Predicting QRS and PR interval prolongations in humans using nonclinical data

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ABSTRACT

Background and Purpose: Risk of cardiac conduction slowing (QRS/PR prolongations) is assessed prior to clinical trials using *in vitro* and *in vivo* studies. Understanding the quantitative translation of these studies to the clinical situation enables improved risk assessment in the nonclinical phase.

Experimental Approach: Four compounds that prolong QRS and/or PR (AZD1305, flecainide, quinidine and verapamil) were characterised using *in vitro* (sodium/calcium channels), *in vivo* (guinea pigs/dogs) and clinical data. Concentration-matched translational relationships were developed based on *in vitro* and *in vivo* modelling and the *in vitro* to clinical translation of AZD1305 was quantified using an *in vitro* model.

Key Results: Meaningful (10%) human QRS/PR effects correlated to low levels of *in vitro* Nav1.5 block (3-7%) and Cav1.2 binding (13-21%) for all compounds. The *in vitro* model developed using AZD1305 successfully predicted QRS/PR effects for the remaining drugs. Meaningful QRS/PR change in humans correlated to small effects in guinea pigs and dogs (QRS 2.3- 4.6% and PR 2.3-10%), suggesting that worst case human effects can be predicted by assuming four times greater effects at the same concentration from dog/guinea pig.

Conclusion and Implications: Small changes *in vitro* and *in vivo* consistently translate to meaningful PR/QRS changes in humans across compounds, and accurate characterisation of concentration-effect relationships therefore require a model-based approach. Assuming broad applicability of these approaches to assess the safety risk for non–arrhythmic drugs, this study provides means to predict human QRS/PR effects of new drugs using *in vitro* and *in vivo* effects observed in nonclinical studies.

NON-APPROVED ABBREVIATIONS

BSV (between subject variability);
ECG (electrocardiogram);
FTIM (first time in man);
hCav1.2 (human cardiac calcium channel);
hNav1.5 (human cardiac sodium channel);
iv (intravenous);
rCav1.2 (rat cardiac calcium channel);
PD (pharmacodynamic);
PK (pharmacokinetic);
PPB (plasma protein binding);
QTc (heart rate corrected QT)

INTRODUCTION

Adverse effects on vital processes involved in heart function are a major cause of drug withdrawal and late stage attrition (Laverty et al., 2011; Redfern et al., 2010). Important biomarkers for heart function include the duration of key intervals in the electrocardiogram (ECG), such as QT, QRS and PR. Identifying effects on these biomarkers in nonclinical studies is vital for the progression of safe compounds into first clinical trials. Numerous investigations provide insights for predicting risk of prolongation of the heart-rate corrected QT (QTc) interval (Chain et al., 2013; Gintant, 2011; Jonker et al., 2005; Parkinson et al., 2013). Much less is known of the nonclinical to clinical translation of drug-induced conduction slowing manifested as QRS and PR prolongations, despite their association with increased risk of CV mortality and morbidity, especially in risk populations (Nada et al., 2013).

QRS complex duration corresponds to conduction through the ventricular myocardium, and is a predictor of sudden cardiac death (Kurl et al., 2012). In addition, treatment with conduction-slowing drugs (type 1C antiarrhythmics) increased mortality in patients with structural heart disease in the Cardiac Arrhythmia Suppression Trial (CAST) trials (Epstein et al. 1993). Drug-induced QRS widening is primarily linked to inhibition of the sodium ion channel Nav1.5. Recent studies suggest that <10% block of the human Nav1.5 (hNav1.5) may lead to QRS widening in humans (Cordes et al., 2009; Harmer et al., 2011). Despite limitations including use-dependency, nonlinear translation to conduction slowing, variability across laboratories and platforms (Gintant, Gallacher & Pugsley 2011), this suggests that small disturbances in the sodium current are of relevance.

PR interval duration represents time of conduction through the atria and the atrio-ventricular (AV) node and prolongations are associated with increased risk of atrial fibrillations and death in risk populations (Cheng et al., 2009). The primary mechanism for drug-induced PR prolongation is AV block through inhibition of the cardiac L-type calcium (Cav1.2) channel (Nada et al., 2013). In addition to PR prolongation, Cav1.2 block can cause bradycardia (slowed heart beat), reduced contractility and sinus arrest. Potential conduction liabilities may be detected by functional human Cav1.2 (hCav1.2) electrophysiology assays (Cao et al., 2010) or radioligand binding to rat Cav1.2 (rCav1.2) (Morton et al., 2014). Radioligand binding to the diltiazem site of rat Cav1.2 is the most predictive of contractility in canine myocytes *in vitro* compared to radioligand binding at the verapamil and nifedipine sites and conventional and to automated functional hCav1.2 electrophysiology (Morton et al., 2014). It is not known why the radioligand binding assay outperforms the functional assay, and as discussed by

Morton and colleagues, the converse might be expected to be true. For example, the radioligand assay was performed using rat brain Cav1.2 while the functional assay was performed using human cardiac Cav1.2. Also, a functional assay should theoretically detect the effects elicited by binding to any site, while the binding assay is site-specific. PR prolongation may also be caused by Nav1.5 block causing slowed conduction through the atria (P wave prolongation) and/or the His-Purkinje system (Vaughan Williams, 1992). Safety margins have to our knowledge not been suggested for hCav1.2 inhibition or rCav1.2 binding.

During lead identification, different series of molecules are investigated to identify candidate compounds for further optimisation. At this stage, *in vitro* Cav/Nav studies may be conducted and the obtained results (IC_{50}) used, in the context with other data, to drive chemistry and select compounds to progress into *in vivo* studies. Later, *in vivo* investigations of drug-induced effects on CV effects such as ECG intervals and haemodynamics are typically conducted in anaesthetised and/or conscious rats, guinea pigs, dogs and non-human primates (Cros et al., 2012; Erdemli et al., 2012; Heath et al., 2011; Marks et al., 2012), although rats are insensitive to hERG-mediated effects (Mcdermott et al., 2002). During lead optimisation, when a final candidate drug molecule is not yet selected, rodent cardiovascular studies may be conducted to evaluate the CV safety risks of a number of often structurally related molecules, alongside other testing such as efficacy studies. Prior to first time in man (FTIM) studies, ICH S7A/B guidance requires a non-rodent (typically dog or non-human primate) telemetry study to assess cardiovascular risk, including QRS/PR changes, as part of the pre-clinical safety package. Qualitative analyses have confirmed links between hNav1.5 inhibition, conduction in isolated rabbit heart tissue and QRS/PR prolongations in dogs and non-human primates (Erdemli et al., 2012). Also, conscious dog studies identified and differentiated QRS effects of two anti-arrhythmics (Heath et al., 2011). In this work, we wish to expand on this knowledge to investigate **quantitative** *in vivo* to clinical translations of QRS widening or PR prolongations, applying pharmacokinetic-pharmacodynamic (PKPD) and translational modelling. In this study, two approaches to translation were adopted to quantify the translational relationships between nonclinical effects and clinical QRS and PR prolongations. Firstly, empirical (top-down) *in vitro* and *in vivo* to clinical translations were investigated for the anti-arrhythmic compounds AZD1305, flecainide, quinidine and verapamil. In the top-down approach, no assumption was made regarding the nature of the translational relationships, and these were visualised by plotting concentration-matched effects for each compound independently. The translational relationships were used to identify nonclinical effects of each compound corresponding to 10% (appr. 10 ms) QRS widening or 10% (appr. 16 ms) PR

