Training Materials Examples for ICH E14 Q&A 5.1

This example shows a data package for a hypothetical drug to support an integrated risk assessment for ICH E14 Q&A 5.1. The data shown are for illustration purposes only.

Table 1. Integrated Risk Assessment

<table>
<tr><td>QT assessment pathway</td><td>☑ Substitute for thorough QT study (5.1)
☐ Alternative QT study when a thorough QT study is not feasible (6.1)</td></tr>
<tr><td>Clinical QT study findings</td><td>High dose (250 mg x 1): 3.3 (90% CI 2.0, 4.5) ms at mean C_{max}; 1.8-fold the high clinical exposure
Therapeutic dose (50 mg QD): 1.7 (90% CI 1.2, 2.2) ms at mean C_{max}
➢ High clinical exposure was achieved, but a sufficient multiple (2x) was not obtained; therefore, a nonclinical integrated risk assessment can be used as supplementary evidence in lieu of positive control (see Table 1-A).</td></tr>
<tr><td>In vitro findings</td><td>
<table>
<tr><td></td><td>Safety Margin</td><td>Reference Drug Safety Margin</td><td>Best Practice Deviations</td></tr>
<tr><td>Parent</td><td>95x</td><td rowspan="2">51x</td><td>Met best practice</td></tr>
<tr><td>Metabolite 1 (9% of total drug exposure)</td><td>>3369x (5% block at 1000 µM)</td><td>No concentration verification - not expected to affect conclusion of hERG safety margin greater than reference.</td></tr>
</table>
➢ hERG safety margin was higher than the threshold defined based on the safety margins computed under the same experimental protocol for a series of drugs known to cause TdP (see Tables 1-B and 1-C).</td></tr>
<tr><td>In vivo findings</td><td>No QTc prolongation in dogs at 2x the high clinical exposure in QTc study with minimal detectable difference of 10 ms.
➢ No QTc prolongation at exposures of parent compound that exceed high clinical exposures (see Table 1-D). Metabolite 1 not quantified in the in vivo study because it is 9% in humans and not hERG active.</td></tr>
<tr><td>Conclusion</td><td>• Integrated nonclinical assessment showed low risk for QTc prolongation at exposures exceeding the high clinical exposure scenario.
• The clinical and nonclinical assessments can be used as a substitute for a TQT study.</td></tr>
<tr><td colspan="2">Abbreviations: C: concentration; CI: confidence interval; C_{max}: maximum concentration; $C_{max,ss}$: steady state maximum concentration; ECG: electrocardiogram; MDD: minimal detectable difference; µM: micromolar; mg: milligram; min; minutes; mL: milliliter; ms: millisecond; ng: nanogram; PK; pharmacokinetic; TdP: torsade de pointes; Tmax; time of Cmax; QD: once daily; QTc: heart-rated corrected QT interval.</td></tr>
</table>

Table 1-A. Clinical QT Assessment	
High clinical exposure scenario	*The high clinical exposure is with co-administration with a potent CYP3A4/5 inhibitor itraconazole (2.7-fold increase in C_{max}). There are no circulating metabolites >10% of total exposure at steady state.*
Exposure multiple	*The highest dose evaluated in the phase 1 study (250 mg x 1) provide exposures that are about 1.8-fold the high clinical exposure. This dose is the maximal tolerated dose in healthy volunteers (HV).*
Design	*Single acending dose study in HV; 5 dose cohorts (10–250 mg) with 6 active, 2 placebo per cohort*
Baseline	*Day 1 pre-dose ECGs*
ECG acquisition and methodology:	
Digital ECGs	☑ *Yes* ☐ *No*
Replicates	*Average of 3 measurement from non-overlapping 10-second ECGs*
ECG collection	*Pre-dose (-45, -30, and -15 min) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing*
Timing of ECG/PK	*Captures Tmax for parent (1.5 h) and metabolite (2 h). All PK and ECG assessments are within 5 min during the first 2 h and within 15 min from 3 to 24 h post-dosing.*
ECG reading methodology	*Centrally read using semi-automatic algorithm. ECG readers are blinded to subject identifier, treatment and time of ECG collection.*
Concomitant medications	*Concomitant medications are not allowed.*
Results: Exploratory and diagnostic plots to support concentration-response modelling (if applicable)	• *No significant C-QTc relationship using White Paper model; model-based predicted ΔΔQTcF of 3.3 (90% CI 2.0, 4.5) ms at C_{max} (524 ng/mL) for highest dose (250 mg x 1).* • *No findings to suggest model misspecification or hysteresis*

