 nM) defined as the concentration giving an average of 20% reduction of plasma $\beta_42$ following an extended release formulation at steady state (vertical dotted line).
**Table 1  Population PK and PKPD parameter estimates in dog**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical parameter Estimate</th>
<th>RSE%</th>
<th>90% C.I.</th>
<th>Between subject variability Estimate</th>
<th>RSE%</th>
<th>90% C.I. *</th>
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<tbody>
<tr>
<td>CL</td>
<td>0.56</td>
<td>15</td>
<td>30</td>
<td>39</td>
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<tr>
<td>Vc</td>
<td>0.80</td>
<td>118</td>
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<td>Ka</td>
<td>0.49</td>
<td>91</td>
<td></td>
<td></td>
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<tr>
<td>Q</td>
<td>1.2</td>
<td>79</td>
<td></td>
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<tr>
<td>Vp</td>
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<tr>
<td>Proportional residual error for PK observations (%)</td>
<td>0.53</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSE%</th>
<th>90% C.I.</th>
<th>90% C.I.*</th>
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<tbody>
<tr>
<td>QTcR baseline (msec)</td>
<td>244</td>
<td>0.43</td>
<td>242-246</td>
<td>-0.0023-0.017</td>
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<tr>
<td>AMP(_{24h}), study 1 (-)</td>
<td>4.7</td>
<td>16</td>
<td>6.0-3.5</td>
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</tr>
<tr>
<td>AMP(_{24h}), study 2 (-)</td>
<td>1.0</td>
<td>73</td>
<td>-0.2-2.2</td>
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<tr>
<td>PHS(_{24h}) (h)</td>
<td>8.0</td>
<td>15</td>
<td>6.0-10</td>
<td>-0.048-0.18</td>
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<tr>
<td>AMP(_{4h}) (-)</td>
<td>3.4</td>
<td>19</td>
<td>2.3-4.4</td>
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<tr>
<td>PHS(_{4h}) (h)</td>
<td>8.1</td>
<td>3</td>
<td>7.7-8.5</td>
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<tr>
<td>AMP(_{2h}) (-)</td>
<td>1.3</td>
<td>26</td>
<td>0.7-1.8</td>
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<tr>
<td>PHS(_{2h}) (h)</td>
<td>2.0</td>
<td>22</td>
<td>1.3-2.7</td>
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<tr>
<td>Ke0 (h-1)</td>
<td>0.34</td>
<td>19</td>
<td>0.23-0.44</td>
<td></td>
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<tr>
<td>Emax (msec)</td>
<td>33</td>
<td>14</td>
<td>25-40</td>
<td>-0.01-0.3</td>
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<tr>
<td>EC(_{50}) (nM)</td>
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<td>22</td>
<td>1.0-2.0</td>
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<td>Proportional residual error for QTcR observations (%)</td>
<td>0.027</td>
<td>4</td>
<td>0.025-0.029</td>
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</table>

Abbreviations: CL, clearance; Vc, central volume of distribution; Ka, absorption rate constant; Q, intercompartmental clearance; Vp, peripheral volume of distribution; AMP, amplitude; PHS, phase shifts relative to the time of dose; Ke0, time delay between drug plasma concentration and QT effect; Emax, maximum drug effect; EC\(_{50}\), drug concentration resulting in half of the maximum effect; RSE, relative standard error calculated by the SR (PK parameters) or R (PD parameters) covariance matrix obtained by NONMEM; C.I. parametric confidence interval (5th and the 95th percentile), calculated using the RSE.

* The C.I. is related to the corresponding variance parameter
Table 2  Population PKPD parameter estimates in human

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Typical parameter</th>
<th>Between subject variability</th>
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<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>RSE%</td>
</tr>
<tr>
<td>QTcF baseline (msec)</td>
<td>363</td>
<td>0.5</td>
</tr>
<tr>
<td>AMP&lt;sub&gt;24h&lt;/sub&gt; (-)</td>
<td>2.0</td>
<td>223</td>
</tr>
<tr>
<td>PHS&lt;sub&gt;24h&lt;/sub&gt; (h)</td>
<td>3.6</td>
<td>23</td>
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<tr>
<td>AMP&lt;sub&gt;4h&lt;/sub&gt; (-)</td>
<td>1.2</td>
<td>21</td>
</tr>
<tr>
<td>PHS&lt;sub&gt;4h&lt;/sub&gt; (h)</td>
<td>0.33</td>
<td>55</td>
</tr>
<tr>
<td>AMP&lt;sub&gt;2h&lt;/sub&gt; (-)</td>
<td>1.7</td>
<td>15</td>
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<tr>
<td>PHS&lt;sub&gt;2h&lt;/sub&gt; (h)</td>
<td>1.5</td>
<td>2.5</td>
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<tr>
<td>Ke&lt;sub&gt;0&lt;/sub&gt; (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>3.2</td>
<td>27</td>
</tr>
<tr>
<td>Slope (nM&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.80</td>
<td>27</td>
</tr>
<tr>
<td>γ (-)</td>
<td>0.38</td>
<td>9</td>
</tr>
</tbody>
</table>

Proportional residual error for QTcF observations (%) 0.012 3 0.012-0.013 22 17 15-28

Abbreviations: AMP, amplitude; PHS, phase shifts relative to the time of dose; Ke0, time delay between drug plasma concentration and QT effect; slope, drug effect; γ, shape parameter for the drug effect; RSE, relative standard error derived by non-parametric bootstrap method of 500 samples; C.I., nonparametric confidence interval (5th and the 95th percentile), derived by the bootstrap method of 500 samples.
Figure 3
Figure 5

Graph showing the relationship between AZD3839 unbound concentration (uM) and % change QTC / APD₉₀ and % inhibition hERG. The graph includes lines for Minimal therapeutic conc., Human, Dog, GP MAP, and hERG.