prolongation in humans. Thresholds of 10% effect in humans were selected as such effects were deemed clinically relevant and quantifiable in clinical studies, in the absence of generally accepted thresholds for concern (Nada et al., 2013). Secondly, mechanism-based translation using the operational model (Black and Leff, 1983) was investigated to identify the system parameters linking ion channel effects (measured *in vitro*) to clinical QRS and PR prolongations induced by AZD1305. In the middle-out approach, the *in vitro* to clinical translation is quantified by assuming a model for this relationship. While the empirical translations were investigated for all compounds, middle-out modelling was only performed for AZD1305, as high quality exposure and ECG data were available from a clinical study. In contrast to the first approach, this approach allows direct simulation of clinical effects given the estimated model and any PK curve. Objectives of this study were to *i)* compare the translational relationships between *in vitro, in vivo* and clinical effects on cardiac conduction for four anti-arrhythmic compounds, *ii)* identify nonclinical effects corresponding to 10% QRS and PR prolongations in humans and *iii)* quantify the systems parameters describing the relationship between ion channel effects *in vitro* and clinical QRS and PR prolongations. Results of these analyses will provide a starting point for predicting QRS widening and PR prolongation in humans based on nonclinical observations.

METHODS

Compounds

Four anti-arrhythmic compounds were investigated: the proprietary small molecule AZD1305 (Sigfridsson et al., 2012) and the three marketed anti-arrhythmic compounds flecainide, quinidine and verapamil. AZD1305 is a mixed ion channel blocker (hERG, hNav1.5, rCav1.2) previously in development for the treatment of atrial fibrillations, which was discontinued due to safety concerns regarding QTc prolongations and TdP risk (Rónaszéki et al., 2011). Quinidine, flecainide and verapamil are class 1a, 1c and 4 anti-arrhythmics, respectively.

Nonclinical data

In vivo data were collected from previous studies in routinely conducted AstraZeneca assays in anaesthetised guinea pig (Marks et al., 2012) and conscious dog (Prior et al., 2009). All animal care and experimental procedures had local ethics committee approval and conformed to the *UK Animals (Scientific Procedures) Act, 1986*. Guinea pig and dog studies were

conducted as part of routine safety pharmacology validation work, and not operator/analyst blinded.

Guinea pig telemetry data were available for flecainide and verapamil as these compounds were assessed during assay validation, while no data were available for AZD1305 and quinidine. Details of the experimental setup are described by Marks and colleagues (Marks et al., 2012). Briefly, exposure and CV biomarkers were investigated in sodium pentobarbitone anaesthetised guinea pigs using parallel study designs. Four male Dunkin Hartley guinea pigs (Harlan UK Limited, weight range 496 to 614 g, age 7-8 weeks) were randomised to each treatment and vehicle group. Baseline variability was minimised by controlled body temperature and respiratory rate. Animals were housed in groups of two in cages with Aspen chip bedding and sizzle nest (supplied by Datesand Limited). Dry pellet (Teklad Global Higher Fibre Guinea-pig Diet 2041, Harlan UK Ltd) and water was offered ad libidum, fresh fruits and vegetables daily and environmental enrichment was provided in the form of chew sticks. Temperature was kept within 16-23°C and 12/12 hour light/dark cycles were maintained. Guinea pigs were prepared under continuous sodium pentobarbitone anaesthesia as previously described (Marks et al. 2012). Guinea pigs were artificially ventilated following a tracheotomy and body temperature was controlled using a homeothermic blanket system. Catheters were inserted to into the jugular veins for administration of drug and anaesthetic and for blood sampling and the carotid arteries for monitoring left ventricular and arterial pressure and contractility. Needle electrodes were placed in a lead II configuration for monitoring the ECG. Guinea pigs were allowed to stabilise for 20 minutes following surgical preparation, monitored continuously during anaesthesia and terminated by an overdose of pentobarbitone at the end of the procedure. Lead II ECGs were monitored continuously by needle electrodes during a 20 minute stabilisation period followed by an intravenous infusion of three 15-minute ascending doses and a 30-minute washout period. Exposure data were collected and 1 minute averages of continuous ECG recordings extracted at 10 time points each. Doses, the achieved exposure and the resulting change in QRS and PR interval durations are summarised in **Table 1**.

Details of the experimental setup for the dog telemetry assays are described by Prior et al. (2009) and Bergenholm et al. (2016). Briefly, exposure and CV biomarkers were investigated in conscious male beagle dogs (Dog Breeding Unit, Alderley Park, AstraZeneca, weight 11.2-18.3 kg, age 19-31 months) using cross-over study designs. Animals were housed in groups of four or less except during recording days and feeding when they were housed individually. Pen

temperature was kept within 20±5°C and 12/12 hour light/dark cycles were maintained. Dry pellet (350g SDS-Dog-D3(E) SQC diet (Special Diet Services Ltd) was offered in the afternoon, water provided ad libitum and toys offered for environmental enrichment. Cardiac effects were monitored using telemetry devices (DSI® PhysioTel) surgically implanted under anaesthesia prior to this study as previously described (Prior et al., 2009). The telemetry transmitter had been placed in the abdominal muscle and the ECG electrodes sutured in a lead II configuration across the chest. A minimum of four weeks recovery was allowed between surgery and each study. Animal welfare was monitored using CCTV cameras, by examining all animals for abnormal signs prior to the start of dosing and at each blood sampling time point, and by recording food consumption. Four dogs were orally administered vehicle and each treatment dose in single ascending doses separated by 2-5 days. ECG were extracted as mean values of 5 ECG complexes and exposure collected from 1h pre-dose and at 13 (CV) or 6 (exposure) time points up to 24h post-dose. Doses, achieved exposure and resulting change in QRS and PR interval durations are summarised in **Table 1**.

The relationships between drug concentration and hNav1.5 inhibition were simulated using estimates of concentrations at 50% inhibition (IC_{50}) and Hill coefficients (γ) measured by automated IonWorks electrophysiology using hNav1.5 transfected Chinese hamster ovary cells (Harmer et al., 2008). This assay is routinely conducted at AstraZeneca, and was consistently evaluated at 8 concentrations using physiological pacing rates (3 Hz) for all compounds. Compound interactions with human Cav1.2 were studied by automated electrophysiology (Morton and Main, 2013; Morton et al., 2014) and with brain Cav1.2 from male Wistar rats by radioligand binding to the diltiazem, verapamil and nifedipine sites (Morton et al., 2014) (Table 1). Data from both assays and all binding sites were initially explored, and the estimated concentrations at 50% binding to the diltiazem site (K_i) were chosen to simulate *in vitro* Cav1.2 effects based on these initial results and the findings by Morton and colleagues (Morton et al., 2014).