Table 1-A Notes

White paper model described in “Scientific white paper on concentration-QTc modeling” (Garnett, C. et al., J Pharmacokinet Pharmacodyn 2017; doi 10.1007/s10928-017-9558-5) and “Correction to: Scientific white paper on concentration-QTc modeling” (Garnett, C. et al., J Pharmacokinet Pharmacodyn 2018; doi 10.1007/s10928-017-9565-6).

Abbreviations: C: concentration; CI: confidence interval; Cmax: maximum concentration; ECG: electrocardiogram; h: hour; mg: milligram; HV: healthy volunteers; min: minutes; ms: millisecond; PK: pharmacokinetic; QTcF: Fridericia heart rate corrected QT interval; Tmax: time of Cmax; ΔΔQTcF: baseline and placebo adjusted QTcF.

Table 1-B. In vitro hERG Assay Evaluation

Analyte: Parent; Protocol 001		
Best Practice Element	**Deviation / Issue**	**Impact of Deviation / Issue**
Temperature (35 -- 37°C)	None	
Voltage Protocol[1]	None	
Recording Quality[2]	None	
IC_{50} Calculation[3]	None	
Concentration Verification[4]	None	
Positive Control[5]	None	
Negative Control[6]	None	
Good Laboratory Practice	None	
Analyte: Metabolite 1; Protocol 001		
Best Practice Element	**Deviation / Issue**	**Impact of Deviation / Issue**
Temperature (35 -- 37°C)	None	
Voltage Protocol[1]	None	
Recording Quality[2]	None	
IC_{50} Calculation[3]	• Concentrations higher than 1000 µM could not be studied due to solubility issues. • Highest concentration was associated with less than 50% block.	• Not possible to estimate IC_{50} due to limited inhibition at highest concentration (5%). • Not expected to impact interpretation due to high multiple over high clinical concentration (3369x) and minimal block observed (5%).
Concentration Verification[4]	Concentration verification was not performed.	• If there is significant drug loss, IC_{50} could be over-estimated. • At 99% drug loss, the highest concentration 1000 µM would correspond to 34x high clinical instead of 3369x. • Since no block was observed at this concentration (5%) it is not expected that the lack of concentration verification could result in a false negative.

Positive Control[5]	None
Negative Control[6]	None
Good Laboratory Practice	None

Table 1-B Notes

1: Approximate the appropriate elements of a ventricular action potential; evoked at adequate frequencies
2: Adequate voltage control; stability at baseline; steady state inhibition
3: Justification if 50% could not be achieved, selective blocker at high concentration, residual background current subtracted
4: Validated analytical method; samples collected from cell chamber; samples collected from satellite or real experiments; concentration-response relationship with nominal or measured concentrations
5: Positive control is one of the "reference drugs" under Q&A 1.2; two or more concentrations 20-80% block; positive control within expected range
6: Vehicle-control included, includes all non-compound materials in the test solution

Abbreviations: °C: degrees Celsius; IC_{50}: half maximal inhibitory concentration; µM: micromolar

Table 1-C. In vitro Assay Results

Investigational Drug						
	In Vitro Assay[1]	High Clinical $C_{max,ss}$ (ng/mL) [2]	Protein Binding, %[3]	Mol Wt (g/mole)	hERG IC_{50} (µM) / (µg/mL)[4]	Safety Margin[5]
Parent	Protocol-001	291 (265, 319)	1	300	100 µM / 30 µg/mL	104x (**95**, 114)
Positive control: moxifloxacin					85 µM	
Metabolite	Protocol-001	97 (89, 106)	2	350	5% block at 1000 µM / 350 µg/mL	>3682x (**3369**, 4013)
Positive control: ondansetron					1.6 µM	
hERG Safety Margin Threshold Defined by Reference Drugs[12]						
Reference Drugs[6]	In Vitro Assay	Critical Concentration (ng/mL)[7]	Protein Binding, %	Mol Wt (g/mole)	IC_{50} Distribution (µM)[8]	Safety Margin[9]
Moxifloxacin	Protocol-001	1866 (1591, 2188)	40 (37, 43)	401	62 (38, 104); N = 10	23x (13, 39)
Ondansetron		249 (152, 412)	73 (71, 76)	293	1.4 (0.8, 2.6); N = 4	10x (4, 27)
Dofetilide		0.37 (0.24, 0.55)	64 (62, 66)	442	0.01 (<0.01, 0.02); N = 4	44x (16, 117)
Pooled Safety Margin for Reference Drugs[10]						22x (9, 51)
Threshold[11]						>51x