Clinical data

Exposure, QRS and PR intervals following AZD1305 treatment were collected from a randomised, double-blinded and placebo-controlled phase I study in 29 healthy male volunteers. Subjects were assigned to a dose group and thereafter randomised to placebo or treatment. This study was performed in accordance with the ethical principles of the

Declaration of Helsinki, is consistent with the International Conference on Harmonisation (ICH)/Good Clinical Practice. Details of this clinical study are described elsewhere (Parkinson et al., 2013). Healthy volunteers were administered two separate doses of placebo or AZD1305 (six oral doses (10-500 mg) and two iv doses (10 and 70 mg)). Lead II ECGs were monitored continuously and extracted at baseline and at 18 specific time points and plasma samples were taken pre-dose and at 14 time points within 24 hours following dose administration.

Literature searches were conducted in Pubmed to identify the clinical effects of flecainide, quinidine and verapamil on QRS and PR. Search criteria and references to the identified studies are described in **Supplementary materials 1**. Measured individual exposures together with QRS and PR intervals over time or at pre-dose were rarely reported. Therefore, associated pairs of exposure and QRS and PR change from baseline were collected, such as pairs of maximal exposure and effect or exposure and effect sampled at the same time point. Information in text, tables and/or figures was used to extract the data and percentage change in QRS or PR intervals was converted to change in ms. Collected additional information included number of subjects, dose, route of administration, dosing history and if the subjects were healthy volunteers or patients. Studies of verapamil effects following iv administration were excluded to increase consistency with dog data as verapamil more potently induces PR prolongations following iv compared to oral administration (Reiter et al., 1982), primarily due to different metabolism and potency of its two enantiomers (Echizen et al., 1985a, 1985b).

Plasma protein binding

Free (unbound) plasma concentrations were calculated using *in vitro* estimates of plasma protein binding (PPB) for each compound in guinea pig, dog and human plasma by a standard equilibrium dialysis method (Banker et al., 2003) for all compounds except flecainide, where dog PPB was acquired from Heath et al. (2011). Unbound fractions originated from AZ laboratories, contracting laboratories and literature sources.

Nonclinical to clinical translation

Two approaches were adopted to quantify the translational relationships between nonclinical effects and clinical QRS and PR prolongations (**Figure 1**).

Translation method 1: Top-down (empirical) translation

Empirical translational relationships between *in vitro*, guinea pig or dog effects and effects in humans were investigated for the anti-arrhythmic compounds AZD1305, flecainide, quinidine and verapamil, following the approach visualised in **Figure 1A**.

Exposure-effect relationships for each compound were characterised in each species using PKPD modelling (all guinea pig and dog data, clinical AZD1305 data) or nonlinear regression (clinical literature data for flecainide, quinidine and verapamil). Monolix 4.3.2 (Lixoft) and MATLAB 2013b (The MathWorks) were used to develop and analyse the models. Detailed methods are described in Bergenholm et al., (2016) (dog PKPD models), **Supplementary materials 1** (human regression models) and in **Supplementary materials 2** (guinea pig and human PKPD models). Briefly, a model was developed to describe baseline and drug-induced effects on QRS and PR intervals for each compound in each species. A single phase cosine function was applied to describe potential circadian variations and an RR interval correction model was applied to describe potential changes due to heart rate variations, both at baseline and due to drug effects. Direct and delayed (effect compartment) proportional and E_{max} drug effect models were evaluated. Estimated drug effect parameters were extracted from the selected models to simulate the predicted change from baseline. Assuming no uncertainty in the baseline was required as this information was not available for the literature models. Ion channel effects were simulated using the collected *in vitro* parameters.

The resulting exposure-effect models and the *in vitro* models were used to simulate QRS or PR prolongations, hNav1.5 inhibition or rCav1.2 binding at 100 evenly spaced, matched concentrations within the supported concentration ranges. Each translation was investigated at matching total and unbound concentrations by converting the estimated drug effect parameters accordingly, and in millisecond and percentage change from baseline by scaling the simulated responses. Uncertainty and variability in the estimated drug effects were estimated and visualised by 95% confidence intervals (CIs) for the typical effects and prediction intervals (PIs) for new observations. The CIs provide a range for the estimated average drug effects as predicted by the model, and are useful for cross-species translation as they represent the typical behaviour. As CIs represent uncertainty in typical effects, they get tighter as the amount of data increases. PIs provide a range for new observations, take both variability and residuals into account, and do not get smaller when the amount of data increases. PIs are therefore wider than CIs, and of importance to predict new data. For the population PKPD models, the CIs and PIs were generated using Monte Carlo methods. CIs were constructed from the covariance matrices of the typical parameters for the PD drug effects and PIs from the estimated typical parameters, between-subject variabilities and residual variabilities. 10000 randomly sampled parameter

sets were simulated and sorted at each concentration, and the 2.5th and 97.5th percentiles were extracted. Non-physiological parameter values (e.g., EC_{50} below 0) were removed. CIs and PIs for the regression models based on literature data were produced using the built-in Matlab function *predict*.

Predicted *in vitro*, guinea pig and dog effects were plotted against the predicted human effect at matched total and unbound concentrations to visualise the translations for each compound. Nonclinical effects corresponding to a 10% change in humans were extracted.

Translation method 2: Middle-out (semi-mechanistic) translation

A middle-out translation method was applied to quantify the *in vitro* to clinical translation, where a mathematical description for the translational relationship was assumed and quantified. AZD1305 was selected for this analysis as high-quality, high-resolution clinical data were available, rather than the literature analyses combining many studies. *In vitro* and clinical AZD1305 data were combined to estimate the signal transductions from effects at the ion channel level to clinical QRS or PR prolongations using the operational model of agonism (Black and Leff, 1983) as visualised in **Figure 1B**. The model was applied according to

$$\Delta ECG_d = \frac{E_m(\tau {c_{e,u}}^{\gamma})^n}{({K_d}^{\gamma} + {c_{e,u}}^{\gamma})^n + (\tau {c_{e,u}}^{\gamma})^n} \quad (1)$$

where $c_{e,u}$ is the predicted unbound drug concentration in the effect compartment, K_d the concentration at 50% bound or inhibited receptor, γ the Hill factor of the drug-ion channel interaction, E_m the maximal QRS or PR prolongations possible in the system, τ the transducer ratio and n the exponent of the sigmoidal relationship between bound/inhibited ion channel and QRS or PR prolongation. The transducer ratio τ is the ratio of the maximum inhibited/bound ion channels to the inhibited/bound ion channels corresponding to the half-maximum response. The E_m values could not be estimated from the AZD1305 data, as maximum prolongations were not reached causing practical identifiability issues. To allow estimation of the remaining parameters, the E_m values were therefore fixed. A range of E_m values (20-100 ms) was investigated by performing parameter estimation and simulating the resulting models. K_d and γ were fixed to the *in vitro* estimates describing the ion channel inhibition or binding. Also, baseline variability was minimised as described in **Supplementary materials 2** and an effect compartment was applied to account for the short delay between exposure and QRS and PR effect.

The operational model was developed using *in vitro* and high-quality phase I clinical data for AZD1305. As the operational model has been shown to be structurally identifiable (Janzen et al. 2016), and assuming that the mechanisms of new compounds are similar, the system-specific parameters of this model (E_m, τ and n) may be fixed and effects of such compounds predicted by incorporating the *in vitro* potency (K_d and γ) of the new compounds. Such predictions were produced for flecainide, quinidine and verapamil by combining their specific *in vitro* potencies with the estimated systems parameters. These predictions were then compared to the collected QRS and PR prolongation data from the literature study. This can be viewed as a form of validation of the system-specific parameters, as this evaluates the performance of the system model to predict new data on which it was not trained.

Finally, the systems parameters were used to predict QRS and PR prolongations at 0-100% inhibition/binding and generate 95% confidence intervals for this relationship using Monte Carlo methods similar to the PKPD models. Also, percentage inhibition/binding corresponding to 10% QRS or PR prolongations were extracted.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan et al., 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander et al., 2015).

RESULTS

Nonclinical and clinical data

The acquired data are summarised in **Table 1**, including effects of the investigated compounds on Nav1.5 and Cav1.2 *in vitro* and QRS and PR intervals in humans, dogs and guinea pigs. Changes in heart rate and blood pressure were also observed. Both were slightly increased following AZD1305 treatment in dogs and humans and decreased following quinidine, flecainide and verapamil treatment in dogs and verapamil treatment in guinea pigs, while only heart rate was decreased following flecainide treatment in guinea pigs.

Translation to QRS complex widening in humans

Translation method 1: Top-down translation to clinical QRS widening

PKPD or regression models were developed to describe drug-induced QRS effects for all compounds, and the parameters describing the drug effects were extracted and simulated to generate the CIs and PIs (**Figure 2, Table 2**). QRS effects of AZD1305, flecainide and quinidine were described by proportional models in dogs, while effects of flecainide in guinea pigs were better described by an effect compartment power model. QRS prolongations by AZD1305 and quinidine in humans were captured by proportional models, while a sigmoid model better described the larger prolongations reached following treatment with flecainide. The wide PIs indicate large variability and residuals in the data sets. Details of the PKPD and regression modelling results are described in **Supplementary materials 1** (human regression models) and **Supplementary materials 2** (human and guinea pig PKPD models) and in Bergenholm et al. (2016) (dog PKPD models).

Simulated QRS widenings in humans were plotted against *in vitro* and *in vivo* effects at matched total or unbound exposures to visualise the translational relationships for each compound. Uncertainty in the mean predictions and variability in the data were visualised by overlaying the CIs and PIs, respectively. Nonclinical effects corresponding to 10% QRS widening in humans were extracted. Typical QRS widenings of 10% occurred at unbound concentrations corresponding to 3-7% (CI range 2-9%) hNav1.5 inhibition *in vitro* (**Figure 3A**). This indicates that conduction liabilities may occur well below the IC_{50} of a compound, where Hill factors have large impact. Hill factors were 0.75-1.2 for the investigated compounds. Assuming Hill factors of 1 resulted in considerably less consistent translational relationships (2-10% hNav1.5 inhibition compared to 3-7% when Hill factors were included). CIs for AZD1305 and quinidine were overlapping, whilst QRS widening by flecainide were larger at equal *in vitro* changes. Accounting for the fractions unbound was vital for consistent *in vitro* to human translational relationships between the compounds.

For the *in vivo* to clinical translations, 10 % QRS widening in humans corresponded to 4.6 % (CI range: 2.1-9.9) in guinea pig (**Figure 3B**) and 2.3-3.3 % (CI range: 0.8-4.5) in dog (**Figure 3C**) at matched total concentrations. The confidence intervals for all three compounds overlapped for the dog to human translation. The guinea pig to human translation was only investigated for flecainide and therefore has lower confidence compared to dogs. Higher sensitivity to detect flecainide changes was observed in guinea pigs compared to dogs, while humans were the most sensitive. QRS interval baselines were shorter in guinea pigs and dogs by approximately 75 and 50 %, respectively. Comparisons of absolute differences therefore

further increased the translational gap. Similar results were acquired for translating effects of total and unbound drug *in vivo* as PPB fractions were similar between the species.

Translation method 2: Middle-out translation to clinical QRS widening Identifiability issues led to high correlation between E_m and τ, and was solved by fixing E_m. Goodness of fit values were improved when E_m was increased from 20 ms to 40 ms, and remained similar up to 100 ms. Simulations of optimised models with fixed E_m values between 40 and 100 ms showed similar predictions up to 20 ms change (Figure in Supplementary Materials 3). As widenings above 20 ms are unlikely to occur in a safety setting (by a drug not intended to cause QRS widening), and highest observed widenings for all investigated compounds were 31 ms, an E_m value of 40 ms was selected. The selected value for E_m influenced the estimated value for τ. The operational model with E_m = 40 ms well described AZD1305-induced QRS widenings (**Figure 4A**). Final estimates for τ was high (8.0±0.4), suggesting an efficient signal transduction with some signal amplification, as the exponent *n* was larger than 1 (1.5±0.1). Baseline and effect compartment parameters were similar to the estimated values in the PKPD models (**Supplementary materials 2**).

In order to test whether the systems properties of AZD1305 could be used in the prediction of other compounds, the systems parameters were combined with *in vitro* potency parameters for flecainide and quinidine and used to predict the QRS widening of these compounds in the measured range of unbound concentrations (**Figure 4B**). QRS widenings induced by quinidine were well predicted while flecainide effects were slightly under-predicted.

The translational relationship between inhibited hNav1.5 and QRS widening in humans was simulated and the CIs and PIs generated (**Figure 4C**). These results indicate that only 6% (CI range: 5-7%) inhibition of hNav1.5 is required to induce 10% QRS widening.

Translation to PR interval prolongation in humans

Translation method 1: Top-down translation to clinical PR prolongation

PKPD or regression models were developed to describe drug-induced PR effects for all compounds, and the parameters describing the drug effects were extracted and simulated to generate CIs and PIs (**Figure 5**, **Table 3**). PR effects of AZD1305 and flecainide in dogs were described by proportional models and verapamil by an E_{max} model, and effects of flecainide and verapamil in guinea pigs were described by effect compartment proportional models. PR prolongations by AZD1305 in humans were captured by a proportional model, while sigmoid

or E_{max} models better described the larger prolongations reached following treatment with flecainide or verapamil. The wide PIs indicate large variability in the data sets. Details of the PKPD and regression modelling results are described in **Supplementary materials 1** (human regression models) and **Supplementary materials 2** (human and guinea pig PKPD models) and in Bergenholm et al. (2016) (dog PKPD models).