Table 1-C Notes

1: In vitro assay protocol evaluated for best practice in Table B.
2: For the investigational product, include high Clinical Exposure scenario is defined as in ICH E14 Q&A 5.1, i.e., Cmax,ss achieved when the maximum therapeutic dose is administered in the presence of the intrinsic or extrinsic factor (organ impairment, drug-drug interaction, food effect, etc.) that has the largest effect on increasing $C_{max,ss}$. Shown as mean (95% CI).
3: If the protein binding is higher than 99%, use 99% when calculating the free fraction (ICH S7B Q&A 1.2).
4: If the concentration range did not allow for estimating IC_{50}, provide the % block and highest concentration studied, e.g., 10% (1 μM).
5: Safety margin calculated as the IC_{50} normalized to the drug's estimated high clinical concentrations (ICH S7B Q&A 1.2). 95% CI computed using the CI of the high clinical C_{max}. Shown as mean (95% CI).

Example to Derive Safety Margin Threshold from Reference Drugs

6: Predominant hERG blockers with known TdP risk and different electrophysiological properties were used as reference drugs.
7: Critical concentration (CC) for each reference drug was computed from the C-QTc relationship, where CC is the mean concentration that gives a 10-ms mean increase in ΔΔQTc [(10-intercept)/slope]. The posterior distribution for model parameters (intercept and slope by study) was used to quantify the uncertainty in the CC.
8: The IC_{50} distribution is assumed to be log-normal, includes both within- and between-laboratory variability. All laboratories used the same experimental protocol (Protocol-001). N indicates the number of laboratories. Shown as 50th (2.5th, 97.5th) percentile.
9: Safety margin for each drug was computed by sampling from the distributions of CC, IC_{50} and protein binding. Shown as 50th (2.5th, 97.5th) percentile.
10: A random effects meta-analysis was used to derive the pooled safety margin across trials and drugs; shown as 50th (2.5th, 97.5th) percentile.
11: Threshold is defined as the upper 2-sided 95th percentile of the pooled distribution.

12: Considerations to use the preestablished hERG safety margin threshold for the Investigational drug:

- The Investigation drug and reference drugs are evaluated under the same experimental protocol (blue shaded cells).
- The concurrent positive control for each assay is one of the reference drugs used to derive the threshold (orange shaded cells).
- The IC_{50} of positive control, computed from two or more concentrations achieving 20–80% block, is similar to the expected range of IC_{50} under the same experimental protocol (yellow shaded cells).
- Directly compare the lower 95% confidence bound of the hERG safety margin of parent and metabolite to safety margin threshold (green shaded cells).

- If the hERG safety margins of the parent and metabolite are higher than the pre-established threshold, then the in vitro assay indicates a low risk for QT prolongation due to direct hERG block.

Abbreviations: C: concentration; CC: critical concentration; CI: confidence interval; $C_{max,ss}$: maximum concentration at steady state; g: gram; IC_{50}: half maximal inhibitory concentration; µM: micromolar; Mol: molecular; N: number; PK: pharmacokinetic; ss: steady state; TdP: torsade de pointes; Tmax: time of Cmax; Wt: weight