Simulated PR prolongations in humans were plotted against *in vitro* and *in vivo* effects at matched total or unbound exposures to visualise the translational relationships for each compound. Nonclinical effects corresponding to 10% PR prolongation in humans were extracted. Typical PR prolongations of 10% occurred at unbound concentrations corresponding to 13-21% (CI range 8-24%) rCav1.2 binding at the diltiazem site *in vitro* (**Figure 6A**). PR prolongations by verapamil were slightly larger compared to AZD1305 and flecainide at equal *in vitro* effects, although the CIs were largely overlapping. Accounting for the PPB was vital for consistent *in vitro* to human translational relationships between the compounds.

For the *in vivo* to clinical translations, 10 % PR prolongation in humans corresponded to a 2.3-4.3 % change in guinea pigs (CI range: 0.3-7.6) (**Figure 6B**) and 2.4-10 % change in dogs (CI range: 1.9-28) (**Figure 6C**) at matched total concentrations. The CIs for flecainide and verapamil overlapped for the guinea pig to human translations and for AZD1305 and verapamil for the dog to human translations, whilst PR prolongations by flecainide were larger in humans for equal prolongations in dogs. Different administration routes were used, and may primarily have influenced the guinea pig to human translation of verapamil, as iv infusion of verapamil induces more PR prolongation in humans compared to oral administration (Reiter et al., 1982).Typical PR interval point baselines were 170 ms in humans, 103 ms in dogs (61 % of human) and 62 ms in guinea pigs (37 % of human), and absolute differences between effects in guinea pigs, dogs and humans were thus larger than relative differences. Similar results were acquired for translating effects of total and unbound drug *in vivo* as PPB fractions were similar between the species.

Translation method 2: Middle-out translation to clinical PR prolongation Practical identifiability issues led to high correlation between E_m and τ, and was solved by fixing E_m. Similar results were obtained, where simulations of optimised models with fixed E_m values between 40 and 100 ms showed similar predictions up to 30 ms change (Figure in Supplementary Materials 3). As highest observed prolongations for all investigated compounds were 56 ms, an E_m value of 60 ms was selected. The operational model with E_m = 60 ms well

described AZD1305-induced PR prolongations (**Figure 7A**) although the high variability in the data led to wider CIs and PIs compared to the QRS model. The final estimate for the system parameter τ was lower for PR compared to QRS and with larger uncertainty (4.0±0.7 vs. 8.0±0.4), reflecting a less efficient signal transduction and reduced precision due to the more variable data. The exponent *n* was estimated to be 2.1±0.2, suggesting some signal amplification. Baseline and effect compartment parameters were similar to the estimated values in the PKPD models (**Supplementary materials 2 and 3**).

The systems parameters were combined with *in vitro* potency parameters for flecainide and verapamil and used to predict the PR prolongation of these compounds in the measured range of unbound concentrations (**Figure 7B**). To account for the different potency and metabolism of the two verapamil enantiomers (Echizen et al., 1985a, 1985b), the efficacy of verapamil was assumed to be mediated only by the more potent S enantiomer. The estimated K_i for verapamil of 0.044 µM was therefore corrected to account to the predicted enantiomer composition *in vivo* by $K_{i,invivo} = K_i*0.5/0.18$ (fraction S enantiomer *in vitro*/fraction S enantiomer *in vivo;* human verapamil ratio: Echizen, Vogelgesang, et al. 1985). PR prolongations induced by flecainide were slightly over-predicted while verapamil effects were well predicted by the model.

The translational relationship between bound rCav1.2 and PR prolongation in humans was simulated and the CIs and PIs generated (**Figure 7C**). These results predict that 15% (CI range: 12-22%) binding of rCav1.2 at the diltiazem site is required to induce 10% PR prolongation.

DISCUSSION AND CONCLUSIONS

Small in vitro interactions lead to relevant QRS/PR prolongations

Translation between *in vitro* effects and QRS/PR change in humans show that relatively low hNav1.5 inhibition (3-7%) and rCav1.2 binding (13-21%) correlate with 10% QRS/PR change (**Figure 3A and 6A**). Translation using the middle-out approach resulted in similar thresholds, strengthening the confidence in the predicted relationships. Since only low inhibition/binding is necessary to induce human QRS/PR changes, using IC_{50} in margin calculations may over- or understate risk when Hill (sigmoidicity) factors are different from 1, as Hill factors have a high impact at these inhibition levels. For example, 10 % inhibition occurs at concentrations 9 times lower than IC_{50} with a Hill factor of 1, but only 4 times lower with a Hill factor of 1.5. Concentrations corresponding to inhibitions leading to a meaningful human change may

therefore provide safer margins, such as 5% hNav1.5 inhibition and 15% rCav1.2 binding. However, technical issues may lead to difficulties measuring these relatively small inhibition levels and to large variability in the range of IC_5-IC_{15} compared to IC_{50}. Considering the full concentration-response curve is therefore important, and extrapolation from IC_{50} values as has been done for unbound C_{max} and hERG channel inhibition (Redfern et al., 2003) may be necessary.

In vitro to clinical translations to human QRS widenings were highly consistent, although QRS widening by flecainide was higher at similar inhibition levels compared to AZD1305 and quinidine (**Figure 3A**). This reflects the mechanisms of action of type 1a and 1c antiarrhythmics (quinidine and flecainide, respectively), which bind to the open state of Nav1.5 (Hondeghem, 1987) and dissociate to the closed states with different rates. Flecainide dissociates slower compared to quinidine (>1500 ms vs. 300-1500ms, Wilde 1998), leading to increased accumulation of Nav1.5 block between heart beats. More Nav1.5 block therefore remains at the beginning of each action potential, causing more QRS widening.

Translation of *in vitro* effects to clinical PR prolongations were relatively consistent between the investigated compounds. Similar inhibition levels resulted in larger PR prolongations for verapamil, possibly resulting from differences in the selectivity of the compounds towards additional binding sites on Cav1.2, as verapamil binds to the verapamil site on Cav1.2 in addition to the diltiazem site (Table 1). Also, while QRS prolongations are strongly linked to the block of a single ion channel, multiple mechanisms contribute to AZD1305-, flecainide- and verapamil-induced PR prolongations that were not taken into account in this work. For example, AZD1305 and flecainide prolong the P wave (by Nav1.5 block) and flecainide also reduces intra-cellular Ca^{2+} release (Bannister et al., 2015; Watanabe et al., 2009).

While the top-down *in vitro* to clinical relationships provide predictions of human effects at specific *in vitro* levels such as the predicted therapeutic C_{max}, they cannot directly be used to predict effects at full PK curves. However, this is possible with the semi-mechanistic approach using the estimated system parameters in combination with *in vitro* (unbound) potency. Such predictions may be used to predict exposure-effect relationships as exemplified in **Figure 4B** and **Figure 7B**, or alternatively over time simulating QRS/PR effects at a predicted PK. While large QRS/PR effects may be under-predicted due to the assumed maximal (E_m) values, such large side effects are unlikely to occur. This approach may also be used to combine all available data (or data only for reference compounds) to estimate systems parameters to predict clinical

effects of unknown entities. The approach has yet to be proven by predicting clinical PR and QRS change using preclinical data of an unknown entity.

QRS and PR prolongations are smaller in dogs and guinea pigs compared to humans

The translational relationships for QRS/PR effects demonstrated smaller changes at matched exposures in the nonclinical species compared to humans. However, across compounds, the effects were consistent, especially for QRS where low percent changes were 3-4 times larger in humans compared to dogs. PR translations were more variable, with human changes 1-4 times larger compared to dogs. Fewer compounds were investigated in guinea pigs, reducing the confidence in these results and limiting the possibility to evaluate the consistency in the translation between compounds. However, guinea pigs did show similar trends as dogs, with lower sensitivity compared to humans.

It is important to note that the levels of effects in dogs and guinea pigs that correspond to meaningful clinical changes of 10% (2-5% for QRS, 2-10% for PR) are well below the effect levels that these studies are typically powered for (guinea pig: 19/21% QRS/PR, Marks et al., 2012). However, this power analysis is based on point-wise statistics, whereas employing a PKPD modelling approach increases sensitivity and specificity (Gotta et al., 2015) as all dose levels and time points are used simultaneously. Conducting PKPD modelling of nonclinical *in vivo* data as a routine analysis is therefore recommended to improve power to identify small QRS/PR effects. Furthermore, nonclinical effects should be evaluated well above the expected therapeutic exposure to ensure that potential side effects in cardiac conduction are developed.

Possible mechanisms for the reduced sensitivity of dogs and guinea pigs

Anatomically, guinea pig and dog hearts are 300 and 6 times lighter than human hearts (Joseph, 1908) and have smaller specialised tissues, e.g. AV node (reviewed in Abolghassem, 2009) resulting in shorter QRS and PR intervals. Therefore, evaluating relative rather than absolute changes from baseline reduces the translational gap between guinea pigs, dogs and humans.

A major assumption is that the *in vitro*, *in vivo* and clinical (unbound) plasma concentrations all are equivalent to the target tissue exposure. For these compounds, the same fraction unbound was applied as the measured PPBs were similar across species, and considered to be within the variability of the assay. However, small errors in these fractions have direct impact on the translational relationships, and high quality data of the free fractions in each species could potentially improve precision in the translational relationships. Errors in PPB are however unlikely to cause the high differences in sensitivity. Exposures at the target

sites may also differ between species due to differences in the distribution to the heart tissue and intra-cellular targets.

The reduced sensitivity of guinea pigs and dogs to conduction slowing is likely to be present at the tissue level as flecainide and quinidine reduce the depolarisation rate more in human atrial tissue compared to guinea pig, rabbit and dog (Wang et al., 1990). It is not known if *in vitro* studies using guinea pig and dog Nav/Cav would indicate reduced potency compared to human Nav/Cav. Cav1.2 is multi-functional with many splice variants (Hofmann et al., 2014) which could potentially differ between species. 94-98% amino acid homology of Nav1.5 between mice, rats, pigs and humans (Zimmer et al. 2002; Blechschmidt et al. 2008) indicate that Nav1.5 is highly conserved between species. However, the relative quantity of different isoforms of Nav vary throughout the conduction system (reviewed in Haufe, Chamberland, & Dumaine, 2007) and between species (Blechschmidt et al., 2008). Also the density of other ion channels may contribute to the differences in sensitivity. This has been suggested for QT prolongation, where higher densities of Kir2.1 and Kmin in dogs increase the repolarisation reserve, reducing repolarisation slowing due to ERG block (Jost et al., 2013). Thus, conduction slowing may differ between species partly due to differences in the relative quantity of ion channel isoforms and splice variants.

Limitations

One major limitation of this work is the low number of compounds investigated the translation to human effects for each endpoint (3 for the *in vitro* and the *in vivo* dog and 1-2 for the *in vivo* guinea pig). Historical studies were used for this analysis, and the number of compounds were therefore limited by the availability of sufficient data in the investigated models. The low number of compounds is a consequence of that compounds with potency against these targets are typically screened out prior to *in vivo* and clinical studies. Also, the data sets were incomplete as data for 2 of the test compounds were not available in guinea pig. Although these limitations may be partly overcome by strengthening each individual translation by applying all *in vitro* and *in vivo* relationships suggested in this work, additional investigations into these translational relationships are important to increase confidence in human predictions. All *in vivo* studies were conducted with small group sizes of at the most four animals per treatment group. However, applying PKPD modelling to analyse these data allow simultaneous analysis of data across treatments and time points, thus increasing both sensitivity and specificity of the analysis (Gotta et al. 2015).

Applying the translational relationships to reduce conduction liabilities

Prior to this study, no quantitative information was available on the relative sensitivity to drug-induced QRS/PR effects in nonclinical species and humans. Although this study is limited by the low number of investigated compounds, it provides a starting point for nonclinical assessment of conduction liabilities and predictions to humans. Improved sensitivity to detect potential liabilities of compounds in drug discovery can reduce animal use, as potentially unsafe drugs can be discontinued at an earlier stage, with clear relevance for the replacement, refinement or reduction (the 3Rs). Compounds with different mechanisms of action were investigated to account for possible compound-specific differences and to achieve a broader applicability of the recommendations and translational relationships of this work. Despite the relatively consistent *in vitro* to clinical translations for the investigated compounds, the influence of drug-ion channel kinetics and other mechanisms on QRS/PR prolongations highlight the importance of also evaluating drug effects *in vivo*.

This study has not defined a threshold for clinical QRS/PR effects that should be avoided, but has considered a 10% change in humans to be meaningful and then observed what the nonclinical *in vitro* or *in vivo* change was at matched concentrations. Resulting nonclinical changes at 10% or any preferred level of change in humans (**Figure 3** and **Figure 6**) may be used as first attempts to define margins for acceptable effects at expected unbound therapeutic concentrations, to be easily applied in early *in vitro* and *in vivo* safety assessment. Before FTIM studies, a more in-depth assessment of the therapeutic dose range may be required, such as clinical simulations of PR/QRS change over time using the predicted human PK. Percent QRS/PR change was up to four times larger in humans compared to guinea pigs and dogs. This suggests that worst case human QRS/PR effects may be predicted by simulating four times larger slopes compared to dogs and guinea pigs, while also accounting for baseline and protein binding differences. To include a measure of uncertainty, a best case scenario may also be predicted by a two times larger (QRS) or the same (PR) slope. Although small distributional delays may be present, QRS and PR effects are likely to be well approximated by a direct effect model. In addition, the *in vitro* system models can complete the risk assessment by predicting QRS/PR effects at the predicted PK. Considering the small compound set (1-3 compounds per nonclinical assay and endpoint), additional analyses should be conducted to strengthen the suggested nonclinical margins and translational relationships. Several independent predictions of clinical effects provides additional confidence and any discordance offers a measure of the uncertainty regarding the human prediction. Therefore, a combined view applying information from *in vitro* and *in vivo* studies is vital to predict cardiac conduction risks before FTIM studies,

using the preliminary translational relationships suggested in this work to build on an integrated package of evidence of clinical QRS/PR risk.