Table 1-D. In Vivo QT Assessment

QT Study							
Exposure	The 10 mg/kg dose provides a 2-fold margin over high clinical exposures						
Design[1]	*Crossover, N=4*						
Species:	*Dogs*						
Historical QTcI Sensitivity:	*MDD: 8 ms (95% CI: 6 ,10)*						
ECG collection	*24-h telemetry*						
ECG reading methodology	*Fully automated*						
PK Collection	*Same study, at 3 h post-dose* *Cmax characterized at same dose levels in Toxicokinetic Study*						
Analysis Methods:							
Data reduction method	*0-3 h, 3-8 h, 8-12 h, 12-18 h, 20-24h after dosing (super-intervals)*						
Analysis methodology	*By-time window using ANOVA*						
HR correction method	*QTcI based on 24 h baseline data in each animal*						
ECG Findings	*No ventricular tachyarrhythmias*						
Summary Findings							
Moiety & Dose	*QTcI Effect Size (ms ± SE)[2]*	*Parent concentration at 3 h (ng/mL)[3]*	*C_{max}-total (ng/mL)[4]*	*C_{max}-free (ng/mL)[5]*	*Protein Binding: Species (%)[6]*	*High Clinical $C_{max,ss}$ (ng/mL)[7]*	*Exposure Ratio[8]*
0.5 mg/kg	1 ± 4	7	10	10	1% (dog) 1% (human)	*291 (95% CI: 265 – 319)*	0.03
3 mg/kg	-3 ± 5	55	60	59			0.2
10 mg/kg	2 ± 3	595	582	576			2.0
MDD[9]	10 ms						

Table 1-D Notes

1: Study design indicates crossover or parallel, sample size, species and historical MDD under same study design. MDD is a statistical indication of the smallest effect size that can be determined in a QTc assay.
2: Indicate unit of effect size: Δ from vehicle (ms). Reference drug effects should be reported in same units
3: Indicate the drug exposure (e.g., mean; total drug) obtained at each dose group in QTc study animals
4: Indicate total drug level (e.g., mean) from a PK study (either in QTc study animals or separate animals)
5: Indicate free (unbound) drug levels (corrected for protein binding in the animal species)
6: Indicate protein binding in the animal species used for the QTc study. If protein binding is higher than 99%, use 99% when calculating the free fraction.
7: For the investigational product, include high clinical exposure as defined in ICH E14 Q&A 5.1, i.e., $C_{max,ss}$ achieved when the maximum therapeutic dose is administered in the presence of the intrinsic or extrinsic factor (organ impairment, drug-drug interaction, food effect, etc.) that has the largest effect on increasing $C_{max,ss}$.
8: Exposure ratio is the ratio of mean $C_{max,}$free: mean High Clinical $C_{max,ss}$ free
9: MDD is calculated from the ANOVA model, *e.g.,* MDD = $t_{\alpha=0.05,df}$*sqrt(2)*Residual/sqrt(N=4)

Abbreviations: ANOVA: analysis of variance; CI: confidence interval; C_{max}: maximal concentration; $C_{max,ss}$: steady state maximal concentration; df: degrees of freedom; h: hour; kg: kilogram; MDD: minimal detectable difference; mL: milliliter; ms: millisecond; ng: nanogram; PK: pharmacokinetic; QTcI: individual heart rate correction

Training Materials for ICH E14 Q&A 6.1

This example shows a data package for a hypothetical drug to support an integrated risk assessment for ICH E14 Q&A 6.1. The data shown are for illustration purposes only.

Table 2. Integrated Risk Assessment

<table>
<tr><td>QT assessment pathway</td><td>☐ Substitute for thorough QT study (5.1)
☑ Alternative QT study when a thorough QT study is not feasible (6.1)