Author Contributions

- L Bergenholm, J Parkinson, N D Evans, M J Chappell and T Collins participated in the design of the modelling research study.
- L Bergenholm, J Parkinson and T Collins acquired the data.
- L Bergenholm performed the literature survey.
- L Bergenholm performed the PKPD and translational modelling research.
- All (L Bergenholm, J Parkinson, J Mettetal, N D Evans, M J Chappell and T Collins) participated in analysing the results.
- All (L Bergenholm, J Parkinson, J Mettetal, N D Evans, M J Chappell and T Collins) participated in writing the manuscript.

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Conflicts of interest

None.

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Figure 1: Two methods for nonclinical to clinical translation. **A.** Top-down translation to empirically assess effects at matched drug concentrations were performed for AZD1305, flecainide, quinidine and verapamil. Resulting translational relationships may be used to identify rough estimates of nonclinical effects that correspond to a clinical safety margin. **B.** Middle-out approach combining compound potency *in vitro* with clinical data to estimate the signal transduction was performed for AZD1305. The estimated signal transduction parameters define the system.

Figure 2: QRS prolongations in guinea pig (top row), dog (middle row) and humans (bottom row) induced by AZD1305 (left column), flecainide (middle column) and quinidine (right column). Data points represent individual healthy animal/human volunteer change from model-predicted QRS baseline against simulated unbound concentration in the plasma (dog) or effect compartment (guinea pig, AZD1305 human) (dots), or associated average unbound exposure-ΔQRS pairs with standard errors where available in healthy volunteers (dark circles) and in patients (light squares). The shaded areas represent the 95% confidence intervals (darker area) and prediction intervals (lighter area). Brown colours represent excluded data (human flecainide).

Figure 3: Top-down translation to QRS widenings in humans from **A.** hNav1.5 inhibition *in vitro*, **B.** QRS widenings in guinea pigs and **C.** QRS widenings in dogs, by AZD1305 (solid lines), flecainide (dashed lines) and quinidine (dashed-dotted lines). Effects of AZD1305 and quinidine in guinea pigs were not available. The shaded areas represent the 95% confidence intervals (CI, darker areas) and prediction intervals (PI, lighter areas) overlaid for all compounds and species.

Figure 4: Middle-out translation of hNav1.5 inhibition to QRS widening in the clinic. **A.** Fit to data and typical parameter estimates for the system parameters for the operational model. **B.** Model predicted and measured effects of flecainide and quinidine in humans. Predictions were generated using the estimated signal transduction parameters and the *in vitro* estimated potency in the hNav1.5 assay. Clinical data were collected from literature studies and represent effects in healthy volunteers (dark green) and patients (light green). **C.** Model predicted translation between hNav1.5 inhibition *in vitro* and QRS widening in humans, highlighting the confidence interval for inhibited ion channel at 10% QRS widening.

Figure 5: PR prolongations in guinea pig (top row), dog (middle row) and humans (bottom row) induced by AZD1305 (left column), flecainide (middle column) and quinidine (right column). Data points represent individual healthy animal/human volunteer change from model-predicted PR baseline against simulated unbound concentration in the plasma (dog) or effect compartment (guinea pig, AZD1305 human) (dots), or associated average unbound exposure-PR pairs with standard errors where available in healthy volunteers (circles) and in patients (squares). The shaded areas represent the 95% confidence intervals (darker area) and prediction intervals (lighter area). Brown colours represent repeated dosing data (human verapamil).

Figure 6: Top-down translation to PR prolongations in humans from **A.** rCav1.2 binding at the diltiazem site *in vitro*, **B.** PR prolongations in guinea pigs and **C.** PR prolongations in dogs, by AZD1305 (solid lines), flecainide (dashed lines) and verapamil (dotted lines). Effects of AZD1305 in guinea pigs were not available. The shaded areas represent the 95% confidence intervals (CI, darker areas) and prediction intervals (PI, lighter areas) overlaid for all compounds and species.

Figure 7: Middle-out translation of rCav1.2 binding at the diltiazem site to PR prolongation in the clinic. **A.** Fit to data and typical parameter estimates for the system parameters for the operational model. **B.** Model predicted and measured effects of flecainide and verapamil in humans. Predictions were generated using the estimated signal transduction parameters and the *in vitro* estimated binding in the rCav1.2 assay. Clinical data were collected from literature studies and represent effects in healthy volunteers (dark blue) and patients (light blue). **C.** Model predicted translation between rCav1.2 binding *in vitro* and PR prolongation in humans, highlighting the 95% confidence interval for percent bound ion channel at 10% PR prolongation.

Table 1: Summary of the acquired nonclinical and clinical data.

Study type		AZD1305	Flecainide	Quinidine	Verapamil
hNav1.5 inhibition in automated patch clamp[a]	IC_{50} (CI, µM)/γ	**34.6** / **0.753**	**5.8** (5.7-5.84) / **1.20**	**8.7** (6.7-11.4) / **1.19**	**8.9** (7.0-11.3) / **1.02**
	n	1	2803	5	4
hCav1.2 inhibition in automated patch clamp[b]	IC_{50} (CI, µM)	>100	18, >33	>33, 57	2.9 (2.7-3.2)
	n	1	2	2	605
Radioligand binding to rCav1.2[b]	K_i (µM) verapamil / nifedipine / diltiazem site	40 / NA / **4.5**	15 / NA / **1.4**	5.6 / NA / **8.4**	0.057 / 3.6 / **0.044**
	n	1	1	1	1
Anaesthetised guinea pig telemetry; parallel design, multiple ascending dose	*n*		4 veh + 4 treat		4 veh + 4 treat
	Dose (mg kg^{-1})		iv: 0, 0.3, 1, 3		iv: 0, 0.1, 0.3, 1
	C_{max} (µM)		2.70 ±0.52		1.97 ±0.26
	QRS_0, QRS_{max} (ms)		24 ±2, 30 ±4		22 ±1, 25 ±2
	PR_0, PR_{max} (ms)		55 ±12, 64 ±12		61 ±6, 77 ±5[c]
	Free drug (%)		57		19.7
Conscious dog telemetry; Latin square, single ascending dose	*n*	4	4	4	4
	Dose (mg kg^{-1})	iv: vehicle, 2.15, 4.3; oral: vehicle, 8.7	oral: 0, 3, 10, 20	oral: 0, 10, 25, 50	oral: 0, 1, 5, 15
	C_{max} (µM)	3.2 ±0.8	4.5 ±2.2	24 ±12 (60)	0.78 ±0.26
	QRS_0, QRS_{max} (ms)	46 ±3, 50 ±5	55 ±5, 64 ±11	54 ±3 (52), 59 ±5 (61)	44 ±2, n.e.
	PR_0, PR_{max} (ms)	108 ±13, 131 ±15	97 ±7, 118 ±11	102 ±10, n.e.	114 ±22, 169 ±45
	Free drug (%)	50	36.9[d]	6.18	18.9
Human telemetry	Study type	Phase I	Literature survey	Literature survey	Literature survey
	n	29	16 studies	15 studies	16 studies
	Dose (*n*)	iv: placebo (4), 10 (4), 70 (3); oral: placebo (14), 10 (4), 30 (4), 90 (4), 180 (4), 360 (5), 430 (4), 500[d] (2) mg	iv: 1.5-2 mg kg^{-1}, 150 mg. oral: 100-600 mg	iv: 3.7-10 mg-kg^{-1}. oral: 3 mg kg^{-1}, 100-2250 mg	oral: 80-480 mg
	C_{max} (µM)	3.4	2.6	12.3	1.7
	QRS_0, ΔQRS_{max} (ms)	97.4, 11.5 ±12.4	92.5, 31	92.5, 18	92.5, -
	PR_0, ΔPR_{max} (ms)	159.4, 14.4 ±12.0	160, 56	160, -	160, 53
	Free drug (%)	63	62.1	12.2	20.7