➢ 6.1 pathway is appropriate because doses higher than maximum tolerated dose cannot administered to obtain high clinical exposures and the tolerability prohibit the use of the product in healthy participants.</td></tr>
<tr><td>Clinical QT study findings</td><td>Therapeutic dose (250 mg QD): 3.3 (90% CI 2.0, 4.5) ms at mean $C_{max,ss}$ (145 ng/mL)
➢ Alternative QT clinical study designs should incorporate ECG assessments with as many of the usual “thorough QT/QTc” design features as possible (see Table 2-A).</td></tr>
<tr><td>Clinical adverse events</td><td>In the pooled database of active-controlled clinical trials, there are no reports of TdP, ventricular tachycardia, ventricular fibrillation or flutter, sudden death, syncope or seizures. None of the subjects reported QTc >500 ms or an increase from baseline QTc >60 ms.
➢ No increased rate of adverse events that signal potential for proarrhythmic effects (ICH E14 Section 4).</td></tr>
<tr><td>In vitro findings</td><td><table><tr><td></td><td>Safety Margin</td><td>Reference Drug Safety Margin</td><td>Best Practice Deviations</td></tr><tr><td>Parent</td><td>95x</td><td>51x</td><td>Met best practice</td></tr></table>➢ A hERG safety margin was higher than the threshold defined based on the safety margins computed under the same experimental protocol for a series of drugs known to cause TdP (see Tables 2-B and 2-C)</td></tr>
<tr><td>In vivo findings</td><td>The minimal detectable difference (MDD) in the assay (10 ms) is similar to the reported MDD from historical positive control; therefore, the exposure ratio should be greater than or equal to 3x to have similar sensitivity to clinical QT study based on historical positive control data.
No QTc prolongation was observed at doses 5.0x the high clinical exposures.
➢ The study at 5.0x exposure and MDD of 10 ms had sufficient sensitivity to detect a QTc prolongation effect of a magnitude similar to dedicated clinical QT studies (see Table 2-D).</td></tr>
<tr><td>Conclusion</td><td>The drug has low likelihood of proarrhythmic effects due to delayed repolarization.
a. The nonclinical studies following best practice considerations for in vitro and in vivo studies showed low risk for QTc prolongation. There are no major metabolites.
b. The high-quality ECG data collected in the alternative QT clinical assessment do not suggest QT prolongation, defined as an upper bound of the two-sided 90% confidence interval around the estimated maximal effect on ΔQTc less than 10 ms as computed by the concentration-response analysis.</td></tr>
</table>

	c. The cardiovascular safety database does not suggest increased rate of adverse events that signal potential for proarrhythmic effects.
Abbreviations: C: concentration; CI: confidence interval; Cmax: maximum concentration; Cmax,ss: steady state maximum concentration; ECG: electrocardiogram; h: hour; MDD: minimal detectable difference estimates the study-specific variability; mg: milligram; min; minutes; mL: milliliter; ms: millisecond; ng: nanogram; PK; pharmacokinetic; TdP: torsade de pointes; Tmax; time of Cmax; QD: once daily; QTc: heart-rated corrected QT interval	

Table 2-A. Clinical QT Assessment	
High clinical exposure scenario	*Therapeutic dose is the maximum tolerated dose (250 mg QD) with $C_{max,ss}$ = 145 ng/mL. Compared to subjects with normal renal function, subjects with moderate and severe renal impairment are expected to have approximately 1.5- and 2-fold Cmax based on physiological-based pharmacokinetic modeling. There are no circulating metabolites >10% of total exposure at steady state.*
Exposure multiple	*The highest dose evaluated in the alternative clinical study (250 mg QD) is the therapeutic dose. The exposure margin is 0.5.*
Design	*Single-arm, open-label pharmacokinetic and safety study in 24 subjects from a related patient population. Subjects with renal impoirment were excluded.*
Baseline	*Day 1 pre-dose ECGs*
ECG acquisition and methodology:	
Digital ECGs	☑ *Yes* ☐ *No*
Replicates	*Average of 3 measurement from non-overlapping 10-second ECGs*
ECG collection	*Pre-dose (-45, -30, and -15 min) and 0.5, 1, 1.5, 2, 3, 4, 6 and 12 h after dosing on Day 1 and pre-dose, and 1, 1.5, 2, 3 and 4 h after dosing on Day 5 (when concentrations are at steady-state).*
Timing of ECG/PK	*Captures Tmax for parent (1.5 h). All PK and ECG assessments are within 5 minutes during the first 2 h and within 15 min from 3 to 12 hours post-dosing.*
ECG reading methodology	*Centrally read using semi-automatic algorithm. ECG readers are blinded to subject identifier, treatment and time of ECG collection.*
Concomitant medications	*QTc prolonging medications are not allowed.*
Results Exploratory and diagnostic plots to support concentration-response modelling (if applicable)	• *No significant C-QTc relationship using White Paper model; model-based predicted ΔQTcF of 3.3 (90% CI 2.0, 4.5) ms at $C_{max,ss}$ (145 ng/mL) for 250 mg QD.* • *No findings to suggest model misspecification or hysteresis* • *No QTc >500 ms or increase from baseline >60 ms* • *No premature discontinuations or dose reductions due to QTc prolongation*

Table 2-A Notes

White paper model: described in “Scientific white paper on concentration-QTc modeling” (Garnett, C. et al., J Pharmacokinet Pharmacodyn 2017; doi 10.1007/s10928-017-9558-5) and “Correction to: Scientific white paper on concentration-QTc modeling” (Garnett, C. et al., J Pharmacokinet Pharmacodyn 2018; doi 10.1007/s10928-017-9565-6).