Data presented as mean ±SD or mean (95% CI). *In vitro* data for the presented translational analyses are marked with bold text. hNav1.5, human Nav1.5 ion channel; hCav1.2, human Cav1.2 ion channel; rCav1.2, rat Cav1.2 ion channel; IC_{50}, concentration at half-maximum effect; γ, Hill factor; K_i, dissociation constant; iv, intravenous; C_{max}, maximal plasma drug concentration (total); QRS_0, QRS at baseline; PR_0, PR at baseline; QRS_{max}, maximal QRS; PR_{max}, maximal PR; ΔQRS_{max}, maximal QRS change from baseline; ΔPR_{max}, maximal PR change from baseline; [a]A. R. Harmer et al., 2008, conventional patch clamp at 3 Hz. [b]Morton et al., 2014. [c]N=2 due to death of 2 animals from compound-related effects. [d]Plasma protein binding data for flecainide in dogs acquired from Heath et al. (2011). [d]N=2 as dose escalation was stopped due to subjects with QTcF>500 ms. One subject was dosed at 360 mg instead.

Table 2: PD/regression models of AZD1305, flecainide and quinidine-induced QRS widenings.

	AZD1305		Flecainide		Quinidine	
	Estimate (SE)	BSV[a] (SE)	Estimate (SE)	BSV[a] (SE)	Estimate (SE)	BSV[a] (SE)
Human	**$\Delta QRS = slope * C_{e,u}$**		**$\Delta QRS = E_{max} C_u^n / (EC_{50}^n + C_u^n)$**		**$\Delta QRS = slope C_u$**	
QRS_0 (ms)	96 (1.08)	5.8 (0.838)				
slope (ms/µM)	11.4 (0.84)	26.1 (1.39)	-		9.57 (1.14)	
E_{max} (ms)	-	-	33.7 (10.8)		-	
EC_{50} (µM)	-	-	0.573 (0.256)		-	
n	-	-	1.65 (0.61)		-	
k_{e0} (h^{-1})	43.1 (27.1)	203 (13.7)	-		-	
Add. res. (ms)	1.02 (0.0241)		-		-	
Dog	**$\Delta QRS = slope * C_u$**		**$\Delta QRS = slope * C_u$**		**$\Delta QRS = slope * C_u$**	
QRS_0 (ms)	46.0 (1.4)	5.9 (2.1)	53.6 (1.5)	5.68 (2.03)	53.3 (1.7)	6.25 (2.23)
slope (ms/µM)	1.93 (0.67)	66.2 (25.2)	5.38 (0.95)	30.8 (14.3)	3.00 (0.25)	-
Add. res. (ms)	1.34 (0.0524)		2.65 (0.11)		2.19 (0.12)	
Guinea pig			**$\Delta QRS = a * C_{e,u}^b$**			
QRS_0 (ms)			21.7 (0.893)	11.6 (2.92)		
slope (ms/µM)			-	-		
a			16.9 (1.66)	-		
b			2.46 (0.365)	23.4 (9.15)		
k_{e0} (h^{-1})			1.6 (0.111)	-		
Add. res. (ms)			0.776 (0.050)	-		

All estimates are mean ±sem. BSV, between subject variability; QRS_0, estimated baseline QRS; *slope*, proportional unbound drug effect; E_{max}, estimated maximal effect; EC_{50}, estimated unbound concentration at 50% effect; *n*, estimated Hill factor; *a* and *b*, estimated parameters of the power model; k_{e0}, estimated rate of distribution to the effect compartment; *Add. res.*, estimated additive residuals for the population models.

Table 3: PD/regression models of AZD1305, flecainide and verapamil-induced PR prolongations.

	AZD1305		Flecainide		Verapamil	
	Estimate (SE)	BSV[a] (SE)	Estimate (SE)	BSV[a] (SE)	Estimate (SE)	BSV[a] (SE)
Human	**$\Delta PR = slope * C_{e,u}$**		**$\Delta PR = E_{max} C_u^n / (EC_{50}^n + C_u^n)$**		**$\Delta PR = E_{max} C_u / (EC_{50} + C_u)$**	
PR_0 (ms)	160 (3.57)	11.7 (1.61)				
slope (ms/µM)	17 (2.57)	51.7 (4.07)	-		-	
E_{max} (ms)	-	-	68.9 (27.2)		53.7 (7.2)	
EC_{50} (µM)	-	-	0.77 (0.43)		0.0317 (0.0144)	
n	-	-	1.57 (0.51)		1 (fixed)	
k_{e0} (h^{-1})	10.5 (2.4)		-		-	
Add. res. (ms)	3.70 (0.087)		-		-	
Dog	**$\Delta PR = slope * C_u$**		**$\Delta PR = slope * C_u$**		**$\Delta PR = E_{max} C_u / (EC_{50} + C_u)$**	
PR_0 (ms)	102 (4)	7.95 (2.85)	95.8 (2.3)	4.76 (1.72)	111 (6.13)	11 (3.91)
slope (ms/µM)	13.8 (1.8)	22.5 (10.0)	11.0 (1.2)	13.3 (10.9)	-	-
E_{max} (ms)	-	-	-	-	105 (9.23)	-
EC_{50} (µM)	-	-	-	-	0.196 (0.0881)	83.6 (30.6)
n	-	-	-	-	1 (fixed)	-
Add. res. (ms)	5.86 (0.23)		4.97 (0.21)		6.84 (0.298)	
Guinea pig			**$\Delta PR = slope * C_{e,u}$**		**$\Delta PR = slope * C_{e,u}$**	
PR_0 (ms)			57 (4.39)	21.8 (5.45)	61.6 (1.64)	7.42 (1.91)
slope (ms/µM)			4.14 (1.84)	82 (31.4)	161 (69.7)	-
k_{e0} (h^{-1})			11.9 (3.62)	-	1.07 (0.801)	86.8 (32.7)
Add. res. (ms)			1.95 (0.124)		2.70 (0.17)	

All estimates are mean ±sem. BSV, between subject variability; PR_0, estimated baseline PR; *slope*, proportional unbound drug effect; E_{max}, estimated maximal effect; EC_{50}, estimated unbound concentration at 50% effect; *n*, estimated Hill factor; *a* and *b*, estimated parameters of the power model; k_{e0}, estimated rate of distribution to the effect compartment; *Add. res.*, estimated additive residuals for the population models.