Abbreviations: C; concentration; CI; confidence interval; Cmax; maximum concentration; ECG: electrocardiogram; h: hour; mg: milligram; min; minutes; ms: millisecond; PK; pharmacokinetic; Tmax; time of Cmax

Table 2-B. In vitro hERG Assay Evaluation

Analyte: Parent; Protocol 001		
Best Practice Element	**Deviation / Issue**	**Impact of Deviation / Issue**
Temperature (35 -- 37°C)	None	
Voltage Protocol[1]	None	
Recording Quality[2]	None	
IC_{50} Calculation[3]	None	
Concentration Verification[4]	None	
Positive Control[5]	None	
Negative Control[6]	None	
Good Laboratory Practice	None	

Table 2-B Notes

1: Approximate the appropriate elements of a ventricular action potential; Evoked at adequate frequencies
2: Adequate voltage control; Stability at baseline; Steady state inhibition
3: Justification if 50% could not be achieved, selective blocker at high concentration, residual background current subtracted
4: Validated analytical method; Samples collected from cell chamber; Samples collected from satellite or real experiments; Concentration-response relationship with nominal or measured concentrations
5: Positive control is one of the "reference drugs" under Q&A 1.2; Two or more concentrations 20-80% block; Positive control within expected range
6: Vehicle-control included, Includes all non-compound materials in the test solution

Abbreviations: °C: degrees Celsius; IC50: half maximal inhibitory concentration; µM: micromolar

Table 2-C. In vitro Assay Results

Investigational Drug						
	In Vitro Assay	*High Clinical $C_{max,ss}$ (ng/mL)* [2]	*Protein Binding*[3]	*Mol. Wt (g/mole)*	*hERG IC_{50} (µM)/ (µg/mL)*[4]	*Safety Margin*[5]
Parent	*Protocol-001*	*291 (265, 319)*	*1%*	*300*	*100 µM / 30 µg/mL*	104x (**95**, 114)
Positive Control: Moxifloxacin					*85 µM*	
hERG Safety Margin Threshold Defined by Reference Drugs[12]						
Reference Drugs[6]	*In Vitro Assay*	*Critical Concentration (ng/mL)*[7]	*Protein Binding*	*Mol. Wt (g/mol)*	*IC_{50} Distribution (µM)*[8]	*Safety Margin*[9]
Moxifloxacin	*Protocol-001*	*1866 (1591, 2188)*	*40% (37%, 43%)*	*401*	*62 (38, 104); N = 10*	*23x (13, 39)*
Ondansetron		*249 (152, 412)*	*73% (71%, 76%)*	*293*	*1.4 (0.8, 2.6); N = 4*	*10x (4, 27)*
Dofetilide		*0.37 (0.24, 0.55)*	*64% (62%, 66%)*	*442*	*0.01 (<0.01, 0.02); N = 4*	*44x (16, 117)*
					Pooled Safety Margin for Reference Drug[10]	*22x (9, 51)*
					Threshold[11]	*>51x*

Table 2-C Notes

There is no new information in this table. See Table 1-C Notes.

Abbreviations: C; concentration; CI; confidence interval; $C_{max,ss}$; maximum concentration at steady state; mol; molecular; PK; pharmacokinetic; ss: steady state; TdP: torsade de pointes; Tmax; time of Cmax; Wt: weight

Table 2-D. In Vivo QT Assessment

QT Study							
Exposure	The 30 mg/kg dose provides a 5.0-fold margin over high clinical exposure scenario						
Design[1]	*Crossover, N=4*						
Species:	*Dogs*						
Historical QTcl Sensitivity:	*MDD: 8 ms (95% CI: 6, 10)* *Sensitivity at critical concentration for moxifloxacin: 3.6 ms*						
ECG collection	*24-h telemetry*						
ECG reading methodology	*Fully automated*						
PK Collection	*Same study, at 3 h post-dose* *Cmax characterized at same dose levels in Toxicokinetic Study*						
Analysis Methods:							
Data reduction method	*0-3 h, 3-8 h, 8-12 h, 12-18 h, 20-24h after dosing (super-intervals)*						
Analysis methodology	*By-time window using ANOVA*						
HR correction method	*QTcI based on 24 h baseline data in each animal*						
ECG Findings	*No ventricular tachyarrhythmias*						
Summary Findings							
Moiety & Dose	*QTcI Effect Size (ms ± SE)*[2]	*Parent concentration at 3 h (ng/mL)*[3]	*C_{max}-total (ng/mL)*[4]	*C_{max}-free (ng/mL)*[5]	*Protein Binding: Species (%)*[6]	*High Clinical $C_{max,ss}$ (ng/mL)*[7]	*Exposure Ratio*[8]
3 mg/kg	0 ± 4	55	60	59	1% (dog) 1% (human)	*291 (95% CI: 265, 319)*	0.2
10 mg/kg	2 ± 5	595	582	576			2.0
30 mg/kg	4 ± 3	1550	1455	1440			5.0
MDD	10 ms						
Historical Positive Control Effect							
Moxi 10 mg/kg	5.9 ± 1.3	ND	2980	2116	29 (dog) 40 (human)	Critical Concentration: 1866 ng/mL (free: 1120)	1.9
Moxi 30 mg/kg	17.4 ± 2.8	ND	6730	4778			4.3

Moxi 100 mg/kg	45.5 ± 3.7	ND	18300	12993			11.6

Table 2-D Notes

1: Study Design: Crossover or Parallel, sample size, species and historical MDD for same study design. MDD is a statistical indication of the smallest effect size that can be determined in a QTc assay. Based on the concentration-QTc relationship for moxifloxacin with crossover design, the QTc prolongation at free CC (1120 ng/mL) is 3.6 ms; where QTc = slope*CC+intercept. Therefore, the study design has 1/3 the sensitivity of a clinical QT study if exposures only cover the high clinical exposure scenario, or it would need an exposure ratio of at least 3x to have similar sensitivity as a clinical QT study based on observed MDD.
2: Indicate unit of effect size: Δ from vehicle (ms). Reference drug effects should be reported in same units
3: Indicate the drug exposure (e.g., mean; total drug) obtained at each dose group in QTc study animals
4: Indicate total drug level (e.g., mean) from a PK study (either in QTc study animals or separate animals)
5: Indicate free (unbound) drug levels (corrected for protein binding in the animal species)
6: Indicate protein binding in the animal species used for the QTc study. If protein binding is higher than 99%, use 99% when calculating the free fraction.
7: For the investigational product, include high clinical exposure as defined in ICH E14 Q&A 5.1, i.e., $C_{max,ss}$ achieved when the maximum therapeutic dose is administered in the presence of the intrinsic or extrinsic factor (organ impairment, drug-drug interaction, food effect, etc.) that has the largest effect on increasing $C_{max,ss}$.
8: Exposure ratio is the ratio of mean C_{max} free: mean High Clinical $C_{max,ss}$ free
9: MDD is calculated from the ANOVA model, e.g., MDD = $t_{\alpha=0.05,df}$*sqrt(2)*Residual/sqrt(N=4)
10: Current assay sensitivity evaluation:

- The MDD of the current assay (10 ms) is similar to the reported MDD from historical values in the same laboratory using the same study design [MDD = 8 ms (95% CI: 6, 10)]
- In the same study design, moxifloxacin (a reference compound tested previously) demonstrated dose-related QTcI prolongation and confirmed sensitivity of the assay. To adjust for the difference in moxifloxacin sensitivity between dogs and humans, the exposure ratio should be greater than or equal to 3x to have similar sensitivity as a clinical QT study.
- No QTc prolongation was observed at doses 5.0x the high clinical exposures.

Abbreviations: ANOVA: analysis of variance; CI: confidence interval; C_{max}: maximal concentration; $C_{max,ss}$: steady state maximal concentration; df: degrees of freedom; h: hour; kg: kilogram; MDD: minimal detectable difference; mL: milliliter; ms: millisecond; ng: nanogram; PK: pharmacokinetic; QTcI: individual heart rate correction